0 0301 0019815 6

THE ADRENALS

The ADRENALS

By

ARTHUR GROLLMAN, Ph.D., M.D.

*Associate Professor of Pharmacology and Experimental Therapeutics
and formerly Associate Professor of Physiology, in the Medical
School of The Johns Hopkins University*

BALTIMORE

THE WILLIAMS & WILKINS COMPANY

1936

COPYRIGHT, 1936
THE WILLIAMS & WILKINS COMPANY

Made in the United States of America

Published March, 1936

COMPOSED AND PRINTED AT THE
WAVERLY PRESS, INC.
FOR
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

For
A. P. G.

CONTENTS

LIST OF ILLUSTRATIONS	ix
PREFACE.....	xi

INTRODUCTION

CHAPTER I

An Historical Résumé	1
----------------------------	---

PART I. ANATOMICAL CONSIDERATIONS

CHAPTER II

Gross Anatomy.....	11
--------------------	----

CHAPTER III

Microscopic Anatomy.....	37
--------------------------	----

CHAPTER IV

The Androgenic Tissue	57
-----------------------------	----

PART II. THE MEDULLA

CHAPTER V

The Chemistry of Epinephrine.....	73
-----------------------------------	----

CHAPTER VI

The Physiology of The Medulla.....	87
------------------------------------	----

CHAPTER VII

The Pharmacology of Epinephrine	113
---------------------------------------	-----

PART III. THE CORTEX

CHAPTER VIII

Adrenalectomy	141
---------------------	-----

CHAPTER IX

The Pathological Physiology of Adrenal Insufficiency.....	161
---	-----

CHAPTER X

The Anatomical Pathology of Adrenal Insufficiency	172
---	-----

CHAPTER XI

The Blood Chemistry of Adrenal Insufficiency.....	179
---	-----

CHAPTER XII

The Adrenals and Carbohydrate Metabolism.....	197
---	-----

CHAPTER XIII

The Relation of the Adrenals to the Metabolism.....	205
---	-----

CHAPTER XIV

The Relation of the Adrenals to Other Endocrine Organs.....	209
---	-----

CHAPTER XV

The Preparation of the Cortical Hormone.....	231
--	-----

CHAPTER XVI

The Assay of the Cortical Hormone.....	248
--	-----

CHAPTER XVII

The Relation of the Adrenals to Toxins, Infection, and Immunity.....	264
--	-----

CHAPTER XVIII

The Adrenals and Surgical Shock.....	271
--------------------------------------	-----

CHAPTER XIX

Grafts and Other Surgical Procedures on the Adrenals.....	278
---	-----

CHAPTER XX

Chemical Constituents of the Adrenals.....	286
--	-----

PART IV. CLINICAL CONSIDERATIONS

CHAPTER XXI

Addison's Disease.....	299
------------------------	-----

CHAPTER XXII

Tumors of the Adrenals.....	329
-----------------------------	-----

CHAPTER XXIII

The Adreno-genital Syndrome.....	339
----------------------------------	-----

CHAPTER XXIV

Other Diseases of the Adrenals.....	356
-------------------------------------	-----

EPILOGUE.....	367
---------------	-----

BIBLIOGRAPHY.....	369
-------------------	-----

INDEX.....	401
------------	-----

LIST OF ILLUSTRATIONS

1. The Kidneys and Adrenals of the Frog.....	17
2. The Adrenals of Various Mammals.....	21
3. The Accessory Adrenals of the Cow.....	33
4. Microscopic Section of the Rat's Adrenal.....	41
5. Microscopic Section of the Adult Human Adrenal.....	45
6. Microscopic Section of an Accessory Interrenal Body.....	49
7. Microscopic Appearance of the Medullary Cells of the Rat's Adrenal...	51
8. Cross-Section Views of the Adrenals of the Mouse.....	65
9. Involution of the Androgenic Zone of the Mouse Adrenal.....	67
10. Changes in Physiological Functions during Adrenal Insufficiency....	165
11. Changes in Blood Chemistry during Adrenal Insufficiency.....	181
12. The Effect of Adrenal Cortical and Pituitary Extracts in Chronic Adrenal Insufficiency.....	214
13. Growth Curves of Adrenalectomized Rats Maintained on the <i>Charcoal-</i> <i>Hormone</i> Combination.....	238
14. The Operative Removal of the Adrenals in the Rat.....	253
15. Assay Curves of the Adrenal Cortical Hormone.....	258
16. Comparison of Oral and Intraperitoneal Methods of Administering the Cortical Hormone and the Effect of Salt Therapy.....	261
17. A Patient Manifesting the Adreno-Genital Syndrome and the Results of Surgical Removal of an Adrenal Tumor.....	345

PREFACE

The adrenal glands have been an object of intensive research since Addison first discovered their relation to a relatively uncommon disease. The mass of literature extant on these relatively tiny glands is scarcely commensurate with our knowledge of their function in the animal economy. Despite their small size, the glands must exert a vital influence in the organism for their removal soon leads to a complete breakdown in the normal functioning of practically all the organs and tissues of the body.

In the present volume an attempt has been made to analyze the great accumulation of literature on the subject of the adrenals and present a working hypothesis from which the reader may start on his own efforts. A study of the adrenals illustrates nicely the dependence of endocrinological advances on many fundamental sciences. Modern Endocrinology has become the leading branch of medical investigation by adopting anatomy, chemistry, physiology, pharmacology, pathology, and clinical medicine as its tools, and thereby has advanced from the state of quackery in which it had long floundered. Our knowledge of the adrenals has resulted from the combined efforts of chemical, physiological, pathological, and clinical investigators. It is necessary to consider all these diverse sources of information in order to obtain an understanding of these glands in their relation to the body as a whole.

The bibliography on the adrenals embraces many thousands of references, inclusion of all of which would be undesirable. I have, therefore, cited only a fraction of the literature, particularly the more recent work.

The adrenals have hitherto been looked upon as glands containing two essentially separate divisions—the cortex and the medulla. In the present volume I have considered the glands

in man and in certain animals as composed in early life of three distinct physiological entities—the medulla, the cortex, and the androgenic tissue. The last named term has been coined to describe that portion of the adrenal which, in certain pathological conditions, gives rise to disorders of the reproductive system. This view of the tripartite nature of the adrenal is supported by many well-established facts. It affords a rational explanation of the relation of the adrenals to certain pathological conditions of the reproductive system and avoids assuming a relation between the cortex proper and the sex glands as previous writers have unjustifiably done.

Many of the views expressed in the present volume are based on the results of experimental studies in which my colleagues, to whom I am deeply indebted, have collaborated. Dr. Warfield M. Firor has performed surgical operations and enthusiastically coöperated in these researches; Dr. Evelyn Howard is jointly responsible for the studies dealing with the relation of the adrenals to the reproductive system; and Mr. Ellis Grollman has aided in the preparation of the adrenal extracts.

I wish also to express my sincere appreciation to the publishers for their cooperation and unfailing courtesies.

A. G.

Baltimore, Maryland.

INTRODUCTION

CHAPTER I

AN HISTORICAL RÉSUMÉ

Probably as a result of their small size and yellowish, fat-like consistency, the adrenals were not recognized as specific organs by the ancients, being overlooked as parts of the perirenal fat. The claim that the adrenals are mentioned in the Bible is based on an error in the Vulgate translation. The single Hebrew word, "*Kalayot*" (kidney) of Leviticus (Chapter III) appears in the Latin of the Vulgate both as "*ren*" and as "*renunculus*." To consider the latter as referring to the adrenals is thus unjustified.^{55*}

Bartholomaeus Eustachius¹⁹⁰ gave the first clear description of the adrenal glands. The sixth chapter of his "*Opuscula Anatomica*" published at Venice in 1563 is entitled "*De glandulis quae renibus incumbunt*." It describes and pictures the adrenals as they occur in man. Despite the clear exposition of Eustachius many of his followers still denied the glandular entity of the adrenals. Thus Piccolomini still spoke of the glands as displaced fragments of the kidney. Others ignored their existence entirely.

Eustachius called the adrenals the "*glandulae renibus incumbentes*," a name, the connotation of which is retained in our modern expression, "suprarenal." Subsequent writers used a variety of appellations to designate the glands. Thus Casserius called them the "*renes succenturiate*" which as "*reins succenturioux*" was used in the French literature until displaced by the modern expression, "*glandes surrénales*." Winslow first

* Superior figures refer to the numbered entries of the bibliography. For the literature before 1850, the reader is referred to the excellent historical reviews of Biedl⁵⁵ and Shumacker.^{56a}

introduced the English term, "suprarenal" which, although descriptive of the glands as located in man, is a misnomer when applied generally, and hence should be replaced by the more generally applicable term, "adrenal." The term "*glandulae renales*," introduced by Dienerbroeck was adopted in the German "*Nierendrüsen*" which has been displaced by the more appropriate term "*Nebennieren*." The Italians refer to the glands as "*giandola*" or "*capsula surrenale*" or as "*soprarrenale*." The Spanish use the expression, "*suprarrenales*."

The seventeenth century contributed little to our knowledge of the adrenals. The literature of this period consists of anatomical descriptions which distorted the actual facts in order to give support to fantastic hypothetical theories. Thus Spigelius considered the adrenals as mere fillers of the abdominal cavity to help support the stomach. This theory was later retained by Highmore who also expressed the view that the adrenals absorbed humid exudates from the nearby blood vessels. Riolan believed that the adrenals supported the abdominal nerve plexuses thereby preventing their weighing on the renal vessels. This theory was refuted by Bartholin and Molinetti who pointed out that the adrenals lay above the nerve plexus and that the latter being firmly attached to the vertebral column were in no need of any support.

The proximity of the adrenals to the abdominal nerve plexus suggested to Thomas Wharton that the glands withdrew something from the nerves. He therefore called them the "*glandulae ad nerveum plexum*." Glisson maintained a similar view while Collins was more specific and suggested that the adrenals transported a fermentative liquor from the nerves to the kidneys.

The association of the adrenals and the kidneys gave rise to Molinetti's theory according to which the adrenals diverted the blood from the kidney in order to avoid the excretion of urine by the fetus. Molinetti's views were supported by Coxe¹³³ of the University of Pennsylvania, the earliest American writer on the subject.^{566a}

Sylvius suggested the idea that the adrenals altered the blood in such a way as to prevent its coagulation in the body. This theory was supported by Boerhave, Deidier, Sauvage, and others.

Bartholin first described the brownish fluid present in adrenals which are removed from the body sometime after death. This fluid results from autolytic processes in the medulla but was considered by Bartholin as an "atubiliary" juice derived from the spleen and liver. He, therefore, called the adrenals the "*capsulae atubiliarae*." Kerkring claimed that the adrenals secrete a juice which gives color and animates the blood and produces fermentation in the heart.

Severinus, apparently mistaking a band of fibrous tissue for a duct, described the existence of an excretory duct leading from the adrenals to the testicles. Valsalva described similar ducts to the ovary and testicle and therefore claimed that the adrenals were necessary for the proper functioning of the reproductive organs. He supported this theory experimentally by removing the adrenal of one side of a dog and the testicle of the opposite side. The animal failed to copulate after this operation which proved to Valsalva that the adrenals were indispensable parts of the reproductive system. Meckel also considered the adrenals as part of the sex glands.^{566a}

The *Academie des Sciences* of Bordeaux in 1716 offered a prize for the best thesis on the subject, "*Quel est l'usage des glandes surrénales?*" Montesquieu, the celebrated author of the "*Esprit des lois*" was appointed to judge the contributions. His subtle irony and apt criticisms exposed the sophistry of the various theories advanced to explain the function of the adrenals. He found no contribution worthy of the prize.⁵⁵

During the eighteenth century, some advance was made in the anatomical knowledge of the adrenals but more fantastic theories were elaborated to explain their function. Thus Senac suggested that the adrenals secreted meconium in the fetus while Von Helmont believed that they elaborated a juice which prevented the formation of renal calculi. Goodsir con-

sidered the adrenals, thymus, and thyroid to be of functional importance only in the embryo, while Riegel suggested that the adrenals were responsible for the secretion of fat into the abdominal cavity.

Morgagni first noted the existence of accessory adrenal bodies in man, an observation already made in the dog by Hartmann. Morgagni also noted the relatively large size of the adrenals in the fetus and deduced that their function was limited to intra-uterine life. He believed that the adrenals filtered a fluid from the blood into the *receptaculum chyli* and thoracic duct in order to ensure the patency of these vessels during fetal life when no chyle was being derived from the gastro-intestinal tract.

Winslow gave an accurate anatomical description of the adrenals. Nevertheless, subsequent writers failed to adjust their concepts of the physiological function of the glands to the anatomical facts. Thus Boerhave thought that the glands diluted the blood leaving the kidney. Haller²⁶³ also disregarded Winslow's description.

With the 19th century began the study of the comparative anatomy of the adrenals. Meckel⁴⁴⁸, whose friend, Cuvier, put at his disposal the vast zoological collection of the Royal Gardens at Paris, examined and described the adrenals of many species. He described the variations in birds and mammals but missed the glands in the reptilia. He noted too the "absence" of the glands in anencephalic monsters⁴⁴⁷ and suggested that the adrenals were related to the reproductive system. Retzius, in 1819, first described the adrenal homologues in the Selachian fishes and Stannius⁵³⁴, in the teleost fishes. Rathke in 1825 described the true nature of the glands in the amphibia, and Nagel⁴⁶⁴ first recognized the adrenal glands in the birds.

Bergmann in 1839 first noted the relation of the adrenal medulla to the nervous system. Meckel⁴⁴⁸, Johannes Müller, and Rokitansky⁵³⁵ finally disposed of the idea that the adrenals were related to the kidneys.

During the middle of the nineteenth century advances were made in the anatomical study of the adrenals. The work of Pappenheim, Schwager-Bardleben, Henle,²⁹³ and others laid the foundation for the comprehensive study of the minute anatomy of the glands.

Ecker¹⁶⁹ first demonstrated the glandular nature of the adrenal cells and concluded that these organs must pour some secretion into the blood either directly or by way of the lymphatics.

Köllicker (1854) in his "*Microscopischen Anatomie oder Gewebelehre des Menschen*" gave a good microscopic description of the adrenals. Arnold¹⁹ first introduced the conventional division of the gland into zones while Leydig's³⁹⁶ studies demonstrated the relation of the adrenals to the nervous system.

The modern study of the physiology of the adrenals may be said to have begun with Thomas Addison.⁷ His classic description of the disease of the adrenals, which has since been associated with his name, initiated a series of researches which have continued unabated to the present day. Addison's description, published in 1855, is one of the classics of medical literature, and remains today an accurate and almost complete account of the syndrome observed in the disease. Although many cases of Addison's disease have been carefully observed by subsequent workers, little has been added to Addison's description of the clinical symptoms and course of the disease. It is remarkable that despite the characteristic symptoms of this morbid condition, only three accounts of the disease are known which pre-date Addison's description. The earliest case on record is the fragmentary account by José de Sigüenza⁴²⁶ in his "*Historia de la Orden del glorioso doctor San Jeronimo*." Not only did Addison recognize the condition as a morbid entity but he also accurately correlated it with a pathological state of the adrenals. Addison's work served as a stimulus for a number of clinical studies as well as for the

physiological investigations on the adrenals which followed the appearance of his classic paper.

Addison's publication prompted Brown-Séquard⁹⁶ in 1856 to extirpate the adrenals from a number of laboratory animals. The rapidly fatal consequences of these operations led him to the correct conclusion as to their indispensability for life. Some subsequent workers failed to confirm Brown-Séquard's conclusions. This controversy, regarding the period of survival of animals following adrenalectomy has continued to the present day.

During the latter half of the nineteenth and early part of the twentieth century, the comparative anatomy of the adrenals was fully investigated. The earlier work of Stannius,⁵⁹⁴ Gray,²³⁹ Leydig,³⁹⁶ Remak, Kölliker, His, and Braun⁸¹ culminated in the researches of Balfour,²⁸ Soulié,⁵⁸¹ Poll,⁵⁰⁸ Vincent,⁶⁴⁸ Kohn,³⁵⁷ and others.

The observations of Oliver and Schaefer⁴⁸⁰ in 1894 of the remarkable pressor effects of extracts of the adrenal medulla turned attention to epinephrine, the existence of which had been noted in 1856 by Vulpian⁶⁵² who described the green coloration occurring when the medulla was moistened with ferric chloride. The high concentration of epinephrine in the gland (as compared to the relatively low concentrations in which other hormones occur) led to its isolation and identification in the course of a decade following Oliver and Schaefer's publication. It was thus the first hormone to be crystallized, identified, and synthesized.

The remarkable pharmacological effects of epinephrine soon found for it a permanent place in therapy. Physiologists looked upon its production as the obvious function of the adrenal glands and numerous studies appeared describing its actions under various conditions. Bitter controversies raged regarding the functional importance of epinephrine to the organism.

The adrenal cortex, in the meantime, was for the most part

neglected. The realization that the cortex of the adrenal, rather than the medulla, was vital to the organism soon turned increasing attention to this part of the gland. With the advance of Endocrinology during recent years, the adrenal has shared in the intensive study which has been applied to the glands of internal secretion. Although the function of the glands is still a mystery and the product elaborated by the cortex still unidentified, much progress has been made. The field has been sufficiently clarified to reveal the problems open for investigation and the prospects of their solution. The preparation of extracts which are capable of furnishing a complete replacement therapy in the adrenalectomized animal has opened new fields of investigation and supplied a new therapeutic agent for the treatment of human disease.

PART I. ANATOMICAL CONSIDERATIONS

The anatomy of the adrenals has been studied very extensively particularly from the comparative and embryological viewpoints. Much confusion still exists regarding the finer details of the microscopic structure of the glands due to the great difficulty of properly preserving and preparing the tissue for histological study. Many descriptions of the microscopic appearance of the cells of the glands are in reality based on artifacts. Despite these difficulties a great body of facts has been accumulated which throws light on the probable function of the adrenals. Many theories which have been advanced on the basis of physiological or clinical evidence must be discarded because they are in conflict with the anatomical facts. On the other hand, the available anatomical data lead to certain definite conclusions which are useful in shaping the course of future physiological investigations.

CHAPTER II

GROSS ANATOMY

The adrenal glands of mammals are compound organs derived by the union of two originally separate types of tissue. Because of their topographical relationship to one another these tissues are referred to as cortical and medullary. These expressions so commonly used in referring to the mammalian adrenal are inappropriate when applied to the corresponding tissues of the lower animals, and hence a nomenclature more suitable from the point of view of comparative morphology is desirable. We shall, therefore, refer to the cortex of the mammalian adrenal and its analogues in other genera as the interrenal body or tissue, a term introduced by Balfour²⁸ in 1878. Although a topographical term, this has the advantage, over the expression, "cortex," in that it is correct for vertebrates in general during the early stages of their development. We shall refer to the medullary portion of the mammalian adrenal and its analogue in other vertebrates as the chromaphil tissue, a term introduced by Stilling⁵⁹⁶ in allusion to the specific staining reaction of this tissue when treated with chromates. Kohn³⁵⁷ introduced the term "chromaffin" and Poll⁵⁰⁸ the expression "phaeochrome" to denote the same tissue. These expressions frequently occur in the literature synonymously with the term "chromaphil."

The teleological significance of the particular relation existing between the cortical and medullary portions of the adrenal is still a matter of conjecture. Their independence in the early stages of ontogeny is a repetition of the condition occurring during phylogeny as indicated in the elasmobranch fishes where the two elements remain separate.

COMPARATIVE ANATOMY

Tissues corresponding to the interrenal body or the cortex of the mammalian adrenal have not been demonstrated anatomically in animals below the cyclostomata (hags and lampreys). The homologue of the chromaphil or medullary tissue has, however, been demonstrated in a number of the lower invertebrates. The ease with which this chromaphil tissue may be identified (staining reactions, pressor effects, *etc.*) have aided in its detection in these lower forms. It is possible that the discovery of a corresponding micro-chemical or biological test for the internal secretion of the interrenal tissue may also lead to the demonstration of the interrenal homologue in the lower genera.

Poll and Sommer⁵⁰⁹ have described cells in the abdominal ganglia of leeches (*Hirudo medicinalis*, *Gnathobdella*, and *Rhynchobdella*) which resemble the chromaphil tissue of the higher genera. That these cells probably secrete epinephrine was demonstrated by Biedl⁵⁶ and by Gaskell²¹¹ who showed that extracts of the ganglia of the leech inhibit the contractions of the uterus of the virgin cat, a reaction typical of epinephrine. In the mollusc (*Paludina vivipara*), Leydig³⁹⁶ described tissues which he thought corresponded to the chromaphil tissue of vertebrates. Vincent,⁶⁴⁶ however, failed to obtain a pressor effect by injecting extracts of this tissue and hence Leydig's opinion is open to question. On the other hand, Roaf, Nierenstein, and Poll⁶⁴⁹ have described tissues in *Purpura lapillus*, and in *Nephtys scolopendroides* which resemble closely the chromaphil tissue of the adrenal.

In none of the above species has any evidence been adduced to indicate the existence of any interrenal tissue. The lowest vertebrates in which both adrenal elements have been shown to exist are the cyclostomata. In the lamprey (*Petromyzon*) Giacomini²¹⁶ described small lobulated structures projecting into the lumen of the posterior cardinal veins and renal arteries

which appear anatomically to be homologues of the interrenal tissue. The chromaphil tissue is represented in the lamprey by a series of thin strips running along the large arteries and their branches and extending from the region of the second gill cleft to the tail.⁶⁴⁹

In the hagfish (*Bdellostoma myxiniidae*) chromaphil cells have been described but no interrenal tissue has been identified.⁶⁴⁹

It is only in the *Amniota* (mammals, birds, and reptiles) that we find the adrenal as a definite organ composed of distinct parts, the chromaphil and interrenal tissues. In the *Anamniota* (amphibia, fishes, and cyclostomes) the compact union of chromaphil and interrenal tissues has not occurred and these tissues are represented by a number of separate bodies. The amphibia are intermediate between the mammals and the lower *Anamniota* for in them we find the union of interrenal and chromaphil tissue into a single organ, but without the penetration of the latter tissue to assume a true medullary position. In the fishes and cyclostomes the homologous tissues of the cortex and medulla of the mammal are present as distinct and separate bodies.

The adrenal system of the Elasmobranchs (sharks, dogfish, and rays or skates) was first described in detail by Balfour.²⁸ He assigned the name interrenal to the homologue of the adrenal cortex of mammals and the name "suprarenal bodies" to the homologue of the medulla. The term "suprarenal bodies" is now replaced by the more suitable term "chromaphil bodies." The interrenal bodies of the elasmobranchs are usually arranged as a pair of ochre yellow, ribbon-shaped structures lying medial to and in the region of the posterior part of the kidneys. They are sometimes contiguous to the midline and fused into a single organ or joined by strands of tissue. The glands are usually paired in the skate⁴⁰⁹ and unpaired in the dogfish and shark. Microscopically the cells

resemble those of the Stannius' corpuscles, (their homologues in the teleost fishes) and the adrenal cortex of the higher vertebrates.

The chromaphil tissue of the elasmobranchs occurs in the form of segmentally paired bodies arranged on branches of the aorta in close relation to the ganglia of the sympathetic chain.⁶⁴⁹

Direct evidence exists for the physiological identity in function of the chromaphil bodies and the adrenal medulla. Thus Vincent^{646, 647, 648} showed that the saline extracts of these bodies in the dogfish had vasoconstrictor and pressor activity similar to that of extracts of the adrenal medulla. Similar extracts prepared from the interrenal bodies manifested no such actions. Vincent's observations were confirmed by Biedl and Wiesel⁵⁷. Lutz and Wyman⁴⁰⁹ demonstrated the mydriatic action of extracts of the chromaphil tissue of certain elasmobranchs, which is indicative of the elaboration of epinephrine by these bodies. Extracts of the interrenal body, on the other hand, were free of any sympathomimetic activity.

The homology of the interrenal bodies of the elasmobranchs with the adrenal cortex has also been proven by physiological experiments. Thus Biedl⁵⁶ demonstrated that removal of the interrenal bodies in the skate and the torpedo results in symptoms (weakness, pallor, cachexia, *etc.*) and ultimate death analogous to that observed after removal of the adrenal cortex in higher animals. Kisch³⁵³ confirmed and extended these observations. The operative difficulties involved in performing interrenalectomy in fishes renders conclusions based on extirpation experiments of doubtful value. Less equivocal evidence was obtained by Grollman, Firor, and Grollman²⁵⁰ who demonstrated that extracts of the interrenal body of the skate (*Raja stabuliformis*, *Raja diaphanes*, and *Raja erinacea*), when administered to bilaterally adrenalectomized rats, manifested a complete replacement therapy.

In the teleost fishes the interrenal bodies occur as small

round or oval pale pink bodies on the spinal or ventral surface of the kidney. They lie either free on the surface of the kidney or embedded in its substance. These structures are known as the corpuscles of Stannius. Interrenal tissue is also present in certain of the teleosts at the cranial border of the head kidney, on the anterior and posterior cardinal veins. This tissue was first described by Giacomini.²¹⁸ Their existence, in addition to the corpuscles of Stannius, account for the fact demonstrated by Vincent⁶⁴⁷ that extirpation of the corpuscles of Stannius in the eel does not result in death.

The chromaphil tissues of teleost fishes were also discovered by Giacomini.²¹⁷ This tissue is found in the wall of the cardinal veins towards the cranial end of the body along the head of the kidney.

In the Dipnoi (lung fishes) the existence of interrenal tissue has not been demonstrated with certainty. Parker and Giacomini⁶⁴⁹ have described a lymphoid tissue in *Propterus annectens* having an epithelial appearance which they suggest has replaced the interrenal tissue of other species. Further study of this tissue is desirable before one can justifiably deny the existence of interrenal tissue in these fishes. The chromaphil tissue of the Dipnoi has been demonstrated by Giacomini²¹⁸ as segmentally arranged bodies around the intercostal arteries and the wall of the posterior cardinal vein and the right azygos vein.

The chromaphil and cortical tissues are first found together in the amphibia. In the frog the adrenal is seen as a thin, golden-yellow ribbon closely attached to the ventral surface of the kidney. In Figure 1 are reproduced the kidneys of a frog with their attached adrenals. The greatest part of the adrenal in the amphibia is composed of columns of cortical interrenal cells, the chromaphil cells occurring at the borders of the columns of the cortical cells. This arrangement is transitional between the complete independence of chromaphil and cortical tissue as observed in the fishes and the inclusion of the chroma-

phil tissue within the rim of cortical cells as observed in the mammal. In Urodela the adrenal occurs as a series of strips which extend along the kidney and project anteriorly as far as the origin of the subclavian artery.⁷⁰⁰

An intimate relation between the chromaphil and interrenal tissues is first encountered in the reptilian adrenal. Here we find the relation of these tissues to vary from that described above for the amphibia to the condition met with in the bird and described in the next paragraph. Thus in the crocodiles and sea turtles (*Chelonia*) the arrangement is practically identical with that seen in the bird while in the tortoise (*Testudina*) the arrangement occurring in the frog is encountered.^{498, 508}

In the birds the adrenals consist of ochre colored bodies situated on each side of the vena cava. The left gland is usually lenticular in shape with its internal border excavated and molded around the vena cava or abdominal aorta. The right gland is pyramidal in shape and also in intimate contact with the vena cava. The adrenals in the bird lie at the anterior end of the kidney in intimate contact with the reproductive organs. Microscopically, the adrenals in the bird consist of an interlacement of the interrenal and cortical tissues. Each occurs in the form of strands of cells which intertwine with one another. These strands have been designated as "chief" and "intermediate" strands to denote the interrenal and chromaphil tissues, respectively.

It is only in the mammals that we find the interrenal tissue as a cortex surrounding a medulla composed of chromaphil tissue. The cortex is the homologue of the cortical or interrenal columns of the reptile, bird, and amphibia, of the corpuscles of Stannius in the Teleosts, and of the interrenal body in the Elasmobranch fishes. The right adrenal in the mammal is situated in close contact with the vena cava. It is usually in close apposition to the kidney but in some species it may be separated from and situated a short distance anteriorly to the kidney. The left adrenal is, in most species, situated away

FIG. 1. THE AMPHIBIAN ADRENAL

The kidneys of a frog (*Rana catcshiana*) with the adrenals attached to their ventral surface ($\times 1$).

from the vena cava but in some species (*e.g.*, the rabbit) it lies in close contact to the large abdominal veins.

GENERAL MORPHOLOGY

The adrenals of the different mammalian species differ in their gross anatomical appearance and topographical arrangement as shown in Figure 2 in which are reproduced the photographs of representative types of glands. The apposition of the glands to the kidneys or the vena cava gives depressions which modify the simple bean-shaped or spherical forms observed in some species.

The weight of the adrenals compared to the body weight or kidney weight in different animals has been the subject of several investigations since Meckel⁴⁴⁸ and Cuvier¹⁴⁴ first initiated this type of comparative study. In general the adrenals of most mammals weigh from 0.01 to 0.02 per cent of the total body weight. The guinea pig and several of its wild relations are exceptional in this respect, their adrenals weighing almost ten times as much per unit of body weight than other animal species. The relatively large gland of the guinea pig is composed chiefly of cortical tissue.¹⁰⁸ The medulla bears the same relative relation to the body weight as it does in other mammals.¹⁸³

Except during the period of degeneration of the androgenic zone, described in Chapter IV, the adrenals increase in size as the body grows (although not proportionately) except in fowls¹⁸³ where there is a marked reduction in adrenal size after maturity despite an increase in body weight. This may suggest the presence of androgenic tissue in the fowl's adrenal.⁷⁹ In the guinea-pig the medulla attains its full development by the end of the first month of life, subsequent growth of the gland being limited to the cortex. In other animals likewise the medulla increases but slightly in size after the first period of life. In birds the medulla forms a very large fraction of the whole gland, the cords of medullary cells being interwoven in a

uniform network with the strands of cortical tissue. The medullary cells, however, are capable of multiplication after adolescence and have been noted to increase in the gland remaining after unilateral adrenalectomy.

The weights of the adrenal glands vary readily in response to the metabolic requirements of the body. Thus the fever of infectious processes, cold, heat, *etc.* call forth an hypertrophy which is very obvious even on casual inspection. The adrenals of ill-nourished or unhealthy animals are much larger than those of normal healthy ones. The adrenals of the grey wild rat are much larger than the glands of the domesticated albino.¹⁶² This increase in size involves the cortex chiefly and the medulla to a much lesser extent. The large gland of the wild rat may be attributed to the less favorable conditions under which it flourishes and its greater vigor and activity which involve a greater metabolic requirement. Domestication reduces the size of the adrenals. In the rat the adrenals are somewhat larger in the female than in the male.²⁸⁶

THE ADRENALS OF MAN

The adrenals in man sit "cap-like" on the upper poles of the kidneys. The right gland is somewhat triangular in shape bearing a resemblance to a "cocked-hat." It is situated behind the inferior vena cava and right lobe of the liver and in front of the diaphragm and upper end of the right kidney. The upper part of its lateral surface is devoid of peritoneum and lies opposite the liver, while the inferior portion is covered by peritoneum reflected onto it from the coronary ligament. Near the anterior border of the gland is a short furrow (the hilum) from which emerges the adrenal vein.

The left adrenal is crescentic in shape with its concavity placed in apposition to the medial border of the upper part of the kidney. The upper area of its anterior surface is covered by the peritoneum of the omental bursa which separates it from the cardiac end of the stomach while the lower area is

FIG. 2. THE MAMMALIAN ADRENAL

The adrenals, showing the diversity of their shape in various mammals. 1, the left gland of a sperm whale (*Physeter macrocephalus*) ($\times 1_3$) (Courtesy of Dr. E. M. K. Geiling); 2, the beef (*Bos taurus*) ($\times 3_4$); 3, the sheep (*Ovis aries*) ($\times 2$); 4, the rat (*Mus Norvegicus* var. *albinus*) ($\times 1\frac{1}{2}$); 5, the pig (*Sus scrofa*) ($\times 3_4$); 6, The monkey (*Macacus rhesus*) ($\times 1$); 7, the cat (*Felis catus*) ($\times 1\frac{1}{2}$); 8, the dog (*Canis familiaris*) ($\times 1$).

devoid of peritoneum and is in contact with the pancreas and splenic artery. Along its posterior surface is a ridge dividing it into a lateral area resting on the kidney, and a medial area on the left crus of the diaphragm.

The surface of the adrenals is surrounded by areolar tissue containing fat and is invested by a fibrous capsule through which pass numerous fibrous processes and vessels entering the organ.

Externally the glands appear yellowish or brownish-yellow. When cut into they are seen to consist of a firm external or cortical portion, deep yellow in color, and striated, forming the greater mass of the gland, and an internal or medullary, soft and pulpy portion, of dark-brownish appearance due to the presence of blood.

The fibrous coat of the adrenals consists of an outer looser, and an inner firmer layer. The latter is intimately connected with the deeper parts of the gland and is continuous with the septa which enter into the formation of the organ. The arrangement of these septa gives rise to the structure characterizing the microscopic appearance of the gland and its division into zones as described later.

The size of the adrenals varies widely depending on the previous life history of the individual from which they were derived. Thus infection increases their size and hence in persons dying after an infectious process enlarged adrenals are encountered. After sudden accidental death, relatively small adrenals are found. Rarely a single gland only is encountered or the two glands are fused.

The average combined weight of the two glands in the human adult varies from 5 to 15 grams.^{444, 514} There is thus a considerable variation in their normal size and it is therefore difficult, by mere inspection of size, to determine whether or not a given gland is normal. The majority of normal glands, however, weigh between 3.5 and 5 grams each. They are on an average about 25 to 30 mms. in height, 40 to 50 mms. in

breadth, and 2 to 8 mms. in thickness, except at the bases where they are twice as thick. The medulla makes up about 10 per cent of the weight of the glands.

Circulation. The blood supply of the adrenal is peculiar in several respects.^{201, 213} There is a separate arterial supply to the cortex and medulla. The cortex, however, has no marked venous system, the blood being drained into the capillary network of the medullary arteries and ultimately drained by the well developed venous system of the medulla. The transition of the arterial network of the cortex to that of the medulla occurs in the reticular zone in which is found an exceedingly fine capillary system with very narrow meshes each of which appears to surround a single cell. It has not been established if this network consists of closed capillaries or of unwallled spaces. The adrenal cortical capillaries behave on vital staining like the reticulo-endothelial cells of the spleen, liver, *etc.* in which loosely built or interrupted capillary systems are found.¹⁰

Aside from the above described circulation there is a second venous circulation of the cortex in which tiny branches from the left adrenal (possibly also from the right) communicate with branches of the splenic and pancreatic veins and thus with the portal system.

The arterial blood reaches the adrenals through channels derived from the aorta, the inferior phrenic, and the renal arteries. Three arteries, one of which supplies the medulla, penetrate into the capsule and break up into capillaries which end in the venous plexus of the medulla. The adrenals are richly vascularized. Houssay and Molinelli³¹⁵ observed a blood flow of 0.35 cc. per minute per kilo of body weight in dogs. Neumann found the blood flow through the adrenals to exceed that of any other organ, *viz.*, 6 to 7 cc. per gram of adrenal tissue per minute. Subsequent workers have found smaller blood flows.⁵⁴⁵

The great muscular content of the adrenal vein is striking.

Some investigators have considered it as capable of blocking the blood leaving the adrenal. It is more likely, however, that this muscular development acts to help propel the venous blood into the caval system thereby ensuring the circulation of the vital products of the adrenal gland.

The adrenal vein receives the blood from the medullary venous plexus and from the cortex. It emerges from the hilum of the gland and opens on the right into the inferior vena cava and on the left into the renal vein.

Under certain conditions, the venous blood from the adrenals reaches the portal vein. According to Cow¹³¹ part of the blood supply to the medulla in the cat goes to the kidney. In the dog this venous anastomosis of adrenal and renal vessels occurs only rarely.

The efferent blood vessels of the adrenals are divided by Ferguson¹⁹⁴ into four types: the sinusoids, the small central vein, the large central vein, and the suprarenal vein. Each of these types presents distinctive features but in all of them circular muscle is conspicuously deficient or absent. The large central vein is characterized by prominent and characteristic muscular ridges.

Lymphatics. The lymphatics of the adrenals run in the trabeculae of the cortex and are connected with spaces between the trabeculae and the cell columns and by clefts between the cells within the columns. They communicate with efferent-valved lymphatics in the fibrous capsule and in the medulla where they form a close plexus around the central vein. The lymphatics of the adrenals drain into the lateral aortic lymph glands.

Nerve supply. The adrenals receive their chief innervation from the splanchnics. It is questionable if twigs from the vagi and phrenics also innervate the adrenals, as some writers claim. In the rabbit the right adrenal is supplied by both right and left splanchnics while the left gland receives fibers from the right splanchnic only. The fibers to the adrenals are derived

from the adrenal (or suprarenal) plexus and are probably all postganglionic and unmyelinated.¹⁷⁹

The efferent nerves of the adrenal are not accurately known. Elliott¹⁷⁹ assumes the existence of a center controlling epinephrine discharge near the bulbar vasomotor centers. Others place it in the subthalamie region.⁴¹⁹

THE CHROMAPHIL TISSUE

The medulla of the mammalian adrenal and its homologues in the lower animals are components of the chromaphil system. The tissue comprising this system is characterized by the brownish discoloration which it undergoes when fixed in a chromate solution. Stilling⁵⁹⁶ in 1890 observed that this chromaphil reaction was given not only by the adrenal medulla but also by groups of cells associated with the sympathetic nervous system. Stilling applied the generic term "chromophil" to these cells which Vincent⁶⁴⁹ altered to the more euphemistic expression "chromaphil." Kohn,³⁵⁷ who applied the term chromaffin to these cells, introduced the term "paraganglia" to denote the cells found in association with the sympathetic nervous system.³⁵⁸ The adrenal medulla itself was indicated by Kohn as the "*paraganglion suprarenale*." Kohn also called attention to the conspicuous strip of chromaphil cells situated ventral to the abdominal aorta and superior to the inferior mesenteric artery. He named this tissue the "*paraganglion aorticum abdominale*." Zuckerkandl⁶⁹⁷ discovered a pair of similarly staining bodies in the region of the inferior mesenteric artery of the new born. These bodies are known as the organs of Zuckerkandl.

The paraganglia are small round masses, 1 to 3 mms. in diameter, placed within or contiguous to the capsules of the ganglia of the sympathetic system. Usually one paraganglion is associated with each ganglion of the coeliac, renal, adrenal, aortic, and hypogastric plexuses. Sometimes they are also associated with the ganglia of the cardiac and inferior mesen-

teric plexuses and have been reported in association with ganglia on the kidney, prostate, ureter, *et cetera*.

In addition to the paraganglia a number of masses of chromaphil tissue are also developed in relation to the abdominal sympathetic plexuses. Of these, the most prominent are the aortic bodies (Zuckerkindl's bodies) which lie on either side of the aorta at the origin of the inferior mesenteric artery. In the new-born they are about a centimeter in length but degenerate and gradually disappear (except under certain pathological conditions) so as to be seen only microscopically from puberty until the age of about forty.

The carotid gland also forms part of the chromaphil system. It is a bilaterally paired organ situated in close relation to the bifurcation of the common carotid artery. It is oval or wedge-shaped depending upon its position in relation to the artery and is about 7 mms. long, and 2 to 5 mms. in diameter. In appearance it is grayish, yellowish or brownish red.^{356, 384}

The coccygeal body has been erroneously referred to by some authors as a part of the chromaphil system. This body does not, however, give the chromaphil reaction.⁵⁹³ It corresponds to the caudal glomeruli of lower animal forms.⁶⁴⁹

The chromaphil tissue of mammals occurring in the paraganglia of the sympathetic trunk is homologous to the segmentally arranged chromaphil bodies of the elasmobranchs. The chromaphil bodies of the abdominal plexuses are, on the other hand, formations peculiar to the higher vertebrates, while the inclusion of chromaphil tissue within the capsule of cortical tissue, as in the adrenal glands, is a development found only in the mammalia. Although similar anatomically, it is questionable if all the component tissues of the chromaphil system are functionally equivalent (*cf.* Chapter VI).

The method for demonstrating the chromaphil tissue as introduced by Stilling⁵⁹⁶ and Kohn³⁵⁷ consisted simply in exposing the tissue to a solution of potassium bichromate and subsequently washing and examining the tissue for brownish

patches. A more satisfactory technique, described by Wislocki,⁶⁸³ consists in staining with bichromate and fixing in 10 per cent formalin. The tissue is then washed and bleached by placing in the sunlight for 6 to 24 hours in a solution of hydrogen peroxide. In this way the chromaphil tissue stands out prominently against the bleached surrounding tissue. Wislocki was able to demonstrate abdominal chromaphil tissue in the dog, cat, rabbit, and man, as described by earlier workers, as well as in the opossum, guinea-pig, squirrel, and monkey. In the rat no abdominal chromaphil tissue could be demonstrated.

Biedl and Wiesel⁵⁷ demonstrated the pressor effects of extracts of the organs of Zuckerhandl to be similar to those obtained from epinephrine. Vincent,⁶⁴⁸ Fulk and MacLeod²⁰⁹, and Kahn³⁴⁴ showed that the accessory chromaphil tissue of the dog, guinea-pig, rat, and other animals also manifested biological effects similar to epinephrine. Kahn showed that the abdominal chromaphil body possesses about one-twelfth to one-thirtieth as much epinephrine as the adrenal medulla. Kahn also claimed to have demonstrated the presence of epinephrine in the venous blood draining the paraganglia, but the method which he used is open to criticism.

There is no evidence to indicate that the accessory chromaphil tissue takes over the function of the adrenal medulla when the latter is extirpated. Wislocki and Crowe⁶⁸⁵ removed one adrenal from dogs, together with one-fourth to three-fourths of the remaining gland, and implanted radium emanations into the remaining fragments. In this way, all medullary tissue was destroyed. Hypertrophy of the abdominal chromaphil bodies did not occur following the operation.

EMBRYOLOGY

The primordial cells which give rise to the adrenals are differentiated at an early stage of embryonic life.⁶⁷⁴ The medulla and the chromaphil tissue in general, and the sympathetic nerve cells arise in common from the neural crest and

are thus ectodermal in origin. Although the chromaphil tissue develops in intimate relation with the sympathetic nervous system, the two are developmentally distinct in origin. The differentiation of chromaphiloblasts from sympathoblasts begins in the embryo but is not complete until late in gestation or in some species after birth. When any cells in a given area of the sympathoblasts differentiate, all do, and hence no intermixture of chromaphil and sympathetic cells results despite their ultimate intimate relations and common origin.

The cortex of the adrenal and the interrenal tissue in general are derivatives of the ventral portion of the coelomic epithelium. They are thus mesodermal in origin. Embryologically, therefore, the cortex and medulla are quite distinct in origin. It is not until we reach the amphibia that the union of the two *anlage* begins to form the compound organ denoted as the adrenal.

In the present section we shall outline briefly the embryological development of the adrenal and its homologues in the elasmobranchs, amphibia, birds, and mammals. For more detailed descriptions the excellent monographs of Balfour,²⁸ Soulié,⁵⁸¹ and Poll⁵⁰⁸ should be consulted.

Elasmobranchs. The development of the interrenal in the dogfish (*Scyllium*) has been studied by Poll.⁵⁰⁸ In this elasmobranch the interrenal first appears in the 7 mm. embryo as a number of irregularly distributed thickenings of the splanchnic mesoderm, ventral to the dorsal aorta, and extending from the pronephros as far back as the cloaca. These rudiments meet and become continuous taking the form, in the 10 mm. embryo, of a cellular rod lying under the dorsal aorta. In the 16 to 28 mm. embryo, the interrenal body gradually becomes separated from the coelomic epithelium and the nephrotomes along which it had previously been in apposition. Only the posterior half of the rudiments take part in the above described fusion to form the interrenal. The anterior rudiments atrophy or develop into small accessory bodies.

The chromaphil bodies of *Scyllium* develop at a much later

stage than the interrenal organ. In the 53 mm. embryo, differentiation of the lateral parts of the sympathetic ganglia begins. The chromaphil cells become smaller than those which are to become ganglionic cells and assume their characteristic reaction with chromic acid. In the 90 mm. embryo the chromaphil organs have assumed their definitive forms. In subsequent development, modification of the segmentally arranged chromaphil masses occurs, some disappearing, others undergoing fusion. There is a marked variety in the final adult arrangement in the different genera.

Amphibia. The development of the adrenals in amphibia has been described by Brauer⁸⁰ for *Hypogeophis*. As in the Elasmobranchs, the interrenal first appears as segmentally paired proliferations of the coelomic epithelium which extend from the pronephros to the cloaca. The chromaphil bodies develop as in the Elasmobranchs but shift and take up their position in intimate contact with the interrenal bodies. In the posterior paired region, they lie on the medial face of the interrenal. Elsewhere they lie dorsal or lateral or even surrounding the interrenal.

Bird. The development of the adrenal in the bird has been described by Soulié.⁵⁸¹ The cortical portion of the gland arises next to the anterior and dorsal part of the germinal epithelium, posterior to the pronephros. At the 96th hour of incubation in the hen's egg, and continuing for 4 or 5 hours, groups of cells bud off into the underlying mesenchyme lying between the mesonephros and aorta. These cells, arranged in the form of epithelial cords increase in number by cell division filling the entire space above and medial to the mesonephros. The chromaphil system of the adrenal begins to form at about the eighth day with the migration of sympathetic ganglion cells into the adrenal to occupy their permanent position as the adrenal medulla.^{288, 516}

Mammals. In man, the first development of the cortical system is noted at the beginning of the fourth week. At

this time there occurs a proliferation of the epithelial cells lying between the mesonephros and the root of the mesentery. The buds of tissue thus formed, as found in a 12 mm. human embryo, lie as a continuation of the suprarenal ridge. In the 20 mm. embryo, the sympatho-chromaphil cells begin to migrate into the developing cortex along the line of the central vein to form the medulla of the adrenal gland. It is not until the embryo is 10 cms. in length that the migrating chromaphil cells have reached the central vein and formed a true medulla. In man this penetration of the epithelial *anlage* by the sympathetic is accompanied by a special formation of folds and a process of invagination.³⁷⁶ On cross-section the cortex appears invaginated deeply at certain points, especially along the central vein, so that this vein is almost entirely surrounded by cortical tissue, particularly in the adult. This peculiar process, which is not limited to man, results in a more intimate contact between medulla and cortex.

Differentiation of the chromaphil cells in man begins in the 18 mm. embryo but is not complete until about birth. The chromaphiloblasts (cells destined to become chromaphil tissue) are larger in size and stain less intensely than the sympathoblasts. Their specific reaction with chromates appears at this stage. Epinephrine does not appear in the human adrenal until after birth.³⁹³

In mice the first appearance of the chromaphil reaction takes place between the fourteenth and fifteenth day of development.³¹⁷ At this time the future medullary cells are just beginning to penetrate into the interrenal tissue leaving the sympathetic ganglion *anlage* which at this time is closely applied to the interrenal ridge. The sympatho-chromaphil mass at this time is made up of cells of embryonic type. The differentiation of the chromaphil cells is accompanied by the acquisition of darker nuclei and the assumption of a chromaphil reaction. The interrenal tissue at this time is surrounded by a layer of fibroblasts. That the chromaphil reaction, as observed at an

early state of differentiation is in reality due to the presence of epinephrine is demonstrated by the inhibition of intestinal movements by extracts of this tissue.^{317, 669}

ACCESSORY ADRENAL BODIES

The tissues of the adrenals are not confined to that which occurs in the main glands. As we have already seen, the medullary tissue is only one part of a widely distributed chromophil system. The cortical tissue is also composed of several masses of glandular tissue located at a considerable distance from the main gland. Previous writers have considered all of this tissue, because of a similarity in its gross histological appearance, as being functionally part of the same tissue. This view, however, is not supported by physiological or pathological findings and we shall, therefore, classify the cortical (or interrenal tissue) into two groups: 1) the true cortical tissue and 2) the androgenic tissue. The cortical tissue proper elaborates a hormone essential for life. It forms the outer layers of the adrenal gland and occurs at times as accessory adrenal bodies in the near vicinity of the adrenal. A second part of the cortical tissue, resembling the first histologically in many respects, occurs as the internal layer of the cortex in certain animals during the early period of development, but soon disappears (except under some pathological conditions). This tissue also appears in the form of temporary accessory bodies which are widely distributed. The second type of cortical tissue shall be designated as the androgenic tissue because of its masculinizing effects and shall be described in the next chapter.

Accessory adrenals were first described in the dog by Hartmann in 1699 and in man by Morgagni in 1740. Du Vernoi in 1751 described their occurrence in man with particular clarity.⁵⁵ Accessory adrenal tissue also frequently occurs as islets in the medulla or in the cortical tissue and was first noted by Nagel⁴⁶⁴ and subsequently by Rokitansky,⁵³⁵ Arnold,¹⁹ Marchetti,⁴³¹ and others. These islets resemble benign adenomata and

FIG. 3. ACCESSORY ADRENALS

An adrenal of the cow (*Bos taurus*) showing an accessory adrenal projecting from its surface and two discrete accessory bodies found in the connective tissue surrounding the main glands ($\times 1$).

represent most probably hypertrophied nodules similar to those often encountered in other secretory glands.

The term accessory adrenal is applied to small islets of tissue which are present in the fat and connective tissue surrounding the adrenal glands. These islets are for the most part microscopic in size except in large animals such as man, the horse or cattle. In the last named they frequently attain the size of a walnut. In the cat, rabbit, or dog they are occasionally visible to the naked eye, but in the rat or mouse they can only be detected in microscopic sections. These accessories at times are said to resemble the main gland in their histological arrangement and contain chromaphil tissue in their medulla. For the most part, however, they consist of cortical tissue only the cells of which resemble in their arrangement those of the Stannius corpuscles of the Teleost fishes (*cf.* Figure 6).

In Figure 3 is reproduced an adrenal of the cow containing an accessory body which projects like a wart from the surface of the gland. This type of accessory is in reality merely an hypertrophic nodule which has formed near the surface of the gland. In Figure 3 are also reproduced two discreet accessory adrenal bodies obtained from the connective tissue surrounding the main glands. These represent true accessory bodies. On cross section they are found to be composed of cortical tissue arranged in columns resembling the fascicular layer of the main gland.

The accessory adrenals situated in the vicinity of the adrenals are commonly found in most animals. Although said to occur with greater frequency in some species than in others, this view is based on indirect evidence derived from extirpation experiments. Thus the accessories are said to be relatively uncommon in the cat or dog because adrenalectomy is fatal in these animals. In the mouse, rat, or rabbit, however, the presence of accessory adrenals is considered to be very common because these animals often survive adrenalectomy. As a matter of fact, even in these animals, meticulous care in

adrenalectomy leads to the same fatal outcome as in the cat or dog. The glands, being relatively large in the cat and dog, may be easily extirpated without leaving bits of tissue which might hypertrophy. In the mouse or rat, on the other hand, the glands being extremely small and friable, it is easy to detach bits of cortical tissue which, although microscopic, suffice to permit regeneration and survival of the animal. In the rabbit, the right adrenal is often so firmly attached to the vena cava as to render its complete extirpation difficult.

That the frequency of occurrence of accessory adrenals in the rat or mouse is not based on sound grounds but is explicable on the basis of the above interpretation has been demonstrated by many recent workers. The relative infrequency of these accessories in the rat and mouse can also be definitely proven by serially sectioning the adrenal sites including all the surrounding connective tissue.³⁸⁵ When this is done one finds surprisingly few accessory bodies and these are always present in the connective tissue investing the adrenals. They are not scattered throughout the abdominal cavity as was formerly believed.

Some authors have claimed that certain breeds of rats contain diffusely scattered accessory bodies and hence survive adrenalectomy while others being devoid of such accessory tissue do not survive the operation. These authors, however, give no specific identification of these breeds but apparently arbitrarily differentiate certain animals as being of a different strain on the basis of their survival following adrenalectomy. All recognized strains of the common rat (*Mus Norvegicus*) have been subjected to adrenalectomy in the author's laboratory without encountering the hypothetical accessory-endowed strain assumed by certain authors.

The true accessory cortical tissue occurs usually only in the near vicinity of the main glands. The accessory tissue which is found in various parts of the abdominal cavity at some distance from the main glands is usually a part of the androgenic tissue and shall be described in Chapter IV.

CHAPTER III

MICROSCOPIC ANATOMY

The adrenals of many animal species have been studied microscopically in great detail by numerous workers. Unfortunately much of the histological literature is conflicting and unsatisfactory because of certain difficulties attendant upon the study of the adrenals. These glands are noteworthy for the rapidity with which they undergo autolytic change after death. Moreover, the adrenals are extremely delicate organs, and, as Hoerr³⁰¹ has pointed out, even the slightest pressure will often cause artifacts to appear in the microscopic sections. It is also difficult to fix the glands so as to avoid the production of these artefacts. As a fixative, Hoerr recommends Bensley-Helly's "Formol-Zenker," prepared by adding nine volumes of an aqueous solution, containing 2.5 gs. of potassium dichromate and 5.0 gs. of mercuric chloride per 100 cc., to one volume of neutralized formalin. In order to study properly the morphological details of the cells Hoerr suggests the use of several fixatives and different stains. Failure to appreciate the difficulties inherent in obtaining good sections has undoubtedly contributed to the confusion and misinterpretations which abound in the anatomical literature. Many of the morphological descriptions of bizarre cell structures are mere artifacts to which no physiological significance is to be ascribed. It is particularly difficult to obtain specimens of human adrenals soon enough after death to avoid *post-mortem* changes.

The adrenals of some species undergo radical changes during certain periods of life. The most striking of these changes involves the development and involution of the androgenic zone to be described in the next chapter. Failure to appre-

ciate this temporary appearance of a new tissue in the gland has led to certain of the discrepancies found in the literature.^{319,671}

As Kolmer³⁶² has indicated, the microscopic appearance of the adrenals of every animal has certain distinctive properties so that an experienced histologist could determine from a given section the animal species from which the adrenal was obtained. On the other hand, it is impossible to say from a study of an adrenal from which order or genera it was derived. Closely related species often have adrenals which differ widely in their appearance.

As we have already seen in the preceding chapter, the adrenals as compound organs are first encountered in the amphibia. In the fishes the interrenal and chromaphil tissue are present as separate organs. In the bird these two types of tissues are intimately mixed. The interrenal tissue (corresponding to the cortex of the mammal) is arranged in the bird in irregular columns surrounding the blood vessels. The latter form a network of irregularly arranged sinusoids throughout the gland. The columns of interrenal tissue are called the "chief strands" because they appear to form the foundation for the other elements of the glands. The chromaphil cells have no regular arrangement but occur in groups of 2 or 3, to 30 to 40 cells which, for the most part, lie in direct contact with the venous sinusoids. The chromaphil elements are denoted as the "intermediate strands."

On cross section the adrenals of the mammal are seen to consist of an external (or cortical) and an internal (or medullary) portion. The former which constitutes the chief part of the organ is usually of a deep yellow color, due to the presence of lipids, while the latter is soft and pulpy and of a dark reddish or brownish color due to the presence of blood. In cattle, the cortex when viewed soon after slaughter of the animal is either ochre-yellow or brownish red, depending upon the age, sex, and nutritive state of the animal, while the medulla pre-

sents a greyish appearance. The medulla is not entirely surrounded by cortical tissue in all mammals. In some, the medullary tissue clusters about the adrenal vein with part of it surrounded by cortical tissue while the remainder is directly under the adrenal capsule. Such a condition, for example, is encountered in certain marsupials (*Metachirus opposum*), in the flying fox (*Pteropus medius*), and in the stone-marten (*Mustela foina*).

The separation of the cortex and medulla is also very variable. In the Monotremes, as in the spiny ant-eater (*Echidna aculeata*) and in the duckbill (*Ornithorhynchus paradoxus*), the medulla and cortex are most imperfectly divided, the former penetrating far into the cortical tissue. On the other hand, in some species, a definite band of connective tissue separates the medulla from the cortex, at least during a certain period of life. This is true of the mouse (Figure 9), little ant-eater (*Tamandua tetradactylus*), porcupine (*Hystrix cristata*), elephant (*Elephas indicus*), armadillo (*Dasypus septemcinctus*), and man. The significance of this band of connective tissue shall be discussed in the next chapter.

There is considerable variation in the relative sizes of the cortex and medulla of different mammals. In man and the anthropoid apes, the medulla is represented at birth by a small strip of undifferentiated tissue. The medulla in these animals is a post-natal development. The medulla forms a relatively large proportion of the gland in the porpoise (*Phocaena communis*), rhinoceros (*Rhinoceros unicornis*), flying fox (*Pteropus medius*), chimpanzee, and spider monkey (*Ateles*). On the other hand, the medulla is relatively small in the guinea pig (*Cavia cobaya*) and porcupine (*Hystrix cristata*).

The adrenals of man and the higher apes (gorilla, orangutan) are characterized by folds or lobulations in the cortex which are reflected in the medulla thus making the point of contact of medullary and cortical tissue much more extensive than it is in the more simply shaped adrenals of other species. The

adrenals of certain other large mammals (rhinoceros, hippopotamus, giraffe) also show this lobulation to a lesser extent. The adrenal of the porpoise is characterized by an irregular surface and when sectioned gives the appearance of cerebellar tissue due to many strands of connective tissue which penetrate the cortex from the capsule.

THE CORTEX

Although considerable variation exists in the cellular arrangement of the adrenal cortex in different species, a more or less general pattern is present according to which the cortex is divided into three zones: the glomerulosa, fasciculata, and reticularis (Figures 4, 5, and 9). The layer beneath the capsule is known as the *zona glomerulosa* and consists of cells arranged in rounded groups with an indication of an alveolar structure. The cells of this zone are usually very granular and stain deeply. The *zona fasciculata* is continuous with the *glomerulosa* and consists of columns of cells arranged radially. This zone in most species is very rich in fat. The innermost zone of the adrenal is known as the *reticularis* because of the irregular arrangement of its cells. This zone is in contact and to some extent interlaced by strands of the chromophil tissue of the medulla.

The above described division of the cortex into zones is not clearly defined in all animal species and is probably only of morphological significance. The cause for this peculiar arrangement of the cells is probably a combined effect of the manner in which the cortical cells are constantly being renewed during life and the restraining influence of the capsule and connective tissue framework of the gland. New cells are constantly being formed in the inner glomerulosar and outer fascicular zones and removed from the reticular zone. The pressure of the newly formed cells in the vicinity of the glomerulosar-fascicular boundary would tend to compress the cells on the surface thus giving the glomerulosa its characteristic

FIG. 4. MICROSCOPIC APPEARANCE OF THE ADRENAL

Section through the adrenal of a wild rat. Note the penetration into the cortical substance of the medullary tissue, shown in the lower center of the photograph ($\times 62$). (*Courtesy of Dr. W. M. Firor.*)

appearance³⁶² (cf. Figures 4 and 5). In the fetus and in the accessory cortical bodies the cells usually all show a fascicular arrangement (cf. Figure 6), without any tendency towards a division into zones. In the opossum (*Trichosurus vulpecula*) the adrenal can not be divided into the conventional zones.⁷⁷ In many other species it is only by a stretch of the imagination that one is able to differentiate the conventional zones. In the monotremes, the glomerulosar type of arrangement is found not only at the surface of the gland but also as islets in the interior of the cortex. Differentiation of the cortex into the conventional zones is not discernible in the members of this order.³⁶²

The glomerulosa is poorly developed in many species as in the rat (cf. Figure 4), the lemur (*Lemur rufifrons*), and monkey (*Cebus albifrons*). In the lemur, the glomerulosa is completely absent at certain parts of the gland, the fasciculata coming directly to the surface at these points. On the other hand, the glomerulosa is developed so well in the hedgehog (*Erinaceus europeus*) as to be differentiable even macroscopically. In the horse (*Equus caballus*) and giraffe (*Camelopardalis giraffa*), the glomerulosar cells are irregularly arranged in radial columns for which reason this zone is often referred to in these species as the "zona arcuata."³⁶²

In man the cells of the glomerulosa are small and round (Figure 5). Each round mass of these cells is surrounded by fine connective tissue with capillaries lying between them. In women the glomerular zone is said to become hypertrophied during pregnancy and to store up lipids so as to render it scarcely differentiable in appearance from the fascicular zone.¹²³ The glomerular layer of the human adrenal usually contains little lipid, which, when present, exists in the form of fine droplets.

The fascicular zone comprises the greatest part of the adrenal cortex. It varies in the arrangement of its cells from strands of a single row of cells as in the rat (Figure 4) and bats (Chiroptera) to thick strands comprised of many rows, as in

the Primates and Ungulates. In man the cells of the fasciculata are filled with fine granules and droplets consisting of cholesterol ethers. During childhood this zone is narrow but after puberty the columns are long, two or three cells in width, and closely packed (Figure 5). In late life, the columns become shorter and less closely packed.¹²³

The outer portion of the fasciculata in man has also been denoted as the "spongy zone" while the inner portion has been referred to as the "siderophile zone" because of the appearance of certain cells in this region.²³¹ These siderophile cells, which possibly represent exhausted cells, are most prominent in the guinea pig (*Cavia cobaya*).³⁰¹

The *zona reticularis*, or innermost layer of the cortex manifests the most diverse cytological appearances in different species. It is the zone in which degenerative changes occur and hence its appearance will depend on the rate at which the cells of the adrenal in a given species are being renewed.

In man, the cells of the *zona reticularis* contain fine droplets of lipid as do also the endothelial cells of the blood capillaries within the reticular layer. The reticular layer of the human adrenal is also characterized after the second decade of life by the presence of a pigment which increases in amount with age. This pigment is usually considered as analogous to the "wear and tear" pigment observed in other organs. It has no special significance or relation to the pigmentation of Addison's disease as was formerly believed. In man the reticularis is clearly differentiated from the fasciculata by the arrangement of its cells and its rich blood supply. It is more open in texture than the medulla (cf. Figure 5). The cells of the reticular zone are the largest in the cortex. They are rounded and well-defined with a coarsely granular and finely vacuolated cytoplasm. The *zona reticularis* becomes wider and more vascular as age advances. In late life it may equal the fascicular zone in width.¹²³

In the guinea pig the *zona reticularis* appears speckled due

FIG. 5. THE HUMAN ADRENAL

Microscopic section of an adult human adrenal showing the capsule, glomerulosa, and fasciculata in the upper, and the reticularis and medulla in the lower part of the photograph ($\times 80$). (Courtesy of Dr. I. Gersh.)

to the unequal staining of two types of cells which have been described as dark and light cells. The former are large rounded cells, with a finely granular pale-staining cytoplasm and contain few mitochondria, little lipid, and little pigment. The latter stain deeply, have a hyperchromatic or pyknotic nucleus and abundant mitochondria, lipid, and pigment.³⁰¹ The rabbit possesses no reticularis, the fasciculate cells being contiguous with the medulla. In the mouse, likewise (Figure 9) no clearly defined *zona reticularis* is distinguishable but in the other rodents (Figure 4) the inner part of the cortex is characterized by an irregular or reticulate arrangement.

In man at birth there is a large zone between the reticularis and the medulla. This zone undergoes involution during the first year of life and is replaced by a very thin "juxta-medullary" zone.²³¹ Both of these zones, as well as their homologues in other animals, shall be described in the next chapter.

The cells of the adrenal cortex in almost all animals are characterized by the presence of large amounts of lipids, the nature and significance of which shall be discussed in Chapter XX. The most frequent point of deposition of this lipid is in the fascicularis. However, in the armadillo (*Dasypus septemcinctus*) the lipid is found in the glomerulosa. In the cat also this zone contains fatty inclusions. In the rabbit, guinea pig, elephant, bear, and rhinoceros, there is a deposition of much lipid in the *zona reticularis*. The adrenals of certain Insectivores (hedgehog, mole) and of the marmot (*Arctomys marmot*) are relatively free of lipid as observed histologically, while in a normal healthy specimen of the emu (*Tragulus javanicus*) Kolmer³⁶² was unable to detect any lipid. This finding is of particular interest since many authors have looked upon the lipid as the specific secretion of the adrenals.

The cytology of the cortical cells has been the subject of frequent study in the hope of elucidating the function of the adrenals. These glands offer a rich field for the microscopist, but it is difficult to evaluate the significance of the results

which have thus far been obtained. Many of the described structures are probably only artifacts resulting from fixation and staining. Particular attention has been paid to the mitochondria which assume an important part in the structure of the cortical cells. In the glomerulosa of the guinea pig they are so abundant as to constitute almost all of the cytoplasm.³⁰¹ They are present throughout the cortex. The significance and function of the mitochondria, however, is still a mystery. The Golgi apparatus of the adrenal has recently been studied by Bourne.⁷⁸

The adrenal of the living frog as observed microscopically⁵⁷⁶ appears filled with small, evenly distributed yellow granules which change to a globular form. By fluorescent light a fine network of fibers are seen around the granular cells which disappear on the appearance of the globular form. Pithing and the injection of curare or mercuric chloride induce changes in the structure of the cells indicative of a diminution of their lipid content. These limited observations of Singer and Zwemer⁵⁷⁶ are deserving of a more thorough investigation with the hope of obtaining a more normal histological picture than can be derived from dead tissue.

THE MEDULLA

The chromophil cells of the medulla differ in their morphological arrangement in different animals. In some species the medullary cells are arranged in compact bundles held together by strands of connective tissue; in others they are irregularly scattered without any semblance of order. The type of cell found in the medulla is also variable ranging from a cylindrical to an irregular polyhedral type.³⁶²

In man and the common laboratory animals the cells of the medulla are irregularly arranged without the suggestive form observed in the cortex (*cf.* Figures 4 to 9). The medulla in man is characterized by a rich content of elastic supporting fibers. It is very vascular and is composed of large irregular

FIG. 6. ACCESSORY INTERRENAL TISSUE

Section through an accessory interrenal body of the white rat to show the arrangement of the interrenal cells ($\times 600$).

FIG. 7. THE MEDULLA

High power view of the chromaphil cells of the medulla of a wild rat ($\times 440$).

polyhedral cells arranged in a network. These cells have a finely granular cytoplasm. In the intercellular meshes are sinusoids which allow an intimate contact between the cells and the venous blood. In many places the endothelial lining of the blood sinuses is in direct contact with the chromaphil cells and, according to some observers, is completely absent. There is a loose network of supporting connective tissue containing smooth muscle fibers throughout the medulla. The medulla is supplied with many non-medullated nerve fibers and an occasional sympathetic ganglion.¹²³

The extra-adrenal chromaphil tissue resembles closely that of the adrenal medulla. Both tissues stain characteristically with bichromate due to the presence of epinephrine. The cell columns are thicker in the adrenal than in the abdominal chromaphil bodies. The blood spaces are also wider in the medulla and, according to Vincent,⁶⁴³ the medulla gives an appearance of a higher organization than does the accessory chromaphil tissue. The cytoplasm of the medulla is also more distinctly granular than that of the abdominal chromaphil body.

Some observers have described the presence of two types of cells in the medulla of certain animals. Such an apparent diversity in the cytological appearance of the cells is due in some cases to poor fixation, as shown by Kolmer,³⁶² for the bats (*Vesperugo noctula et pipistrellus*). However, Kolmer describes two types of cells in the hamster (*Cricetus frumentarius*), the horse (*Equus caballus*), and other species. Both types of cells show the chromaphil reaction. It is difficult to say what significance, if any, is to be attached to the morphological difference between these two types of cells.

The constant regeneration of new cells so characteristic of the cortex is not observed in the medulla. However, mitotic activity is observed, particularly in the hedgehog (*Erinaceus europeus*) in which the cytological detail of the chromaphil cells is most striking.³⁶²

One often encounters islets of cortical cells imbedded in the medulla, particularly in those species in which the division of cortex and medulla is not sharply defined.

The adrenal medulla of man and the higher anthropoid apes is a post-natal development. During early infancy the extra-adrenal chromophil tissues degenerate simultaneously with the rapid growth of the medulla which occurs at this period.

PATHOLOGICAL CHANGES IN THE MICROSCOPIC APPEARANCE OF THE ADRENALS

The microscopic appearance of the adrenals is markedly affected by a number of pathological conditions. There is a loss of lipids with the formation of vacuoles in certain infectious diseases, particularly diphtheria. Similar changes occur following endogenous toxicoses as in peritonitis or burns, and following metabolic disturbances as in diabetes or pernicious anemia. In septic processes, as in scarlet fever, there is often an edema of the cortex with the accumulation of an exudate between the cells of the fascicular layer.^{21, 156, 157}

In chronic starvation, as Landau³⁷⁶ first showed, there is an increase in the cholesterol store of the cortex, probably due to the liberation of this substance from the catabolized cells. The changes in the appearance of the adrenals with the state of nutrition must be taken into account before ascribing any observed abnormality to other causes. Thus the changes ascribed to epilepsy, dementia praecox, and other mental diseases can be explained solely on the basis of the altered nutritive condition of the patients suffering from these diseases.

Although the characteristic doubly refractive substance of the cortex increases with rest, vanishes in exhaustion, and undergoes characteristic changes in other conditions,¹⁸¹ it cannot be an essential factor in cortical activity, for it is not present in the adrenal of the sheep and certain other animal species. In pathological states, exhausting changes of the medulla and cortex, as noted histologically, move in close parallelism.

After stimulation of the splanchnic nerves, however, the medulla alone appears to be chiefly affected.¹⁸³

The cells of the adrenal cortex move towards the center to replace the innermost cells near the medulla which are constantly dying. Degenerative processes can be seen in many cells of the normal reticular zone with mitosis in the outer portion of the cortex³⁶² The process of degeneration can be accentuated by a number of toxic agents—phenol, chloroform, mustard gas, bacterial suspensions, metallic poisons, malnutrition, starvation, *etc.*—thus permitting the experimental study of the regeneration of the destroyed tissue. The guinea pig has been particularly useful in the study of these processes for its adrenals are extremely sensitive to toxic agents.³⁰¹ As von Behring showed in 1895, hyperemia and hemorrhage of the adrenals of the guinea pig follow the injection of diphtheria toxin before any other organ is affected.

The reticular zone is the site of the normal degeneration of the cortical cells and invariably suffers the most damage in infectious or toxic processes. It is also the site of most marked congestion, hemorrhages, focal degenerations, or other results of injury. In older animals the area of degeneration is greater and there is less evidence of mitotic activity than in young individuals.³⁰¹

The normal and pathologically induced degenerative changes observed in the adrenal are repaired by mitotic activity, which is found mainly in the outer portion of the fasciculata or in the border level between the glomerulosa and fasciculata.

The connective tissue of the adrenal also proliferates rapidly after hemorrhage, trauma, or other severe injury. The necrotic area becomes enveloped by connective tissue, the cellular debris is gradually absorbed, and finally only a scar remains at the site of injury.

The removal of the constantly degenerating cells of the reticularis is brought about by infiltrated polymorphonuclear leucocytes in severe lesions and by macrophages, plasma cells,

monocytes, and lymphocytes after moderate degrees of injury. The reticulum cells may be transformed into macrophages and the endothelial cells may become phagocytic. In the human adrenal the plasma cells are most common and lymphocytes second in order of their frequency. After severe experimental injury of the adrenal in guinea pigs, the macrophages are most abundant. The infiltrated small cells noted in the adrenal occur in perfectly normal animals and although they may be increased in numbers in disease, their presence is not of any pathological significance as some authors have assumed. These cells probably serve the physiological function of removing the debris normally resulting from degeneration of the reticular cells.³⁰¹

The areas of infiltration of leukocytes and lymphocytes observed in the adrenals of tumor-bearing rats represent a reaction to the necrosis occurring in the large tumors. Similar infiltrations occur in other pathological conditions and are not indicative of any relation of the adrenals to cancer.⁴¹²

CHAPTER IV

THE ANDROGENIC ADRENAL TISSUE

Besides the interrenal (or cortical) and the chromaphil (or medullary) tissues described in the preceding chapter, one finds in the adrenals of man and certain other animals a third type of tissue, which has been designated as "embryonic," "boundary zone," "fetal" or "x-zone" but to which we shall refer as the androgenic tissue. This tissue differs from the remainder of the cortical tissue not only in its morphological and histochemical properties but also in the fact that it normally exists only during a certain period of life. The hypertrophy of this tissue under certain pathological conditions in man (*cf.* Chapter XXIII) gives rise to a symptom-complex characterized by masculinization of the female. Because of this property, the author has designated the tissue as androgenic (from the *Greek*, *ἀνδρόγενος*, to masculinize).

This differentiation of the adrenal cortex into two functionally distinct moieties has not hitherto been made. Although experimental evidence remains to be adduced to prove definitely the exact relation of the function of the androgenic zone to the reproductive system, the facts at hand suffice to indicate that the cortex comprises two tissues, one of which (the interrenal tissue) produces the hormone which is essential for life, and the other (the androgenic tissue) which has some as yet incompletely defined function relating to the reproductive system.

The most characteristic property of the androgenic tissue is its persistence normally for only a relatively short period of time. In man it develops during fetal life and disappears almost entirely during the first year of life.¹⁸² In the mouse, it develops before puberty and disappears in the male at

puberty, while in the female it undergoes involution shortly after ovulation is well established.³¹⁸ This disappearance of the androgenic tissue is not a result of a decreased demand of the organism for interrenal tissue, for removal of one adrenal does not prevent the observed involution of the androgenic tissue in the remaining gland.³²⁰ The probable relation of the androgenic tissue to the reproductive system is indicated by the changes which it undergoes at puberty, during pregnancy,²³¹ after castration in the male,³¹⁸ and by the disorders of the reproductive system, described in Chapter XXIII, which are accompanied by hypertrophy of this tissue.

THE ANDROGENIC TISSUE IN MAN

During early embryonic life the human adrenal exceeds the kidney in size; at birth it is one-third as large as the kidney. This large size of the adrenal at birth is due to the presence of a relatively thick zone of tissue separating the true cortex (or interrenal tissue) from the medulla. This tissue has been considered by previous authors as part of the interrenal system and as exercising the same function as the rest of the cortex. Such a view, however, fails to take into account the facts to be presented below and we shall therefore consider this tissue which comprises what we have designated as the androgenic zone, as a glandular entity, specific in its function, and to be differentiated from the rest of the cortex.

Much of the tissue which has in the past been described as accessory cortical tissue is, in reality, also a part of this androgenic tissue and completely independent functionally from the cortex proper and the true adrenal cortical accessory bodies. This accessory tissue is very common in man at birth, disappearing during infancy with the androgenic zone of the main gland but persisting in cases of hermaphroditism (*cf.* Chapter XXIII) and occasionally giving rise to tumors which in the female cause adrenal virilism. These accessory adrenals are situated at some distance from the main glands and are re-

ferred to as androgenic tissue, in the present book, because like the androgenic zone they are transient and disappear at an early age, hypertrophy when associated with certain hermaphroditic conditions, and give rise to virilism when the site of a cancerous growth. The term androgenic is introduced to indicate their functional masculinizing potentiality. These accessory bodies were described by Marchand⁴³⁰ and are often referred to in the literature as Marchand's bodies.

The accessory androgenic tissue occurs in various parts of the organism, beneath the lower pole of the kidney,⁴³⁰ along the internal spermatic artery,⁴³¹ on the ileopsoas muscle,⁴⁵³ in the solar⁵⁰⁷ or renal plexuses,⁵⁵⁶ between the transverse colon and the spleen, in the right lobe of the liver,⁴⁷⁸ in the pancreas,⁵³⁵ and particularly in association with the reproductive organs. This tissue when associated with the reproductive organs of the male occurs along the spermatic cord, between the testis and epididymis, in the rete testis, and in the paradidymis.⁴⁵³ In the female it is observed in the *ligamentum latum*,⁶²³ on the Fallopian tubes, and in the ovaries.^{363, 656} Schmorl⁵⁵⁶ found such androgenic tissue in 92 per cent of autopsies. Wiesel⁶⁷⁸ found it in 76 per cent of the genitalia of newborn children and Aichel⁸ found it in the broad ligament of every newborn female child which he examined. Aschoff²¹ denies this, however, and thinks Aichel confused chromophil with interrenal tissue.

On the basis of his embryological investigations, Aichel⁸ concluded that Marchand's bodies or as we have termed them, the accessory androgenic bodies, are entities to be differentiated from the true accessory bodies which lie in close proximity to the main glands. According to Aichel the androgenic bodies arise from the degenerating tubules of the paraoöphoron and the epoöphoron and are thus embryologically distinct from the true accessory adrenals. Aichel's views have received little attention from subsequent authors who have inclined to the view that the androgenic bodies represent adrenal tissue dis-

placed from its original position by the migration of the reproductive tract during embryonic development. Physiological experiments as well as pathological observations are, however, in accord with Aichel's conclusions.

INVOLUTION OF THE ANDROGENIC TISSUE IN MAN

The post-natal involution of the androgenic zone was first described in 1911 almost simultaneously by Thomas,⁶²⁶ Elliott and Armour,¹⁸² and Kern.³⁵² In the embryo of eight weeks the adrenal lies anterior to and in contact with the kidney. The two organs at this stage of development are of equal size. The peripheral cells are filled with lipids and represent the cells which will subsequently form the cortex of the adult adrenal. The central part of the gland is composed of larger, angular cells filled with granular cytoplasm. These cells which are loosely arranged constitute the androgenic zone of the adrenal. During most of the period of intrauterine development, the true cortex remains as a comparatively thin rim of closely packed small cells with deeply stained nuclei. The androgenic zone grows rapidly and forms the main bulk of the gland. At birth the true cortex has assumed its division into definite zones. The androgenic zone begins to involute soon after birth and this involution continues during the first years of life. It is not until about the third year of life that the gland assumes the characteristic appearance of the adult gland.¹²³

The androgenic zone in the new-born differs from the cortex proper in several respects. It does not contain the characteristic doubly refracting substance which characterizes the true cortical tissue. It is characterized by an extremely abundant vascular supply with some extravasation of blood. This appearance of hemorrhage has been attributed to injury during birth but the existence of this hemorrhage in the adrenals of newborn delivered by Caesarian section and during fetal life demonstrates that it is not a result of injury.³⁷⁶

Soon after birth involution begins, assuming a massive form during the second week of life. During this period the cells of the androgenic zone are found in all stages of nuclear and protoplasmic degeneration. Fat droplets appear in the degenerating cells which are displaced and compressed by the concomitant hyperaemia and growth of the medulla which occurs rapidly at this stage. Phagocytosis of red blood cells with pigmentary degeneration occurs with thickening of the intercellular reticulum.¹⁸² The degenerating androgenic zone is sharply marked off from the true cortical tissue which remains intact, and from the growing medulla.

At the end of the first year the above involutionary processes are almost complete and the androgenic zone is replaced with connective tissue in the meshes of which may still be found degenerating pigmented cells. This connective tissue by compression becomes narrow and ultimately assumes the layer separating the medulla from the adult cortex.

Neither prematurity nor inanition were found by Lewis and Pappenheimer³⁹⁵ to affect the involution of the androgenic zone, but syphilis markedly retarded its disappearance. Infection by pneumonia caused an increased size of the true cortex while the involution of the androgenic tissue continued, which is evidence for the functional independence of the two tissues. Lewis and Pappenheimer also showed the simultaneous disappearance of the "accessory cortical tissue" or accessory androgenic bodies, as we have called them, which is evidence for their physiological homology with the androgenic zone of the adrenal.

The significance of the involution of the androgenic zone in man has often been the subject of speculation but the theories heretofore advanced to describe the phenomenon are easily refuted. The idea that it results from pressure by the growing medulla was shown by Lewis and Pappenheimer³⁹⁵ to be untenable since a similar degeneration occurs in the accessory bodies where there is no growth of medullary tissue. Ma-

rine's⁴³³ suggestion that the involution represents a physiological response due to diminished demands for cortical tissue because of the antagonistic action of the thyroid takes no account of the morphological features of the process nor of the results in the mouse where the degeneration occurs sometime after birth. In pneumonia also, as we have already seen, an actual stimulation of the growth of the true cortex occurs while degeneration of the androgenic zone proceeds at an undiminished rate.

The peculiar deposition of lipids characteristic of the involution of the androgenic zone is evident in early fetal life (4th month) in the cells of the innermost cortical layer which surround the medullary vein.⁶⁹⁴ This lipid, as judged from its staining reactions and appearance under the polarizing microscope, differs from that which later characterizes the cortex proper. In those animals in which no involution occurs, the appearance of the lipoidal deposits in the cortex at birth resembles that of the adult gland.

It is interesting to note that in hemicephaly, the adrenals at birth, as Morgagni first observed in 1723, are extremely small.⁶⁹⁶ This is due to the absence of an androgenic zone associated with maldevelopment of the reproductive system.¹⁸² In *spina bifida* or hydrocephalus no abnormalities of the androgenic zone are observed. The period of infancy during which the androgenic zone is at its height of activity is also a period of activity of the interstitial cells of the testes which is in accord with the view that the androgenic tissue exerts a masculinizing effect analogous to that exerted by these cells.

In the adult human adrenal one can observe a thin juxta-medullary zone which differs from the rest of the cortex by the disposition of the cells and their chemical and morphological properties.²³¹ These cells are osmophilic and pigmented and are denoted by some writers as part of the reticularis. Others have described these cells as "embryonic in character." As pointed out by Goormaghtigh²³¹ these cells which border the

medulla resemble the cells of the androgenic zone of the infant and are probably a remnant of this involuted tissue. Goormaghtigh observed an hypertrophy of this juxta-medullary tissue in a woman at the menopause who developed hirsutism, in an old woman, and in a third woman during pregnancy.

It is thus probable that the androgenic tissue may remain throughout life as a small inactive group of cells, which hypertrophy when the female reproductive system undergoes fundamental changes. When this hypertrophy becomes pronounced the adreno-genital syndrome described in Chapter XXIII results. According to this theory remnants of the androgenic zone which have not undergone degeneration are responsible for the pathological manifestations of the reproductive system which are associated with certain tumors of the adrenal. The true interrenal tissue is not involved in the genesis of these disorders (*cf.* Chapters XXII and XXIII).

THE ANDROGENIC ZONE IN THE MOUSE

Howard³¹⁸ first demonstrated an area in the mouse, which she designated as the X-zone and which she considered to be homologous to the human androgenic zone. In the young female, this tissue may comprise two-thirds of the entire cortex. Although appearing during extra-uterine life, this zone is temporary in its existence and apparently is the functional homologue of the human androgenic zone. Howard showed that the androgenic zone in mice (or X-zone as she called it) disappeared at 38 days (on an average) in male mice. In females the zone underwent involution during the early part of pregnancy, persisting, if the animal did not conceive, for 3 to 6 months before it disappeared. Castration caused a prolonged persistence of the zone in the male but was without effect in the female. Deansley¹⁵³ and Whitehead⁶⁷² have also made detailed studies of the development and involution of the androgenic⁵⁷ tissue in the mouse adrenal.

In Figure 8 are reproduced cross sections of the adrenals of

12 week-old male and female mice, showing the presence of the androgenic zone in the latter. In Figure 9 are shown the histological appearances of the androgenic zone in the mouse, showing the manner of its involution and its ultimate replacement by a band of connective tissue.

The androgenic zone of the mouse is characterized histologically by its being more deeply stained by Hemotoxylin-Eosin than is the adjacent true cortical tissue of the *zona fasciculata*. It also does not stain with Sudan due to the absence of the doubly refracting lipids which are characteristic of the permanent cortical tissue. In shape, the cells of the androgenic zone are superficially like those of the *fasciculata* except that they are smaller and at the boundary of the *fasciculata* they are elongated and flattened. After its degeneration, this zone assumes the appearance of alveolar connective tissue which replaces the previously existent glandular tissue.^{153, 318, 673}

The above anatomical considerations would not in themselves characterize the androgenic zone as a specific tissue from the standpoint of its functional capacity in the body. Obviously the different staining reactions and cell shapes might be mere morphological deviations independent of any functional significance. It is in this respect that the anatomical methods manifest their limited value. However, the changes occurring in the androgenic tissue indicate the abolition of a function previously fulfilled by the androgenic zone.

Failure to appreciate the true nature of the androgenic zone in mice has led to several flagrant misinterpretations of experimental observations. Thus the observations of Cramer¹³⁴ and his conception of the rôle played by epinephrine under various conditions are vitiated by his failure to note the transient existence of the androgenic zone in mice.^{319, 671}

ANDROGENIC ZONE IN OTHER ANIMALS

Attempts have been made to find the involution characteristic of the human and mouse adrenal in other animal species.

FIG. 8. THE ADRENAL OF THE MOUSE

Cross-section views of the adrenal of the mouse (*Mus musculus*). On the left is a gland from a 12 week-old male, showing the appearance of the adult gland after involution of the androgenic zone. On the right is a gland from a 12 week-old female showing the full development of the androgenic zone ($\times 50$). *m*, medulla; *z.g.*, zona glomerulosa; *z.f.*, zona fasciculata; *z.r.*, zona reticularis; *z.x.*, androgenic zone; *b.v.*, blood vessel. (Reproduced through the courtesy of Dr. R. Deansley¹⁵³ and the Proceedings of the Royal Society of London.)

1 2 3

FIG. 9. THE INVOLUTION OF THE ANDROGENIC ZONE

The adrenal of a female mouse showing the stages of involution of the androgenic zone (X). In 1, this zone is intact. A band of conspicuous nuclei separates the androgenic zone from the fasciculata (F) and glomerulosa (G). In 2, the androgenic zone is in process of involution, the cells being almost completely vacuolated. In 3, this zone is replaced by a band of connective tissue which separates the cortex from the medulla (M) ($\times 200$). (Reproduced through the courtesy of Dr. Evelyn Howard¹³ and the American Journal of Anatomy.)

None has been demonstrated in the dog, cat, rabbit, or cow. It is very improbable that this tissue is limited to man and the mouse, and future studies shall undoubtedly reveal an analogous structure in other animals.

The accessory bodies observed by Wiesel⁶⁷⁶ in the reproductive tract of young rats are possibly androgenic tissue for they disappear, according to Wiesel, with age and do not hypertrophy or support life in adrenalectomized animals¹⁹³ as they should if they comprised part of the interrenal system. No one has, however, confirmed Wiesel's description of these bodies.³³⁵

A tissue has also been described in the pig embryo⁹⁵ which resembles the androgenic tissue of man and may represent its homologue in this animal. Incomplete studies⁶⁹⁴ on the anthropoid apes (Orangutans and chimpanzees) also indicate the occurrence of a post-natal involution similar to that of man.

Kolmer³⁶² from his histological study of the adrenal of a nine-month old elephant (*Elephas indicus*) was led to believe that a process of involution similar to that which occurs in the human adrenal, also takes place in this animal. His description of the adrenals of certain other species also suggests the presence of an androgenic zone in the little ant-eater (*Tamandua tetradactylus*), the armadillo (*Dasypus septemcinctus*), and the porcupine (*Hystrix cristata*). The thick layer of connective tissue which surrounds the medulla in these species is analogous to that seen in the mouse after the involution of the androgenic zone. The unique structure in the adrenal of the opossum (*Trichosurus vulpecula*), described by Bourne,⁷⁷ is also suggestive of the development of an androgenic zone in this species. Further work with these species will be necessary to determine if an androgenic zone is present during the early development of these animals.

It is questionable if the involution of the cortex recently described by Whitehead⁶⁷³ represents the degeneration of androgenic tissue in the rabbit.*

*Cf., however, Jour. of Anatomy, vol. 70, p. 126.

PART II. THE MEDULLA

The medulla of the adrenal is characterized by the presence within it, in rather high concentration, of epinephrine, a substance whose remarkable pharmacological actions has made it the subject of intense study. The existence of this chemically potent substance was first observed by Vulpian,⁶⁵² who, in 1856, noted the green coloration which occurred on moistening the medulla with a dilute solution of ferric chloride. Henle²⁹³ first observed the "bichromate" reaction in which the medulla assumes a characteristic brownish discoloration when treated with a solution of a salt of chromic acid. Because of this reaction, which is shared by certain extra-medullary tissues, the generic term "chromaphil" tissue is applied to these structures.

Oliver and Schafer⁴⁵⁰ in 1894 and Szymonowicz,⁶¹⁶ independently, first called attention to the remarkable blood pressure rise which followed the injection of an extract of the adrenal medulla. Lewandowsky,³⁹¹ in 1898, and Langley,³⁷⁸ in 1901, called attention to the similarity in the pharmacodynamic effects of epinephrine and the effects elicited by stimulation of the sympathetic nerve fibers. With rare exceptions this similarity in function has been confirmed, and epinephrine is classed as a sympathomimetic substance because it mimics in its effects the results of sympathetic nervous stimulation.³⁶

The iron chloride reaction of the adrenal medulla (Vulpian's reaction) and the relatively high concentration in which epinephrine occurs in the adrenals rendered it relatively easy to isolate and identify this substance. The discovery of epinephrine, its isolation, and the demonstration of its powerful pharmacodynamic action focused all attention on this substance as the important product of the adrenal gland. For the following two decades much work was done on its pharma-

cological effects. Numerous experiments were devised and theories elaborated concerning its supposed physiological action in the body. This preoccupation with all phases of interest in connection with epinephrine left relatively little attention for the problems associated with the cortex. It is only in recent years that this part of the adrenal gland has received its due share of attention.

In the present section we shall consider the chemical, physiological, and pharmacological actions of epinephrine.

CHAPTER V

THE CHEMISTRY OF EPINEPHRINE

Epinephrine is found in highest concentration in the medullary portion of the adrenal gland. Considerable controversy once raged as to its occurrence in the cortex, some authors maintaining that it was actually elaborated in the cortex and merely stored in the medulla. This view was based on the demonstration of epinephrine in the cortical cells.²⁷⁸ This presence of epinephrine in the cortex is attributable, however, to its rapid diffusion, *post mortem*, from the medulla. If proper care be taken to remove the gland from the body before the circulation has stopped and immediately cut away the cortical tissue, no epinephrine will be found in the cortex. The occurrence of epinephrine in other chromaphil tissues besides the adrenal, its presence in the chromaphil bodies of certain Selachian fishes in which the cortical tissue is present as a separate organ, and finally its presence in the embryo, before the fusion of the chromaphil and interrenal tissues, all speak against the view that the cortex is involved in the elaboration of epinephrine. Nor is there any valid evidence for the existence of a pharmacodynamically more potent precursor from which epinephrine is formed.

The epinephrine content of the adrenals varies from about one-half to three milligrams per gram of adrenal tissue. Thus a pair of human adrenals contains about 8 mgms. of epinephrine. Both adrenals of the beef contain about 40 to 70 mgms., the pig, 9, dog, 2, cat, 0.5, and rat, 0.15 mgms. The epinephrine content of the adrenals is variously given in the literature depending upon the methods used for its determination.⁶³⁸

Epinephrine is also present in the chromaphil tissue in other

regions of the body besides the adrenal. According to Elliott¹⁸¹ the paraganglion aorticum of the newborn contains twenty-four times as much epinephrine as both adrenals despite the twenty-five-fold size of the latter.³⁹³ In the dog, Kahn³⁴⁴ found one-twelfth to one-thirtieth as much epinephrine in the extra-chromaphil tissue as in the adrenals.

Many sympathetic ganglion cells also contain small amounts of chromaphil tissue.²⁵⁹ Whether or not these contain epinephrine is doubtful.²⁰⁹ The carotid sinus, although a chromaphil tissue, gives only a feeble reaction for epinephrine.³⁸⁴ It would thus appear that although chromaphil tissues may contain epinephrine, this substance is not invariably present and hence is probably not an indispensable mediator for their activity.

Epinephrine is not limited in its occurrence to the chromaphil system of the sympathetic ganglia but occurs in certain secretions of reptilia. The skin-glands of the toad, *Bufo aqua*, contain about 5 per cent *levo*-epinephrine as Abel and Macht² demonstrated. Certain poisonous chinese toads also contain epinephrine in their skin glands.³³⁹ The skin glands of the European and North American toad contain none. Histological investigations also indicate epinephrine to be present in many invertebrates; *e.g.* in leeches in which six chromaphil cells are present in the ganglia of the central nervous system. In certain molluscs as in the mantle of *Purpura lapillus*, chromaphil tissue, extracts of which manifest an epinephrine-like action, are also present.⁶³⁹

ISOLATION AND STRUCTURE OF EPINEPHRINE

Abel¹ first applied the term epinephrine to a crystalline compound which he obtained from the adrenal gland and which he considered to be its active principle. Subsequent work showed Abel's compound to be the *N*-benzoyl derivative of the active principle, but the term epinephrine has been retained for the base itself. Aldrich⁹ and Takamine⁶¹⁸ in 1901 first isolated epinephrine as the free base. They called their

compound "adrenalin," the name recognized officially in the British Empire and European countries and used extensively in the United States despite the official preference for the term "epinephrine." "Suprarenin," another commonly used term for this compound, was introduced by von Fürth²⁰⁸ who prepared insoluble metallic salts of the active principle but failed to isolate the free base in pure form.

The correct empirical formula of epinephrine ($C_9H_{13}O_3N$) was first derived by Aldrich.⁹ The existence of a catechol nucleus

in this compound was suggested by the green coloration given when epinephrine is treated with ferric chloride. This color reaction is common to catechol and its derivatives. Takamine⁹ showed that fusion of epinephrine with KOH yielded proto-catechuic acid.

More direct evidence of the position of the side chain of the catechol nucleus was given by Jowett³⁴² who methylated the hydroxy groupings in the benzene ring of epinephrine (to protect the aromatic radical) and on oxidation of the resulting methyl ether obtained the dimethyl ester of veratic acid.

This reaction showed the position of the side chain relative to the hydroxyl groupings in the epinephrine molecule. The fact

that methylamine could be split off from the compound suggested the presence of a methylamino group in the side chain. Friedmann²⁰⁷ oxidized the tribenzenesulphonyl derivative of epinephrine to a ketone, thereby demonstrating the existence of a secondary alcoholic grouping in the side chain. It thus became probable that epinephrine was represented by the structural formula

which conforms to the requirements of the reactions cited above. The existence of an asymmetric carbon atom in this formula accounts for the optical activity manifested by epinephrine solutions, as first observed by Pauly.⁴⁸⁷

The systematic name for epinephrine as represented by the above formula is dioxypheylethanolmethylamine, α -methyl-amino- β -hydroxy- β -(3, 4-dihydroxyphenyl) ethane, or methyl-aminoethyl-(3, 4-dioxypheyl)-carbinol.

The ultimate proof of the correctness of the above described formula was furnished by Dakin¹⁴⁶ and Stolz⁵⁹⁹ who synthesized epinephrine. This synthesis, which has received commercial application for the preparation of the compound, consists of the following steps:

The last compound on reduction yields epinephrine.

Synthetic epinephrine prepared as just described is optically inactive. To obtain the levo-rotary form which occurs naturally, the racemic compound is resolved by Flächer's¹⁹⁹ method in which the *d*-tartaric acid salt of *dl*-epinephrine is extracted with methyl alcohol in which the dextro-rotary salt is soluble, leaving behind the insoluble *l*-epinephrine-*d*-tartrate, from which in turn the *l*-epinephrine is liberated by treatment with alkali. In this way a compound identical with the natural product is obtained.

Since *d*-epinephrine has little pharmacological activity, it is also converted to the *l*-form in the synthetic preparation of epinephrine. This is accomplished by racemizing the *d*-form by heating with mineral acid and then repeating the above described process for resolving the racemic mixture into its *l*- and *d*-rotatory constituents.³⁵

At present both the natural and synthetic sources are utilized in the manufacture of the epinephrine of commerce.

PREPARATION OF EPINEPHRINE

Epinephrine is prepared in large quantities for medicinal use either synthetically or by extraction from adrenal glands. Its preparation from glandular material is relatively simple and consists in extraction of the minced glands with dilute acid, removal of proteins (after concentrating the aqueous extract) with alcohol, and precipitation of the free base by the addition of ammonia after evaporation of the alcohol. In manufacture on a large scale the theoretical yield, about 0.2 per cent of the weight of the fresh glands, is obtained.³⁵

A convenient laboratory method for preparing pure epinephrine, as described by Bertrand,⁵³ is as follows: 600 grams of finely minced defatted adrenal glands are mixed with 5 grams of oxalic acid and sufficient 95 per cent ethyl alcohol to fill a two liter flask. The flask should be well filled to exclude air, stoppered, and shaken at intervals for two days. The contents are then filtered through muslin and well pressed. The filtered extract is concentrated *in vacuo* until the alcohol is re-

moved. Lipoidal substances separate out at this stage. To eliminate further these lipoidal substances, the aqueous residue is cautiously shaken with petroleum ether. The aqueous layer is separated and precipitated by adding neutral lead acetate, avoiding any excess of this reagent. The lead precipitate is removed by centrifugalization and the remaining fluid concentrated *in vacuo* to about 200 cc. A slight excess of NH_4OH is added to precipitate the epinephrine. To repurify the crude product thus obtained, it is dissolved in $2\frac{1}{2}$ times its weight of 10 per cent H_2SO_4 and an equal volume of ethyl alcohol is added. After filtering, the epinephrine is again precipitated by NH_4OH .²⁰⁴

The racemic form of epinephrine as obtained synthetically is only half as active as the naturally occurring levo-rotatory form. This is due, as Cushny¹⁴³ first showed, to the fact that *d*-epinephrine is only about one-fifteenth as active, for a given weight of substance, as the naturally occurring *levo*-form. Due regard of this fact has not always been taken and certain synthetic products have been utilized whose pharmacological action was much inferior to the natural product.

To determine the activity of a given preparation one must resort to the biological assay described in the next section. The degree of racemization of a chemically pure preparation can be determined more easily by the use of a polarimeter.⁶²⁷

Epinephrine is usually supplied in commerce as a solution of one part per thousand of the hydrochloride dissolved in physiological salt solution. To stabilize the solution, about $\frac{1}{2}$ per cent of chloretone is added. The solution is saturated with carbon dioxide to exclude dissolved oxygen and stored in amber colored containers. In faintly acid solution, the drug is stable for a long period. However, in using such preparations, one must consider the possible pharmacological effects of the free acid and the chloretone. Failure to do so has caused some of the discrepancies recorded in the literature.⁶³⁷ For refined work, obviously, a fresh solution of the crystalline base should be used.

PHYSICAL PROPERTIES

Epinephrine is a white, micro-crystalline, hygroscopic compound which rapidly decomposes when exposed to the air.¹³⁵ Ultraviolet light, particularly, hastens its oxidation. Hermetically sealed, however, it may be permanently stored. Epinephrine melts with decomposition at about 216° or at 263° on *Maquenne's* block. The natural epinephrine is levorotatory, $(\alpha)_D^{20}$ being variously given by different authors, as -50.72° to -53.3° .

Epinephrine is scarcely soluble in water, a saturated solution containing 0.095 milligrams per cc. at 20° to 25°. It is practically insoluble in alcohol and insoluble in chloroform, petroleum ether, benzol, or ether.¹³⁵

Despite the relative insolubility of epinephrine in water, aqueous solutions of certain salts have a marked solvent action, (*e.g.*, the borates) and epinephrine hydrochloride itself exerts such an action. The phenolic groupings of epinephrine form water-soluble salts with strong alkalis. The alkaline carbonates or ammonia can not effect this salt formation and hence may be used, as in the isolation of the compound, for precipitating epinephrine as the free base.

The NH_2 -grouping of epinephrine imparts to it a feebly basic character in virtue of which it forms salts which are readily soluble. Epinephrine hydrochloride, thus formed, is the compound used in medicine.

CHEMICAL PROPERTIES

The chemical properties of epinephrine are inherent in the phenolic, alcoholic, and amino groupings which it contains. Like derivatives of catechol (compare, for example, the action of pyrogallol), epinephrine is easily oxidized in neutral or alkaline solution. In fact, epinephrine in aqueous solution is stable only at a pH of 5 or less. In a more alkaline solution oxidation on exposure to air rapidly occurs as evidenced by the gradual change in color of the solution to pink, then red,

and finally brown, with the formation of an insoluble brownish precipitate. Blood according to Maiweg⁴²² stabilizes epinephrine so that despite its alkalinity (pH 7.4) epinephrine is not as rapidly destroyed as might be anticipated from its reaction in aqueous solution. In the animal body, indeed, a number of substances are present (glutathione, cysteine, ascorbic acid and other reducing agents) which prevent the irreversible oxidation of epinephrine and account for its pharmacological effects obtained at points distant from the site of its injection.⁶⁶⁶

Epinephrine, like catechol, when oxidized is probably first converted to an ortho-quinone. This quinone is destroyed with such extreme rapidity that it is impossible to form an oxidation-reduction system with epinephrine.²⁹

Epinephrine forms relatively few stable salts. The hydrochloride which is the salt of commerce has already been mentioned. Boric acid forms a stable complex, $C_{15}H_{27}O_{11}N_2B_3$, freely soluble in water. This complex is claimed not to be decomposed by alkalis. A dibenzoyl derivative of epinephrine and a chlorbenzoyl derivative have also been described.³⁴

COLORIMETRIC ANALYSIS

The numerous reactions by which epinephrine forms colored solutions has led to their utilization for its colorimetric analysis. These methods for determining epinephrine are limited in their applicability due chiefly to their non-specificity. Other catechol derivatives as well as certain reducing substances also give the same color reactions so that their presence in the solutions to be tested lead to erroneously high results. Only the most widely used methods will be discussed here.

The green coloration produced by epinephrine or other catechol derivatives when reacting with ferric chloride has been used at times for their colorimetric determination. The reaction is very sensitive to pH changes and gives unsatisfactory results. According to most recent studies, the Vulpian

reaction should be carried out in slightly alkaline solution, in the presence of an excess of ferric chloride.⁵⁵³ The purplish coloration thus produced is proportional to the molar concentration of the catechol derivative present in the solution tested.

The ease with which epinephrine is oxidized to give a red coloration has given rise to a number of methods for its analysis. For this purpose, many oxidizing agents have been suggested—iodine, iodic acid, chlorine, mercuric chloride, persulfates, osmic acid, *et cetera*.⁵³⁹ Iodic acid has been used by a number of workers.⁵⁶⁴ The addition of sulphanilic acid increases the sensitivity of this method so that a dilution of as little as 1 part of epinephrine in 5,000,000 is detectable.³⁴¹ Unfortunately this delicacy is not specific to epinephrine.

The use of persulphate for the estimation of epinephrine has recently been investigated by Barker, Eastland, and Evers.³⁷ These authors consider this reagent the most accurate for the colorimetric determination of epinephrine.

The use of iodine as an oxidizing agent in the colorimetric determination of epinephrine has been revived by Euler¹⁸⁸ who has worked out an exceedingly accurate procedure using the spectrophotometer. This method is not interfered with by the presence of reducing agents such as ascorbic acid.

Perhaps the most widely used colorimetric method for the determination of epinephrine is the method of Folin, Cannon and Denis.²⁰² This consists in reducing tungstic acid to its blue oxides. This is an exceedingly sensitive method capable of detecting as little as 0.003 milligrams of epinephrine. Although yielding accurate results when applied to pure solutions, it is so non-specific as to yield erroneously high values when other reducing substances are present. Uric acid, for the determination of which the reagent was originally devised, and ascorbic acid react like epinephrine to give the blue color. Their presence in many biological fluids thus vitiates the results obtained in analyses of epinephrine. Although epinephrine is destroyed by the passage of air through its alkaline

solution, the oxidation products formed still reduce phosphotungstic acid. Folin's method is also inapplicable in the presence of cocaine or procaine. The use of the Folin-Denis colorimetric method can not therefore be relied upon as a test for the presence of epinephrine. Thus extracts of the cortex often show the apparent presence of as much as a milligram of epinephrine per gram of tissue when biological assay reveals the absence of any epinephrine.

Other less important color reactions are also given by epinephrine and have been suggested for its determination. Thus it gives a permanent red color with CuSO_4 and KCN . The Fränkel-Allers color reaction depends on the formation of a rose-color when epinephrine hydrochloride is heated with KIO_3 and dilute H_3PO_4 . Seidell suggested the use of the red color produced on oxidizing epinephrine with MnO_2 .⁶³⁹

Whitehorn⁶⁷⁵ has recently described a method for the determination of epinephrine in biological fluids which depends upon its adsorption by silica. The adsorbed epinephrine is eluted by sulfuric acid and determined colorimetrically. The method is sensitive to one part of epinephrine in 50 million parts of solution.

As may be seen from the preceding comments the colorimetric determination of epinephrine, despite its simplicity and accuracy in pure solution, is fraught with errors in most conditions in which it is desired to be applied. Careful check by one of the biological methods, to be described in Chapter VI, is thus always necessary^{601, 659} if one is to obtain reliable results.

THE ORIGIN OF EPINEPHRINE IN THE BODY

The origin of epinephrine in the body is still unproven although several suggestions have been offered to account for its probable origin. The close structural relation which epinephrine bears to the two amino acids, tyrosine and phenylalanine, suggests that either might give rise to epinephrine by

successive oxidation, methylation of the nitrogen atom and decarboxylation. Attempts to carry out experimentally these processes have, however, not been successful. Tyrosine is oxidized in the presence of tyrosinase to give 3,4, dihydroxyphenylalanine (acrostically abbreviated to dopa), which represents the first stage in the above described series of reactions by which tyrosine would be expected to be converted into epinephrine. Moreover, dopa, although never demonstrated in animals, has been found in the pods of the bean (*Vicia faba*) and in certain insects. If tyrosinase is allowed to act on the methyl ether of dopa (3,4-dihydroxyphenyl-*N*-methylalanine) a small amount of a pressor base is formed whose activity is much enhanced by reduction. Heard and Raper²⁹⁰ consider this pressor base to be adrenalone, the ketone of epinephrine (adrenaline), represented by the formula,

which on reduction gives epinephrine. The above reaction occurs simultaneously with the main series of reactions which give rise to indole derivatives and ultimately to melanin.³⁵

Although the intermediate compounds have not been isolated, we can, according to Heard and Raper,³⁵ represent the probable changes occurring in the transformation of tyrosine to epinephrine by the following formulae:

Attempts to carry out the above reactions by perfusing the adrenal gland have heretofore failed. This could be accounted for by assuming a very rapid loss of its synthetic powers after removal of the gland from the body or that certain of the intermediate steps of the reaction occur in organs other than the adrenal. Repetition of the experiment by perfusion of the organ *in situ* and insertion of the necessary cannulae before interrupting the normal blood supply of the gland would promise greater success than the use of a "chilled" gland removed from the abattoir to the laboratory as employed by previous workers.

The earlier theories concerning the origin of epinephrine in the organism, as described above, presuppose its derivation from tyrosine or from dopa. However, no evidence has ever been adduced to show that either of these substances can be converted to epinephrine in the adrenal. On the other hand, as Nikolaeff⁴⁷² first demonstrated, perfusion of the surviving adrenal with a solution containing tyramine results in the production of a substance having the properties of epinephrine. Schuler and Wiedemann⁵⁵⁷ using slices of fresh adrenals were able to demonstrate the formation from tyramine of a substance which, like epinephrine, reduces the Folin-Dennis reagent and also raises the blood pressure when injected into

an animal. This formation of an epinephrine-like substance did not occur when an emulsion of the gland was used and hence is a property of the intact cell. The addition of other substances, related structurally to epinephrine, such as dopa, tyrosine, hordenine, phenylalanine, or phenylethylamine did not result in the formation of an epinephrine-like product. It would appear from these experiments that the adrenal medulla forms epinephrine from tyramine. The latter substance, according to Schuler and Wiedemann, is formed in the kidney by decarboxylation of tyrosine.

THE DOPA REACTION

The pigmentation observed in Addison's disease has been attributed by Bloch to an abnormal metabolism of epinephrine or its precursors. Bloch⁶³ showed that an isolated piece of skin if soaked in dioxyphenylalanine (Dopa) assumes a pigmented form similar to that observed in Addison's disease. Since dopa is closely related chemically to epinephrine and may be considered as a precursor of epinephrine in the body, Bloch assumed that disease of the medulla, by preventing the formation of epinephrine from its assumed precursor, dopa, allows the latter to discolor the skin. This discoloration of the skin is assumed to be brought about by a specific enzyme (dopase) which converts dopa into melanin.

Raper³⁵ has studied the reaction whereby tyrosine is converted to dopa which on further oxidation yields an indole carboxylic acid which can be transformed into dihydroxyindole,

which in turn condenses to a black pigment similar to the melanin of the skin. The black appearance of bean pods on

exposure to the air is also due to their content of dopa. Epinephrine, too, may be oxidized in the presence of certain oxidases (such as that found in the ink-bag of the cuttle fish, (*Sepia officinalis*) or in toadstools) to give a black pigment resembling the natural melanin found in the skin.⁴⁶⁶

In view of the above described reactions, it has been assumed that epinephrine, tyrosine, phenylalanine, or dopa, are precursors of the melanin pigment of the skin. However, in considering the chemical theories which have been elaborated to account for the abnormal deposition of melanin in the skin of persons afflicted with Addison's disease, it must be remembered that the characteristic pigmentation also occurs in cases in which the medulla appears to be normal. This would indicate that disease of the cortex is primarily responsible for the pigmentation and that any abnormalities in epinephrine metabolism must be only secondarily involved (*cf.* Chapter XXI).

OTHER COMPOUNDS RELATED TO EPINEPHRINE

The important pharmacological properties of epinephrine have led chemists to prepare a number of related compounds some of which have interesting actions, similar to those of epinephrine. None of these has, however, seriously rivalled the natural product and we need only refer to them here. Besides ephedrine, a closely related naturally occurring compound, the most important of these compounds are dihydroxyphenylethanolamine (arterenol) or epinephrine without the methyl group and dihydroxyphenylpropanolamine (homo-arterenol). Of interest in connection with the last named compound is the fact that its levo-rotatory isomer is over 30 times as active as the dextro-rotatory form. The study of numerous synthetic and natural products related more or less to epinephrine has formed an interesting contribution to our knowledge of the relation of chemical structure to biological activity.^{35, 36, 204, 284}

CHAPTER VI

THE PHYSIOLOGY OF THE ADRENAL MEDULLA

No product of the glands of internal secretion has been studied with such thoroughness as has been epinephrine. This intense study has been made possible by the fact that this substance can be detected and assayed in extremely minute concentrations. It has thus been possible to determine the rate of secretion of epinephrine under various conditions. The results of these studies shall be considered in the present chapter with particular reference to their bearing on the functional significance of epinephrine in the organism.

Epinephrine manifests its presence, even in extremely dilute solution, by a number of pharmacological reactions. These have been utilized for determining the concentration of epinephrine present in the blood leaving the adrenals. Despite their apparent simplicity and great sensitivity, the methods used in the study of epinephrine secretion are open to a number of criticisms particularly as regards the physiological significance of the data obtained. It is not surprising, therefore, that authors, utilizing different techniques, have obtained conflicting data and come to diametrically opposed conclusions as regards the significance of their results. In order to evaluate properly the existent data it is necessary to consider first the various methods utilized for determining the rate of epinephrine discharge from the adrenals.

For determining the rate of discharge of epinephrine from the adrenals at any given time, it is obviously necessary to know the concentration of epinephrine in the blood of the adrenal veins and the amount of blood leaving the glands in unit time.⁵²⁵ Many of the earlier workers, unfortunately, neglected to take the last factor into consideration, assuming

that an increase in the concentration of epinephrine in the blood leaving the adrenals was in itself evidence of an increased rate of secretion. Since many physiological conditions are accompanied by a decrease in the rate of blood flow through various organs,²⁴³ neglect to consider this factor will invalidate the conclusions based solely on concentration changes in the blood.

In the following sections we shall consider the various procedures used in the biological assay of the epinephrine content of the blood and the methods used for determining the output of epinephrine from the adrenals. Besides the biological methods of assay listed below, certain of the more sensitive chemical methods already described have also been utilized in studies on the physiology of the medulla. Instead of determining the rate of secretion by analysis of the blood, attempts have been made to obtain this data by analysis of the epinephrine content of the adrenals in various conditions. Depletion of the epinephrine store indicates a stimulation of the rate of secretion. However, if the rate of synthesis follows changes in the rate of secretion, such depletion will obviously not occur.

METHODS FOR THE BIOLOGICAL ASSAY OF EPINEPHRINE

1. *The intestinal strip method.* The inhibition of intestinal muscle by epinephrine serves as a very delicate test for epinephrine. Cannon and Hoskins¹⁰⁵ claim this test to be sensitive to epinephrine in a dilution of 1 to 400 millions; Stewart and Rogoff⁵⁸⁸ claim 1 to 800 millions. The blood to be tested is added to Ringer's solution in which is suspended a strip of intestine (rabbit or guinea pig) arranged to record on a kymograph. The resulting inhibition is compared with that observed after the addition of a known quantity of epinephrine.

Although extremely sensitive, this method suffers from the defect that interfering substances present in the blood may also inhibit intestinal movements.⁵⁸⁷

2. *Blood pressure method.* This method was first suggested by Houghton and carefully worked out by Elliott.¹⁷⁷ Al-

though less delicate than the intestinal strip method it is advantageous for many purposes. The spinal animal is more sensitive for the application of this method and hence the animal is pithed and maintained under artificial respiration. Successive injections may be made, each of which causes a rise proportional to the epinephrine concentration of the sample tested. The pressor response of the unknown solution is compared with that elicited by the injection of a known amount of epinephrine.⁶³⁹

The method just outlined is vitiated if depressor substances are present in the fluids to be tested. This is particularly true of choline and acetylcholine, which as Hunt³²⁴ showed, antagonize the pressor effects of epinephrine. Since choline may be a constituent of extracts prepared from the adrenals, the use of this method alone for assaying the epinephrine content of such extracts is not justified.

The contraction of a strip of artery, arranged as in the "intestinal segment method" has also been used for epinephrine assays. This method is relatively insensitive, and not frequently employed.

3. *The perfusion method.* The assay of epinephrine by determining its effect on the caliber of the blood vessels was described by Lwen³⁷⁵ and perfected by Trendelenburg⁶³⁶ as a highly sensitive method. A frog is decapitated and its spinal cord thoroughly destroyed. It is placed on its back and the abdominal wall reflected. The bladder and intestines are removed and the renal vein ligated. The remaining viscera including the heart are removed, care being taken to preserve the abdominal aorta intact. A cannula, connected to a Mariotte bottle containing frog Ringer's solution is introduced cephalwards into the abdominal aorta. An outflow cannula is introduced into the anterior abdominal vein which lies exposed on the reflected abdominal wall. A drop recorder marks the rate of outflow of the perfusing fluid. A sensitive preparation permits the detection of a change in the rate of the perfusion

when as little as 1 part of epinephrine is present in 100 million parts of the perfusing fluid.

Pisemsky's method is similar in principle to the Læwen-Trendelenburg procedure just described. It utilizes the rabbit's ear as test object. Krawkow³⁶⁷ finds this method to be sensitive to epinephrine in a dilution of 1 to 10 to 50 million.

4. *The uterine strip method.* Epinephrine inhibits the uterus of the rat and stimulates the uterus of the rabbit. Concentrations of one part in 20 million inhibit uterine movements of the non-pregnant cat.¹⁴⁸

As shown by Cannon and Hoskins¹⁰⁵ the blood in asphyxia may contain a substance other than epinephrine which inhibits intestinal contractions. This substance inhibits the contraction of the non-pregnant rabbit's uterus, while epinephrine increases the tone of this preparation. Hence by utilizing both the intestinal strip and uterine contraction methods, one can determine if the observed effect is really due to epinephrine.

5. *The excised-eye method.* Epinephrine exerts a mydriatic action on the enucleated eye.¹⁷⁴ A dilution of one part of epinephrine hydrochloride per million causes maximal expansion of the pupil. One part in 10 million causes a detectable mydriasis.⁴⁵¹

6. *The anastomotic method of Tournade and Chabrol.*⁶³¹ In this method the adrenal vein of the experimental animal is anastomosed with the jugular vein of an adrenalectomized dog. The decrease in volume of the spleen of the recipient, as determined by an oncometer, serves to detect an increase in the epinephrine content of the blood of the donor.

Besides the effect on the splenic volume one can also utilize other pharmacodynamic changes, induced by epinephrine; e.g., the rise in blood pressure, dilatation of the pupil, tachycardiac response, etc.

7. *Auto-assay method.*¹⁰² Instead of the anastomosis, just described, the animal's blood may be allowed to act on its own organs and the epinephrine determined by its effect on 1) the

blood pressure, 2) the pupil (in cats) of an eye (sensitized by previous extirpation of the superior cervical ganglion), 3) the rate of the denervated heart, or 4) on the volume of the spleen or limbs.

The auto-assay method has been widely used particularly with the denervated eye or heart as described in the following sections.

8. *Denervated iris method.* The so-called paradoxical eye reaction⁴⁵¹ for assaying epinephrine depends upon the fact that the pupil dilates after an intravenous injection of epinephrine. The eye of the cat is utilized for this reaction after sensitizing it to the action of epinephrine by extirpating the superior cervical ganglion. At least several days should elapse after the ganglionectomy before utilizing this preparation. The transverse diameter of the pupil is measured and the dilatation caused by the injection of the unknown solution is compared with that obtained by injecting a known concentration of epinephrine.

Stewart and Rogoff⁵⁸⁷ demonstrated the diversity in results obtained in the assay of epinephrine by the auto-assay method (using the paradoxical eye reaction) as compared to the intestinal segment method. Although Cannon and Rapport¹⁰² have criticized the conclusions of Stewart and Rogoff, the experiments of Sugawara⁶⁰¹ and others, also indicate that these two methods of assay do not give concordant results. Sugawara showed that the auto-assay method gave results indicating the presence of only about two-thirds as much epinephrine in a given sample of blood as is obtained by using the isolated intestinal segment of the rabbit. Similar disparities are also obtained when the blood is assayed by the paradoxical eye reaction of a second cat and compared with the results obtained by the intestinal segment method. The blood of the adrenal vein must exert, therefore, a more powerful inhibiting effect upon the movements of the intestinal strip than can be accounted for by its epinephrine content or else

this blood must contain a substance which inhibits the normal epinephrine effect on the denervated pupil.

Kojima and Saito³⁶⁰ also found that the intestinal segment method gave results which were higher for epinephrine than the colorimetric method of Folin, Cannon, and Denis.

9. *Denervated heart method.* In their earlier experiments Cannon and Hoskins¹⁰⁵ obtained blood from the adrenal vein by means of a catheter passed through the femoral artery and pushed forward until it reached a point near the entrance of the adrenal vein into the vena cava. The blood obtained was assayed by the intestinal segment method. This method of Cannon and Hoskins although applicable to the unanesthetized animal suffers from the fact that it cannot give quantitative results and was therefore discarded for an auto-assay method depending on the use of the denervated heart.

Cannon and his collaborators¹⁰² have utilized the denervated heart as a sensitive test object for the assay of epinephrine. This preparation is prepared by severing all the nervous connections of the heart. After recovery from the operation, the heart rate is considered by Cannon to be under hormonal control and to react only to changes in the blood concentration of epinephrine or related substances.

Stewart and Rogoff⁵⁸⁷ criticized the method of Cannon on the basis of their experiments in which they showed that an increase in the rate of the denervated heart may be elicited even after extirpation of the adrenals.

Cannon and Rapport,¹⁰² using the denervated heart as an indicator, found that stimulation of the splanchnics caused an increase of 29 beats in the pulse rate. After removal of the adrenals the increase was only 6 beats and they concluded, therefore, that the difference was caused by secretion of epinephrine in response to splanchnic stimulation. The increase noted after adrenalectomy was attributed to the liver, for section of the hepatic nerves abolished this increase. Since removal of the adrenals caused a slowing of the pulse rate

which was counteracted by the injection of 0.0007 mgs. of epinephrine per kilo of body weight per minute they concluded that the normal output of epinephrine is about 0.0035 mgs. per kilo per minute which is more than ten times the amount found by Stewart and Rogoff in their more direct measurements, and fifty times greater than the results of Sataké and his collaborators to be described in a subsequent section.

10. *The "cava-pocket" method.* This method originated by Stewart and Rogoff⁵²⁵ for determining the rate of secretion of epinephrine from the adrenal glands involves the determination of the amount of blood leaving the glands per minute and the concentration of epinephrine in this blood. The veins emptying into the vena-cava are ligated or clamped with the exception of the adrenal veins so as to form a pocket by the occlusion of the vena cava beneath the diaphragm and below the entrance of the adrenal veins. Into this "cava-pocket" only blood from the adrenals can enter. By releasing the clamp at the diaphragm the blood enters the general circulation and its epinephrine content may be determined by an auto-assay method. By releasing the lower clamp on the vena cava, through a cannula, the rate of blood flow is measured. The epinephrine content of the blood can also be determined by assay on a segment of rabbit's intestine and confirming, if necessary, on a segment of non-pregnant rabbit's uterus.

11. *The method of Sataké, Sugawara, and Watanabé.*⁵⁴⁵ Since anesthesia, pain, operative manipulations, etc. all may modify the normal rate of secretion of epinephrine from the adrenals, it is obvious that the methods already described are inapplicable for determining the normal resting rate of epinephrine discharge. The results obtained by these methods may also be rendered abnormal due to the interference of the above mentioned factors. In order to avoid the effects of these vitiating factors, Sataké, Sugawara, and Watanabé devised a method whereby the rate of epinephrine secretion in the dog could be determined without necessitating anesthesia, laparot-

omy, pain, or even fastening the animal. In order to do this, these authors in a preliminary operation cut the dorsal roots from the ninth thoracic to the third lumbar vertebra, thereby rendering the area in the vicinity of the adrenals anesthetic to manipulation. After about one month when the animal had recovered from this preliminary operation, an incision was made below the costal margin and the adrenal vein cannulated. As pointed out by Sataké and his collaborators, their method obviously will interfere with the normal blood flow of the adrenal. Despite this objection their experiments offer a decided improvement on those of their predecessors and the results obtained are undoubtedly of greater physiological significance. To avoid the interference with the normal blood flow occasioned by introducing the cannula into the adrenal vein, it would be of interest to repeat the experiments utilizing the *thermostromuhr* method for determining the blood flow. Measuring the rate of blood flow by this procedure and removing samples of blood for analysis with a syringe and fine needle inserted into the exposed adrenal vein, would enable one to obtain results which more closely represent normal physiological conditions. The average blood flow through each adrenal observed by Sataké *et alii* was 0.3 cc. per kilo per minute. The authors used the rabbit's intestine for the assay of epinephrine.

12. *Euler's method.* Euler¹⁸⁸ has utilized a method for determining the epinephrine content of blood serum based on the activating action of this substance on the reduction of methylene blue added to muscle extract. Although, as Euler showed, such activation by blood serum is not entirely due to epinephrine, the method has been found useful under various conditions.

THE NORMAL RATE OF EPINEPHRINE SECRETION

Considerable work has been done to determine the rate at which epinephrine is normally secreted from the adrenal glands.

It has been found that epinephrine is detectable in the blood of the adrenal veins so long as the nerve supply to the glands is intact. There is thus presumably a steady secretion of epinephrine into the circulation. The exact amount of this secretion has been a matter of controversy. The ease with which an increased secretion is stimulated renders difficult the attainment of truly normal basal values for the unavoidable manipulations (particularly anesthesia) would be expected to give too high values. The lowest values obtained are those of Sataké and his collaborators whose method, as we have seen, involves less abnormal conditions than those of other authors.

In a series of twenty dogs, Sataké and his collaborators⁵⁴⁵ found the epinephrine content of the blood from the adrenal vein to vary from 0.00005 to 0.000225 with an average of 0.0001 mgms. per cc. The total output from both glands was equal to 0.00007 mgms. per kilo of body weight per minute.

The results just quoted are of a much smaller magnitude than the oft-quoted results of Stewart and Rogoff.⁵⁸⁷ The rate of epinephrine secretion as measured by the latter authors was about 0.00025 milligrams per minute per kilogram of body weight. This rate was approximately the same in cats, dogs, monkeys, and probably (judging from the values obtained by other authors) in many other animal species. The results obtained by Stewart and Rogoff are probably abnormally high because of the stimulating effect of the operative procedures involved in preparing the "cava-pocket." The preparation of the "cava-pocket" as Sataké and his collaborators showed stimulates the secretion of epinephrine unless the animal is deeply anesthetized.⁵⁴⁶

Except for the blood of the adrenal vein, the epinephrine content of the blood stream is so slight as to be undetectable by most methods of analysis.⁹² Thus Whitehorn,⁶⁷⁵ using a method capable of detecting one part of epinephrine in fifty million parts of blood, was unable to detect this substance in the peripheral venous blood of man. Euler¹⁸³ using his method

based on the stimulating effect of epinephrine on oxidation in Thunberg's *methylene blue-muscle extract* system, found the peripheral venous blood of man and the rabbit to contain about 0.000,000,0001 mgm. of epinephrine per cc.

THE NERVOUS CONTROL OF EPINEPHRINE SECRETION

The rate of secretion of epinephrine has been shown to be readily affected by impulses from the splanchnic nerves and hence it may be assumed that these nerves normally control the rate of secretion. Stimulation of the nerves to the adrenal increases the rate of epinephrine secretion; sectioning the nerves, abolishes the secretion. Elliott¹⁷⁸ showed that the epinephrine store was protected against depletion by anesthesia and certain other stimulants to secretion if all the fibers coming to the semilunar ganglion were cut. If the spontaneous discharge of epinephrine be under nervous control, we must explain Elliott's results by assuming that anesthesia and certain drugs which ordinarily deplete the adrenals of epinephrine act by preventing the formation of epinephrine and at the same time do not interfere with its discharge.

The blood pressure rise observed on stimulating the splanchnics is only partly due, however, to the liberation of epinephrine, for this pressure effect is in great part obtainable after occlusion of the adrenal veins. The second peak of the blood pressure curve obtained during splanchnic stimulation usually disappears after exclusion of the adrenals from the circulation. The occurrence of this peak has, therefore, been attributed to the release of epinephrine into the general circulation under the influence of the splanchnic stimulation.^{270, 639}

Continued stimulation of the splanchnics may cause the discharge of large amounts of epinephrine from the adrenals. Thus Stewart and Rogoff⁵²⁵ found that 0.4 mgms. of epinephrine was discharged from one adrenal of a cat during 4 hours of intermittent faradic stimulation of the splanchnic nerve. This quantity represents about twice the normal

content of a cat's adrenals. Despite the great amount of epinephrine discharged, the adrenal in the above cited case still contained 0.14 milligrams at the conclusion of the experiment. This indicates the rapidity with which the epinephrine store of the adrenals is restored. After an hour's stimulation, the epinephrine content of an adrenal may still be found to be but little reduced below normal.

Several attempts have been made to localize a center for the nerves regulating epinephrine secretion, but the results of different observers are discordant. Stewart and Rogoff⁵⁸⁷ claimed the center to exist in the thoracic cord since section of the cord at the level of the last cervical vertebra did not reduce the rate of secretion of epinephrine. Elliott,¹⁷⁸ on the other hand, found that anesthesia no longer produced a discharge of epinephrine after cutting the spinal cord at the level of the first thoracic vertebra. Removal of the brain anterior to the *corpora quadrigemina* did not affect the secretion. Elliott, therefore, concluded that the center existed at a point just posterior to the *corpora quadrigemina*.

The existence of a center at the base of the fourth ventricle has been assumed on the basis of the results of piqûre in this region of the brain stem. Following piqûre, a number of reactions typical of epinephrine are induced. The hyperglycemia attendant on piqûre is not entirely due, however, to liberation of epinephrine but is in part a result of nervous reflexes.⁴¹⁹ Stimulation of other parts of the brain stem results in only slight hyperglycemia and apparently in little reflex stimulation of epinephrine secretion. Stimulation of the hypothalamus gives only a slight and transient hyperglycemia. Stimulation of the cerebral cortex causes an epinephrine secretion only when convulsions are incidentally elicited.⁴¹⁹

Feldberg, Minz, and Tsudzimura¹⁹² have recently shown that a substance manifesting the pharmacological actions of acetylcholine is liberated from the adrenals during splanchnic stimulation. The previous injection of eserine greatly en-

hances the epinephrine discharge caused by splanchnic stimulation, which is what one would expect if the discharge of epinephrine caused by splanchnic stimulation were due to the liberation of acetylcholine. The action of acetylcholine or of splanchnic stimulation of the adrenal medulla manifests the two types of peripheral effects which the choline esters show generally; viz., 1) a "nicotine" action which is abolished by large doses of nicotine and 2) a "muscarine" component abolished by small doses of atropine. Feldberg and his collaborators conclude that acetylcholine is the humoral transmitter of splanchnic impulses to the adrenal medulla. This cholinergic action of the preganglionic sympathetic fibers is not limited to the splanchnic nerves.

FACTORS AFFECTING EPINEPHRINE SECRETION

Sensory stimulation. After stimulation of sensory nerves, such as the central end of the sciatic, brachial, or vagus, the blood of the adrenal vein induces the characteristic reactions of epinephrine. It has, therefore, been claimed that such stimuli, by reflex action, cause a discharge of epinephrine. The evidence adduced by earlier workers was criticized by Stewart and Rogoff.⁵⁹¹ As these authors pointed out, observations made without regard to changes in the rate of blood flow through the adrenals and without quantitative determinations of the amount of epinephrine liberated per unit of time offer no criterion for determining the influence of stimulation of afferent nerves.

Stewart and Rogoff's failure to demonstrate that sensory stimulation does not increase the secretion of epinephrine was due probably to the experimental methods which these authors utilized. As shown by Sataké *et alii*,⁵⁴⁶ with the "cava-pocket" procedure on anesthetized dogs or cats, one does not obtain an increased secretion of epinephrine. However, in the non-anesthetized dog the output of epinephrine is increased from two to five times by stimulation of sensory nerves. Narcosis

apparently inhibits the reflex output of epinephrine and it is necessary to view with scepticism all experiments on determining epinephrine secretion which were performed under anesthesia.^{548, 617}

In any case, the mobilization of carbohydrate observed after stimulation of sensory nerves is not completely due to the action of liberated epinephrine for some hyperglycemia is obtained even in adrenalectomized animals.^{345, 346}

Bazett and Quinby⁴⁸ found that the pressor response of cats under urethane anesthesia to sciatic stimulation was almost entirely nervous in origin and not affected by adrenalectomy.

Fastening an unanesthetized dog to the table, struggling, and barking were all found by Sataké, Sugawara, and Watanabé⁵⁴⁶ to be accompanied by a marked increase in the epinephrine secretion from the adrenals.

Anesthesia. Anesthesia markedly affects the rate of secretion of epinephrine from the adrenals. The epinephrine content of the adrenals may be reduced to a half or a third of its normal value. Section of the splanchnic nerves prevents this depletion of the epinephrine stores.^{178, 548, 617}

Asphyxia. That asphyxia affects the secretion of epinephrine as claimed by Anrep was not confirmed by the experiments of Pearlman and Vincent,⁴⁸⁸ nor by Stewart and Rogoff.⁵⁸⁷

Hartman *et alii*,²⁷⁰ on the other hand, found that asphyxia causes mydriasis in the cat after excision of the superior cervical ganglion. Adrenalectomy prevented the reaction. Houssay and Molinelli³¹⁵ also noted a decrease in the volume of a denervated limb during asphyxia, an effect not elicited after cutting the splanchnic nerves or excising the adrenals. The conflicting results of these various authors are to be attributed to the different experimental methods utilized. The type of asphyxia is also a matter of importance for although asphyxia caused by inhalation of carbon monoxide stimulates the secretion of epinephrine, low oxygen tension of

the inspired air may not induce the same result.¹⁰⁵ The hyperglycemia of asphyxia is only partially due to epinephrine discharge for it occurs to some extent after removing the adrenals.

Hemorrhage. Applying the "cava-pocket" method to dogs under ether, Saito⁵⁴² found that it was necessary to deplete the blood about one-fifth of its total volume to elicit an increased secretion of epinephrine by hemorrhage. In the unanesthetized dog, on the other hand, the loss of only one-tenth the total blood volume caused the liberation of two to ten times the normal amount of epinephrine. This hypersecretion continued for about three hours. If one-third the total blood volume was lost by hemorrhage, an output of epinephrine 10 to 30 times the normal occurred and continued for some hours.

The hyperglycemia observed after hemorrhage is due in part only to this increased epinephrine discharge.

Temperature. Hartman and Hartman²⁷⁷ found an increase in the epinephrine discharge from the adrenals (as measured by the denervated iris) of cats when immersed in cold water as a result not of the fall in body temperature but of the peripheral stimulation of cooling. Cannon and his collaborators¹⁰⁷ working with the denervated heart corroborated Hartman's results and also found an augmentation in epinephrine discharge during experimental fever, induced by the injection of killed typhoid bacilli. They therefore inferred that epinephrine has a calorigenic function which in conjunction with shivering serves to protect the organism against a drop in body temperature.

Saito,⁵⁴² on the other hand, in non-anesthetized dogs detected no increase in the epinephrine discharge from the adrenals of dogs subjected to cold. The application of excessive heat (giving a rectal temperature of 41°C) resulted in an augmentation of the epinephrine discharge.

In man the cardio-vascular responses to cold are not such

as would lead one to assume any calorigenic function for epinephrine. The available evidence is thus against the supposition that epinephrine (in man and the dog at least) plays any important calorigenic function in the reaction to cold.²⁴³

Exclusion of the carotid sinus. Ligation of both carotid arteries of dogs under deep anesthesia causes an increased secretion of epinephrine into the blood stream.¹⁸⁹ The increased oxygen consumption which accompanies this occlusion does not take place after adrenalectomy and hence has been attributed to the liberation of epinephrine.

THE EFFECT OF DRUGS ON THE SECRETION OF EPINEPHRINE

Many drugs when injected into the body exert an influence which, in part at least, is due to the secretion of epinephrine from the adrenals. Morphine, chloroform, ether, strychnine, nicotine, strophanthus, camphor, *etc.* cause a discharge of epinephrine which in turn exerts an influence on the organism. Thus, as Dale and Laidlaw¹⁴⁸ showed, while nicotine and pilocarpine stimulate the isolated uterus of the non-pregnant cat, these same drugs inhibit uterine movements when injected into the living animal. In the latter case the discharge of epinephrine caused by these drugs preponderates in its effect on the uterus over the action of the small amount of the drugs which reach the uterus through the circulation. Similarly insulin causes a decrease in the amino acid content of the blood, as a secondary effect of the epinephrine secretion which it stimulates.¹⁵²

Convulsants. Strychnine^{590, 657} causes a marked increase in the output of epinephrine, which persists for over an hour. There is no evidence that this increased secretion is accompanied by any appreciable diminution in the epinephrine store of the medulla. The effect of strychnine occurs after section of the spinal cord in the cervical region but is prevented by division of the efferent secretory pathway and is thus the result of a central

action on the cord. As Watanabé⁶⁵⁷ showed, small doses of strychnine do not cause an augmentation in the rate of epinephrine secretion. Moderate doses ($\frac{1}{4}$ to 3 mgms. per kilo of body weight) cause an increase of four to five times the normal rate of secretion.

Other centrally acting convulsive drugs also cause a marked discharge of epinephrine when injected in doses below the convulsive level.⁵⁹⁰ Thus picrotoxin⁶²⁴ is more effective than strychnine in stimulating epinephrine secretion. Santonin, in sub-convulsive doses, camphor, in convulsive doses, caffeine,⁶⁵⁸ theobromine and guanidine⁶⁰⁴ also cause a discharge of epinephrine from the adrenals. All of the above mentioned drugs apparently act by stimulating the higher centers controlling epinephrine secretion.⁶³⁷

Sympathetic paralyzers. Nicotine, and its related drugs, coniine, quaternary ammonium bases, etc. paralyze sympathetic ganglia and, as might be anticipated, exert an analogous action on the adrenal medulla. Gley²²³ concluded that the pressor effect of nicotine after destruction of the spinal cord and bulb in dogs is practically entirely due to an increase in the epinephrine output. Dale and Laidlaw¹⁴⁸ showed that the effects of nicotine on the non-pregnant uterus of the cat and on the eye after removal of the superior cervical ganglion are in part due to the liberation of epinephrine for they do not occur when this liberation is prevented.

The action of nicotine and its related drugs is directly on the medullary cells and is elicited in the denervated organ. As Stewart and Rogoff⁵⁹⁰ showed, after the injection of nicotine there is first a marked increase in the output of epinephrine which lasts for less than half a minute. This stimulation is followed by a period of depression at the maximum of which no epinephrine is liberated and finally the normal output is gradually resumed. It is probable that nicotine not only releases epinephrine from the medulla but also sets free the epinephrine store of the extra-adrenal chromophil tissues, for

after adrenalectomy mydriasis of the pupil (after a previous excision of the superior cervical ganglion) is still elicited by nicotine injections. It has been suggested that the hyperglycemia which accompanies tobacco smoking is due to the liberation of epinephrine. Other pharmacologically related compounds behave similarly to nicotine, *e.g.*, the tetramethylammonium salts, neurin, hordenin methyl iodide, hydrastinin, and β -dimethyl telluronium salts. Cystisine, coniine, arecoline, spartein, gelsemin and lobelin also cause a discharge of epinephrine. Choline and acetylcholine do not manifest an appreciable effect on epinephrine secretion.⁶³⁹

Sympathetic and parasympathetic stimulants. Tetrahydro- β -naphthylamine, which is a powerful stimulant of the sympathetic nervous system, also stimulates the centers controlling epinephrine secretion. The hyperglycemia following the injection of this drug is probably due to the liberation of epinephrine for it does not occur in adrenalectomized animals or after cutting the splanchnic nerves. The hyperpyrexia following the injection of tetrahydro- β -naphthylamine occurs in adrenalectomized animals, and is thus not due to the liberation of epinephrine.^{75, 602}

Although a sympathetic stimulant, epinephrine itself apparently does not stimulate the medulla for its administration does not increase the rate of discharge of epinephrine from the adrenals.^{603, 635}

Of the parasympathetic stimulants, physostigmin is an active stimulant of epinephrine secretion. Pilocarpin has only a feeble action.¹⁷¹ Atropin does not inhibit epinephrine secretion but appears to stimulate it. Quinine causes an increased secretion.

Curare in doses sufficient to paralyze the skeletal muscles of the cat markedly depresses the output of epinephrine from the adrenals.

Morphine. Morphine may reduce the epinephrine content of the cat's adrenals to one-seventh of their normal value.⁵⁹⁰

An injection of 10 to 20 mgms. of morphine may elevate the blood sugar to 250 milligrams per 100 cc. After exclusion of the adrenals the blood sugar does not rise above 115 milligrams per 100 cc. Hence in the cat, in which morphine is an excitant, morphine produces a marked stimulation of epinephrine secretion. In the dog, in which (unlike in the cat) morphine is not an excitant, this drug also increases epinephrine discharge, by central stimulation. However, the hyperglycemia which follows morphine injections in the dog is not prevented by excluding the adrenals from the circulation.

Histamine. The intravenous injection of 0.01 to 0.1 mgm. of histamine into a cat, after excision of the superior cervical ganglion causes dilatation of the pupils but not if the adrenals have been excluded from the circulation.¹⁴⁷ This effect is attributed to the discharge of epinephrine and may be sufficiently great to prevent the capillary damage caused by histamine.⁴⁵⁷

OTHER FACTORS AFFECTING THE SECRETION OF EPINEPHRINE

Besides the instances cited above, many other conditions and the injection of a variety of substances into the blood stream result in an increased output of epinephrine. The epinephrine store is thus a very labile and sensitive entity which responds to numerous stimulants.

Shock induced in unanesthetized dogs by injections of peptone⁶⁵⁷ is accompanied by an increased secretion of epinephrine. In anaphylactic shock^{118, 547} this increased secretion is slight, for it does not result in a diminution of the epinephrine content of the adrenals nor is the hyperglycemia of shock absent in the adrenalectomized guinea pig.

Many other substances when injected into the blood stream cause reactions which indicate an increased discharge of epinephrine. Thus the hyperglycemia following injections of various salts, which is slight after section of the splanchnics, is attributed to epinephrine discharge. Mercury salts, arsenic,

phosphorus, and similar poisons cause a diminution in the epinephrine content of the adrenals but it is not certain if this be due to an increased discharge or to injury of the mechanism responsible for epinephrine synthesis in the gland.

Starvation may reduce the epinephrine of the adrenals to one-fourth to one-half of their normal content. After infectious and debilitating diseases, the epinephrine content may also be low but in these cases we must consider the effects of the accompanying cachexia, the agonal drop in body temperature, the asphyxia, *etc.* Diphtheria toxin, is particularly potent in reducing the epinephrine content of the adrenals, but in dogs poisoned by this toxin or by tetanus toxin there is no evidence of an increased epinephrine secretion.⁵⁹⁰

Burns are accompanied by an increased secretion of epinephrine. Injury to the kidneys or excision of the kidneys also reduces the epinephrine content of the adrenals but there is no evidence that epinephrine discharge is responsible for the hypertension of renal disease.⁶³⁷

Irradiation with X-rays causes an increased discharge of epinephrine.⁶³⁹

The epinephrine content of the adrenals in persons dying of various diseases has been the subject of numerous investigations.¹⁸¹ Different observers have reported extremely variable results. The difficulty of obtaining the glands sufficiently soon after death to avoid *post-mortem* changes and the reliance upon analytical methods of doubtful accuracy account for the confusing results found in the literature. Schmorl and Ingier³²⁹, in their extensive study, did not find any noteworthy decrease in the epinephrine content of the adrenals of patients dead of various acute infections including diphtheria.

In infectious fevers of man, Euler¹⁸⁷ found the epinephrine content of the peripheral venous blood to be 1 to 500 million to a billion which represents an increase over its normal content.

Herring²⁹⁴ found the epinephrine content of the adrenals to

be increased by thyroid feeding but Kuriyama³⁶⁹ was unable to confirm this. Gley²²³ found that thyroidectomy or pancreatectomy did not affect the epinephrine store of the adrenals.

THE FUNCTION OF EPINEPHRINE

Despite the extensive studies which have been carried out on epinephrine, we are still in ignorance of its function in the organism. Many theories have been advanced to explain the functional significance of the adrenal medulla and its secretion, but the validity of most of these theories is easily disproven and none of them will satisfactorily explain all our chemical, physiological, and anatomical knowledge concerning the medulla.

That epinephrine serves an important function in the organism is an assumption which can scarcely be denied when one considers the facts presented in the preceding sections. It would seem most reasonable, therefore, to adopt the view that we are ignorant of the function of epinephrine rather than omnisciently to disclaim any importance for this substance, and consider it as a waste product of the animal economy, as some have done.

One conclusion is inescapable: *viz.*, that the deprivation in the mammal of the epinephrine store (by adrenalectomy) does not result in any perceptible deficiency. Nor can it be satisfactorily argued that the loss of epinephrine is compensated for by the relatively small stores of epinephrine present in the extra-medullary chromophil bodies. In the rat, such extra tissue has not been demonstrated and this animal can grow and flourish after adrenalectomy when furnished with an adequate supply of the cortical hormone. That the extra-medullary chromophil tissue does not replace the normal secretion of the medulla is also demonstrated by its failure to hypertrophy after adrenalectomy. We can thus safely say that the function of epinephrine is dispensable to the organism.

One of the early theories of the function of epinephrine was

that it exerts a continuous tonic influence on the structures under sympathetic control, particularly upon the blood vessels. That the vascular tone is not sustained by a steady discharge of epinephrine is demonstrated by the fact that ligation of the adrenal vessels, cutting the splanchnic nerves, or otherwise removing the supply of epinephrine has no effect upon the blood pressure.³⁰⁷ Adrenalectomized animals may be maintained in perfect health indefinitely without the administration of epinephrine. Since small doses of epinephrine have a depressor effect one would, as a matter of fact, expect the minute quantity normally secreted into the blood stream to cause a drop in blood pressure rather than any rise.²²²

The view advanced by Elliott¹⁷⁷ that epinephrine is necessary for maintaining normal sympathetic impulses has also been thoroughly disproven. The response to sympathetic impulses remains unimpaired in adrenalectomized animals, nor does the infusion of epinephrine facilitate the passage of such impulses.²⁶⁹

Gley and Quinquaud²²⁴ in a series of papers have also vigorously attacked the tonus theory of medullary function. They demonstrated that exclusion of the adrenals from the circulation did not influence the effects of vagal, depressor, or accelerator stimulation on the heart rate; nor did it influence the effects of splanchnic stimulation or asphyxia on the blood pressure.

The view that epinephrine exerts a continuous effect on the heart and blood vessels has been more recently advocated by Tournade and Chabrol,⁶³⁰ and Heymans.²⁹⁷ The first mentioned authors⁶³⁴ found that the blood pressure of an adrenalectomized dog under chloralose anesthesia rose when its jugular vein was anastomosed with the right adrenal vein of a second dog whose adrenals were intact. If the left adrenal of the second dog was now excised and the right splanchnic nerve cut, thus cutting off the supply of epinephrine, the blood pressure of the first animal dropped from 160 to 100 mms. of Hg.

Heymans²⁹⁷ also, on the basis of his experiments on the carotid sinus, considered the adrenals as playing an important rôle in regulating the cardiac rate and blood pressure. The carotid sinus according to Heymans reflexly regulates the vagal cardiac tone, the neuro-vascular tone, and the secretion of epinephrine from the adrenals. Changes in these functions take place in response to alterations in the blood pressure to which the carotid sinus is exceedingly sensitive. In Heymans' experiments the carotid artery of a dog, B, whose carotid sinus had been isolated, was anastomosed to the jugular vein of a dog, A. The lumbo-adrenal vein of B was in turn anastomosed with the jugular vein of a third dog, C, whose adrenals had been extirpated. Changes in the volume of the spleen of dog C served to detect epinephrine secreted by dog B in response to changes in the blood pressure of its carotid sinus. A drop in the arterial blood pressure of the sinus of B, called forth vasoconstriction and a reflex stimulation of epinephrine secretion in this animal, while a rise in pressure resulted in vasodilation and an inhibition of epinephrine secretion.

As pointed out by Cannon,¹⁰³ the disturbed experimental conditions of the above quoted experiments deprive them of their validity. The abnormally excessive amounts of epinephrine secreted under the experimental conditions cited undoubtedly affect the circulation. The amounts secreted in the normal resting animal, however, could exert no such profound effects. Cannon and his collaborators in their studies of quiet unanesthetized cats, with hearts denervated for some time, could detect no difference between the basal pulse rate before and after exclusion of the secretion of the adrenal medulla by denervation or excision of these glands.

Because of the remarkable pharmacological effects elicited by epinephrine when injected into the blood stream, numerous theories have been suggested to explain a variety of clinical conditions as being dependent on pathological changes in the medulla. The hyperglycemic action of epinephrine led

Zuelzer⁶⁹⁸ to assume the existence of an antagonism between the adrenal medulla and the pancreas. The idea that hypertension is due to overactivity of the medulla is not borne out by determinations of the epinephrine content of the blood.⁶³⁷ Excision of one gland or denervation, as practiced surgically,¹³⁶ is not justified by the available physiological evidence. The operation is based on no sound evidence, is fraught with danger to the patient, and could, in any case, yield only transient effects since compensatory hypertrophy of the remaining gland readily ensues. The claims of a number of authors regarding the presence of an excess of epinephrine in the blood under various conditions,—Graves' disease, renal disease *etc.*—have also not been substantiated when carefully retested.⁶³⁷

There is no evidence that epinephrine regulates urinary secretion, muscular activity, the development of fatigue, and the many other physiological actions for which it has been credited.⁵²⁵

In emotional disturbances there is a discharge of nervous impulses *via* the sympathetic. Since epinephrine when injected induces the same action as the sympathetic, it would be logical to conclude that epinephrine is secreted in times of stress to coöperate with the nervous impulses. This is the crux of Cannon's theory of the "Emergency Function" of the adrenals.¹⁰² Cannon and de la Paz found that the dilatation of the pupils and upstanding of the fur which accompany sudden alarm or excitement are associated with an increased discharge of epinephrine.¹⁰³ Subsequently, Cannon and his collaborators claimed to show that fear, rage, pain, cold, asphyxia *etc.* caused a discharge of epinephrine from the adrenals. They attempted to show that epinephrine is serviceable in lessening muscular fatigue, in accelerating the coagulation of the blood, and in otherwise aiding the organism in an emergency.^{104, 105}

The theory and experiments of Cannon and his collaborators have been the subject of an intense controversy. Stewart and

Rogoff⁵⁸⁷ and Gley and Quinquaud²²² particularly have attacked the validity of the experimental evidence presented by Cannon and his co-workers as well as the interpretation of their experiments. It is unnecessary to repeat again the details of this controversy. In view of the results already quoted it must be admitted that Cannon and his collaborators are probably correct in assuming an increased output of epinephrine under the various conditions in which epinephrine by its pharmacodynamic reactions would be useful to the organism in an emergency. It is very doubtful, however, if the amounts of epinephrine secreted under these conditions would suffice to be of any significance to the organism as demanded by Cannon's theory. Moreover, adrenalectomized animals maintained on an adequate dose of cortical hormone are able to respond to emergencies as efficiently as normal animals do. Such animals respond to fright, become enraged, and fight as well as unoperated animals and hence any assumed emergency function of the epinephrine must, at best, be an easily dispensable one. Cannon's theory also fails to take into account the phylogenetic and ontogenetic facts in the development of the mammalian adrenal. As an emergency organ, one can see no teleological significance in the migration of the chromophil into the interrenal tissue.

All the physiological work on epinephrine secretion has been carried out on mammals. Any conclusion based on our present knowledge must be limited therefore to the mammalian body. We must admit our complete ignorance of the function of epinephrine in the lower animals.

As suggested by Stewart,⁵⁸⁷ epinephrine may be only a survival which though of little significance in the higher animals is important in lower forms where hormonal control retains a more fundamental influence than nervous control. Epinephrine may have assumed in the mammal an entirely different function than it has in the lower vertebrates.

A number of facts speak for an intimate relation between the

functions of the medullary and cortical tissues in the mammal. Ontogenetically and phylogenetically one finds an intimacy which can not be dismissed as a mere vagary of nature. The peculiar circulatory system of the adrenals by which, as we have seen (Chapter II), the vessels from the cortex continue into the medulla is further anatomical evidence for suspecting some relation between the medulla and the product of the cortex. The peculiar chemical properties of epinephrine in its oxidation-reduction potentialities would render it ideal as a protective agent for the vital and destructible cortical hormone. Has epinephrine, therefore, in the mammal some function which links it to the adrenal cortical hormone? Does our ignorance of the function of the latter shroud also that of epinephrine?

The view that the function of epinephrine is to protect or otherwise act synergistically with the adrenal cortical hormone is supported by several other important facts. Sjöstrand⁵⁷⁸ found that those agents which cause an increased output of epinephrine from the adrenals also increase the blood flow through the cortex. This finding led him to suggest that epinephrine was linked functionally with the activity of the cortex. As we shall see later, the agents which stimulate epinephrine secretion are also those which stimulate the utilization and hence the demand for the cortical hormone. One would thus be justified in concluding that the observed stimulation of epinephrine which we have described as occurring under various conditions is in reality only secondary to the increased secretion of the cortical hormone which these conditions call forth.

If the theory outlined above is valid one must conclude that in the mammal only the medulla is of functional significance. The extra-medullary tissue is of little or no usefulness and merely a remnant of its occurrence in the lower animals. That the extra-adrenal chromophil tissue is of little function in the organism as demanded by this hypothesis is

supported by the following facts. Non-fatal adrenalectomy in dogs, rats, or rabbits causes no hypertrophy of any extra-adrenal chromaphil tissue.^{357, 685} If the medulla subserved an important function other than that involving the cortex, one certainly would expect some hypertrophy of this chromaphil tissue. On the other hand, after unilateral adrenalectomy, Stewart and Rogoff⁵⁸⁸ found a compensatory hypertrophy of the medulla of the remaining gland.

The major part of the chromaphil tissue of the mammal in embryonic life occurs outside of the adrenals. This tissue degenerates after birth while the adrenal medulla develops, which speaks for the assumption that the medulla is the functionally active part of the chromaphil tissue while the extra-adrenal chromaphil tissue is of little use to the adult organism. These observations indicate that the medulla takes over the function of the extra-medullary chromaphil tissues. The relative inactivity of the extra-medullary tissue is also indicated by Ingier and Schmorl's³²⁹ observation that in persons dead of diphtheria, the adrenals may be free of epinephrine while the paraganglia still contain their normal store of this substance.

CHAPTER VII

THE PHARMACOLOGY OF EPINEPHRINE

The pharmacology of epinephrine has been studied very thoroughly, the wide variety of its actions having invited attention from numerous investigators. Some of the discrepancies between the work of different authors may be attributed to their failure to appreciate that, not only quantitatively but qualitatively as well, the results obtained from epinephrine will depend upon the dosages employed. Most authors have been content to use commercially available epinephrine solutions instead of the pure crystalline material for their work, thus disregarding the effects of the acid and chloretone which these solutions contain. Chloretone exerts particularly poisonous effects on smooth muscle.⁶³⁹

Almost without exception the effect of injecting epinephrine is the same as stimulating the sympathetic nerves and hence epinephrine is spoken of as a sympathomimetic substance. Epinephrine affects practically all tissues which are supplied with nerves of the sympathetic system. On the other hand, its action on tissues not innervated by the sympathetic is slight. Thus, contrasted to the marked activity which epinephrine exerts on blood vessels, it has no action on the placental vessels which are not under sympathetic control. In the embryo, tissues are not affected until they have become innervated by sympathetic fibers. However, epinephrine is said to affect the heart of the embryo fish and the blood vessels of the chick embryo before their innervation.⁶³⁹ In the chick's amnion, likewise, although not known to contain any nerves, the rhythmic contractions of the unstriated muscle are inhibited by epinephrine.³⁷⁹ The action of epinephrine, however, is not upon the nerves or their endings, for sectioning the nerves not only

does not prevent the action of epinephrine, but actually (after the fibers are degenerated) renders the tissue more easily excitable.

Although most of its actions are sympathomimetic, epinephrine exerts certain effects which indicate a parasympathetic-like action. Thus in the frog and turtle, epinephrine constricts the pulmonary blood vessels which are supposed to be under parasympathetic control.⁴⁰⁵ The effects of epinephrine which follow its injection with neurine, acetylcholine, and related drugs or in the presence of a low calcium concentration, are also explained by assuming some parasympathomimetic action of epinephrine.²⁷⁰

The action of epinephrine is characterized by the absence of any measurable latent period; by its dependence upon the epinephrine concentration, which if maintained constant, may elicit an unabated reaction for a long period of time; and by the rapid subsidence of its effects when the drug is removed from its site of action.^{410, 537}

In general, the effects of epinephrine are dependent upon a number of conditions. The state of tone of the organ may determine the extent of the action. Thus a normally dilated bronchiole is scarcely affected by epinephrine while the constricted bronchiole is readily dilated. As Auer and Meltzer⁴⁵¹ first showed, interruption of the sympathetic pathways increases the effect exerted by epinephrine. Thus the denervated iris is 10 to 40 times as sensitive to the drug as the intact organ. The ionic concentration of the inorganic constituents of the medium also affects the action of epinephrine. The calcium ion and the potassium ion act synergistically with epinephrine; other ions (hydrogen, hydroxyl, sodium, magnesium, *etc.*) also affect the action of epinephrine.⁶³⁹

The alkaloids of ergot (ergotamine and ergotoxine) have the peculiar property of inhibiting the effects of epinephrine even in doses which in themselves induce no great functional changes. Although this is true of most actions of epinephrine,

the dilatation of the pupil is a notable exception, for the mydriatic action of epinephrine is still elicited after ergotamine. Yohimbin, hydrastinin, quinine, and apocodeine share with the alkaloids of ergot the property of abolishing the stimulating effects of epinephrine. They do not, however, paralyze the inhibiting effects of epinephrine nor the inhibiting effects of sympathetic excitation. Hence, when either contraction or inhibition can normally be induced under different circumstances through the sympathetic nerves or by epinephrine, if the normal effect be stimulation, this will be abolished by ergotoxine or apocodeine, and the inhibiting effects alone will appear on subsequent injection of epinephrine. This is spoken of as a reversal action. If the normal effect be inhibition, this remains unaltered.

Originally it was thought that the reversal effect of ergotoxine was due to a double innervation from the sympathetic. The capillary dilatation observed by Dale and Richards²⁴³ after epinephrine led them to believe that the reversal is due to the persistence of the capillary dilatation when the constricting effects on the arteries have been paralyzed by ergotoxine. Schafer⁵⁵⁰ has, however, criticized this view.

Where the parasympathetic acts as an antagonist to the sympathetic, paralysis of the former results in an augmented action by epinephrine. Thus the preliminary injection of atropine (by paralyzing the parasympathetics) results in an enhanced response to a subsequent injection of epinephrine. Epinephrine also acts as an antagonist to other parasympathetic drugs—pilocarpine, physostigmine, choline, *etc.* Nicotine, which paralyzes sympathetic ganglia, does not affect epinephrine action for epinephrine does not act through any effect on the ganglia.^{36, 270}

Histamine, by its action in paralyzing smooth muscle, acts as an effective antagonist to epinephrine. Cocaine and novocaine sensitize organs to the action of epinephrine. Thus the pupil is more widely dilated by epinephrine after a preliminary

application of cocaine. This action is due to the paralysis of the antagonistic fibers from the superior cervical ganglion by the cocaine, for the effect is not seen after extirpation of the ganglion. Caffeine is also an antagonist to the stimulating effects of epinephrine on the blood vessels, heart, and uterus.

EFFECTS OF EPINEPHRINE ON THE CIRCULATION

The stimulating effects of epinephrine on the mammalian heart may be demonstrated on the isolated organ or on strips of cardiac muscle. Addition of epinephrine to the perfusing fluid results in a marked acceleration of the pulse rate, an increase in the strength of beat, and an increased rate of conduction when electrically stimulated. These stimulating effects are more pronounced in a heart poisoned by potassium chloride, chloroform, atropine, cyanide, *etc.* After chloral poisoning, on the other hand, epinephrine is ineffective in stimulating the cardiac contractions.

When administered intravenously to mammals, the preliminary acceleration of the pulse is followed by a marked slowing and often by an irregularity in rate. This slowing is brought about reflexly by impulses arising from the elevated blood pressure in the vagus center, for it can be abolished by previous administration of atropine. In chloroform poisoning, epinephrine still reduces the pulse rate even after vagotomy. This is probably due to stimulation of the vagal endings.²⁹² This reflex slowing of the heart may even result in complete cessation of the auricular beats due to inhibition by the cardio-inhibitory center. If the vagi are cut or paralyzed by atropine the inhibitory effects are prevented, and the heart after epinephrine injections maintains its more forceful and rapid rate of contraction. This cardiac activity still further increases the blood pressure. With large doses of epinephrine the heart becomes dilated to such an extent as to be fatal unless the pericardium be opened.^{550, 639}

Epinephrine increases the irritability of the heart. This in-

creased irritability manifests itself in the appearance of extrasystoles and is not prevented by vagotomy.¹⁰ It is favored by chloroform narcosis. Under chloroform anesthesia ventricular fibrillation is easily induced. Barium salts and strophanthin also sensitize the heart to these effects of epinephrine. The central vagal stimulation, which, as we have seen, arises reflexly as a result of the hypertension, also acts on the sinus and on the bundle of Tawara to give heart block or other derangements of rhythm.

According to Stella,⁵⁸⁶ the reflex bradycardia which follows the injection of epinephrine is due to the effects of the elevated arterial pressure acting on the sensitive regions of the carotid sinus and the territory of distribution of the depressor nerves. The degree of this action is conditioned by a state of increased reflex excitability of the cardiac vagus centers induced by the injected epinephrine.

Oliver and Schafer⁴⁸⁰ first demonstrated the immediate and marked rise of blood pressure produced by injecting an extract of the adrenal medulla. Contraction of the peripheral arteries is so marked as to cause a shrinkage of many organs, as recorded by a plethysmograph. Several seconds after an intravenous injection (subcutaneous administration is ineffective) of 0.05 mgm. per kilo of epinephrine into an animal, the arterial blood pressure rapidly rises, reaches a maximum in about 15 seconds, continues to rise slightly for the next $\frac{1}{2}$ to $1\frac{1}{2}$ minutes, and then gradually recedes. In the dog, the pulse rate may be increased to double its value at first but is slowed as the pressure rises and often becomes irregular. The reduced pulse rate continues for sometime after the blood pressure has returned to normal.

The maximum pressor effect elicitable by epinephrine varies in different animals. In the rabbit the normal pressure is rarely doubled. In the cat and dog, on the other hand, pressures as high as 250 to 300 mms. are obtainable after vagotomy. The rise in pressure is proportional to the natural logarithm

of the amount injected; *i.e.*, it follows the Weber-Fechner law.⁴¹⁰ Since repeated injections are reproducible one may use the pressor response to assay epinephrine.⁶³⁹ A good pressor response is obtained in the cat after an injection of 0.001 to 0.002 milligrams of epinephrine. The pressor effect of epinephrine is still elicited after cutting the sympathetics and allowing the nerve fibers to degenerate. Hence the pressor action is not mediated through the nervous system. Oliver and Schafer⁴⁸⁰ in their original paper deduced this from their own experiments. Langley³⁷⁹ postulated that the drug acts on the smooth muscle directly. However, Brodie and Dixon⁹³ showed that after the administration of apocodein (which blocks sympathetic fibers and in large doses causes vasodilation and a drop in blood pressure), epinephrine fails to give a pressor response. The smooth muscle is still reactive for the application of barium chloride elicits a reaction. Hence the point of stimulation lies between the sympathetic nerve terminals and the muscle cells; *i.e.*, on the hypothetical "receptive substance" of Langley or, as it is more commonly denoted, the myo-neural junction.

There is no support for the view that epinephrine exerts its pressor effects through action on the ganglia of the sympathetic vasomotors as has been claimed. When applied locally to the cervical ganglion, no sympathetic effects are elicited.^{110, 177}

Subcutaneous injections (even in doses which if administered intravenously would be fatal) do not exert pressor effects on cats, dogs, or rabbits, although a very large dose (37 mgs. per kilo) raises the blood pressure in the dog.¹³

In animals with intact centers, a drop in blood pressure follows the return to normal. In rabbits, after the injection of $\frac{1}{3}$ mgm. of epinephrine, this drop persists for several days⁶³⁹ due probably to reflex stimulation of the vasodilator center. This reflex is also held accountable for the notch observed in the ascending limb of the blood pressure curve for it is avoided by deep narcosis or by cutting the cord, and is accentuated

by the preliminary injection of strychnine. This reflex according to Heymans²⁹⁷ is initiated in the carotid sinus.

In cats and dogs, small doses (.0001 mgm. per second) give a fall in blood pressure of 20 to 40 mms. of mercury.²⁷⁰ This is due to vasodilatation in the extremities by stimulation of sympathetic vasodilator peripheral fibers.²⁴³ Whether the arterioles or the capillaries are involved in this reaction is questionable. It does not occur in decerebrate unanesthetized or in normal unanesthetized animals.⁶³⁹

The fact that very small doses of epinephrine cause a drop in blood pressure instead of a rise was first noted by Moore and Purinton²⁷⁰ in 1900. Dale³⁶ showed that ergotoxin had the property of paralyzing those sympathetic fibers which have a stimulating function while unaffected those having an inhibitory function. After poisoning with ergotoxin, epinephrine in a dose which normally evokes a rise in blood pressure produces only a fall.

The effects of epinephrine on the peripheral blood vessels have usually been determined by direct observation, by plethysmographic measurements, by determining the rate of perfusion, or the rate of venous outflow. If the blood pressure rises markedly, passive dilatation of the arteries may result with a consequent increase in the plethysmographic volume. This is, for example, taken to be the case in the kidney where the decrease in size of this organ which follows an injection of epinephrine is sometimes preceded or followed by an increase.

The action of epinephrine differs as regards its effects on different vessels. Despite its general constricting action, certain vessels, particularly the splanchnics, the arteries of the skeletal muscles, the coronary, and the pulmonary vessels are, under certain circumstances at least, dilated by moderate doses of epinephrine.¹¹³ According to Orahovats and Gotsev,⁴⁸⁴ epinephrine causes dilatation of the splanchnic vessels in two-thirds of all cases and constriction in the remainder. The arteries of the brain according to Dixon and Halliburton¹⁵⁹ are

only slightly affected or dilated by epinephrine but Wiggers⁶⁸⁰ claims that they are constricted. Similarly divergent results have been obtained on isolated strips of cerebral arteries.

It is difficult to ascertain the effect of epinephrine on the coronary circulation of the intact heart, since the acceleration of the pulse rate, the increased strength of ventricular contraction, and the increased blood pressure would all tend mechanically to increase the blood flow through these vessels. According to Barbour and Prince³² the coronaries of man and the monkey are contracted by epinephrine while those of other animals are dilated.

The results of numerous studies on the effect of epinephrine on various groups of vessels, have, as in the cases cited, invariably given the most diverse and conflicting results. These discrepancies, as obtained in studies on the pulmonary vessels, have been reviewed by Daly,¹⁴⁹ who has pointed out the many factors which may lead to conflicting results: (1) the epinephrine preparation used; (2) variations in dosage; (3) the perfusion pressure and temperature; (4) the composition of the perfusion fluid; (5) the tone of the blood vessels; (6) the animal species experimented upon; (7) seasonal variations; (8) the interpretation of the observations. The same factors undoubtedly have disturbed the experiments on other arteries.

It must also be remembered that the results obtained on dismembered anesthetized animals or on isolated tissues are not indicative of the changes occurring in the normal animal in which numerous reflexes allow compensatory adjustments, which are absent in the anesthetized or operated animal, to occur. The marked increase in the cardiac output following epinephrine injections would speak for extensive dilatation of the splanchnic and pulmonary vessels in the intact organism.²⁴³

The action of epinephrine is not limited to the arteries. Many capillaries are constricted as may be noted by direct observation. On the other hand, Dale and Richards²⁴³ described a capillary dilatation. The action of epinephrine on

the capillaries is manifested by its ability to counteract the swelling induced by application to the skin of mustard oil. The leakage through the capillaries caused by histamine and the cutaneous edema caused by paraphenylenediamine may also be prevented by the injection of epinephrine.⁶³⁹

Epinephrine when applied directly causes constriction of veins similar to its action on arteries. It is particularly effective in diminishing the calibre of the superficial cutaneous veins. Quiescent rings from the superior vena cava near the heart are stimulated to beat rhythmically when immersed in Ringer's solution containing epinephrine.²⁵⁸

The pulse rate of certain invertebrates (*Limulus*, *Daphnia*, *Pecten*, *Maja*, *Aplysia*, and *Salpen*) are increased by epinephrine, while in others (the crab and the snail) the pulse rate is not affected. With large doses the pulse rate of the horseshoe crab (*Limulus*), lobster, and snail is decreased. In fish an increased rate and strength of contraction as well as vasoconstriction are observed. In the frog the pressor effect is slight and the effect on the heart rather uncertain unless previously damaged by drugs, in which case it is markedly stimulated.⁶³⁹

In man the subcutaneous injection of 0.5 to 1 cc. of a 1:1000 solution of epinephrine causes a slight elevation of blood pressure, pallor of the face and extremities, and an accelerated pulse rate. The intracutaneous injection of one cubic centimeter of a 1 to 10 million solution gives a detectable pallor. A 1 to 1 million solution gives an area of anemia surrounded by a zone of hyperaemia. The subcutaneous injection of 1 mgm. of epinephrine in man causes a rise in the systolic pressure of the brachial artery of 10 to 30 mms. of mercury. The maximum rise is reached after about one-half hour and ends after an hour or two. The pulse rate is increased 10 to 20 beats. Extrasystoles may occur. Euler and Liljestrand²⁴³ found the cardiac output of man to increase from 4.3 liters per minute to 7.5 liters following the subcutaneous injection of

0.7 mgm. of epinephrine. The pulse rate increased from 60 to 66 per minute; the systolic output from 71 to 120 cc. The blood pressure rose from 103/68 to 120/64. These results would indicate that in man the injection of epinephrine leads to marked stimulation of the cardiac activity and a diminished peripheral resistance.²⁴³

RESPIRATION

Epinephrine, as noted by Oliver and Schafer,⁴⁸⁰ causes some diminution in the depth of respiration and at times a temporary apnea. This effect disappears before the pressor action has ceased to manifest itself.³⁸³ That this apnea is not due to vascular constriction in the *medulla oblongata* was shown by Bouckaert⁷⁴ who found that it was not abolished in the cat by previous administration of ergotoxine which, as we have seen, abolishes the vascular constricting effect of epinephrine. Mellanby and Huggett,⁴⁵⁰ however, could not confirm this result. In the dog, Langlois and Garrelon³⁸³ found either a diminution or an increase of respiration after epinephrine, depending upon the state of the respiratory center, while Nice, Rock, and Courtwright⁴⁷⁰ found the effect to depend upon the dose. Small doses stimulated, while large doses inhibited the respiratory center.

Small doses of epinephrine cause an increase in the depth of respiration. In the cat a reduction is produced by 0.3 cc. of a 1:1000 solution. These effects are independent of the accompanying blood pressure changes or vagotomy.

Direct perfusion of the medulla has shown that epinephrine acts directly on the respiratory center. Small doses cause an increase in the depth and rate of respiration while large doses diminish the depth and rate.⁶⁸⁷ In man Hartman²⁷⁰ also observed an increase in rate from 24 to 42 per minute after the intravenous administration of 1 cc. of a 1 to 50,000 solution of epinephrine.

It is now generally agreed that the apnoea which follows

an injection of epinephrine is not due to bulbar anemia.⁶⁸⁷ That the apnoea is reflex in origin was demonstrated by Heymans and Heymans²⁹⁸ who obtained a cessation of respiration in the head of a dog united to the body only by the vagi, after injection of epinephrine into the trunk of the animal. The apnoea is thus dependent upon afferent impulses transmitted by the vagus or the nerve of Hering. The apnoea may be abolished by cutting both vagi and denervating the carotid sinus.²⁹⁷ The reflex which leads to apnea is set up by the elevation of blood pressure in the carotid sinus.

When the bronchioles are in a state of tonic contraction, epinephrine causes their relaxation.⁶⁰⁸ This action accounts for its therapeutic use in bronchial asthma. When the bronchioles were not fully relaxed Dixon and Ransom¹⁶⁰ obtained further dilatation after epinephrine injection, but Golla and Symes⁶³⁹ obtained constriction except in cases where it had already been established by the previous injection of some other drug. The results of various experimenters differ widely, due probably to differences in the state of tone of the bronchioles. After paralysis of the constrictors by ergotoxine, epinephrine produces dilatation of the bronchioles with greater ease than normally.

STOMACH AND INTESTINES

Not only the plain muscle of the vascular system but other involuntary muscle is also acted upon by epinephrine. Contraction or relaxation occurs depending upon the effect of sympathetic stimulation under the same conditions.

Epinephrine causes inhibition of the rhythmic movements and the tonus of the alimentary tract, an observation first made by Boruttau.⁷³ This is used as one of the most delicate physiological tests for detecting and assaying epinephrine (*cf.* Chapter VI). The relaxation of the stomach and intestines by epinephrine is in accord with their innervation, for stimulation of the splanchnic fibres arrests peristalsis and causes re-

laxation of the gut. Certain parts of the intestinal tract, however, receive motor fibres from the sympathetic and these (the pyloric, ileo-colic, and internal anal sphincters and the *muscularis mucosae*) are contracted by epinephrine. Pressor doses in man do not affect the intestinal movements.¹⁶⁵

Although inhibition is the typical reaction of the gut to epinephrine, excitatory effects are occasionally obtained. This is explained by assuming an occasional preponderance of motor over inhibitory sympathetic fibers. Hoskins³⁰⁸ showed that doses below the threshold for inhibition will frequently cause an increased tone and rhythmical activity. In the rabbit epinephrine causes relaxation of the internal anal sphincter and the oesophagus as does also stimulation of the sympathetic nerves to these tissues.

In birds and amphibia where the sympathetic nerves are mainly motor in function, epinephrine causes contraction.

THE EYE

The intravenous injection of epinephrine causes elevation of the lid, retraction of the nictitating membrane, and dilatation of the pupil. These effects are, however, much more pronounced after extirpation of the superior cervical ganglion with subsequent degeneration of the post-ganglionic fibers. Likewise the application of epinephrine to the conjunctiva³²² does not affect the pupil unless the superior cervical ganglion has been extirpated.

The reaction of the enucleated frog's eye immersed in isotonic saline has been used as a test for epinephrine.¹⁷⁴ Although sensitive to epinephrine in a dilution of 1 to 20 million, the method is not specific.

In rabbits after superior cervical ganglionectomy, dilatation of the pupil results from the subcutaneous injection of 0.6 cc. of a 1:1000 solution of epinephrine. Occurring within 15 minutes after the injection, the reaction lasts for over 2 hours. During its maximum dilatation, the pupil does not react to

light or to eserine.⁴⁵¹ The reaction by which stimulation of the *dilator pupillae* and inhibition of the *sphincter iridis* occurs in response to epinephrine is ten times more sensitive in the cat than in the rabbit. In the dog, due to stimulation of the oculomotor center, one often obtains contraction of the pupil instead of dilatation.

Müller's muscle is contracted by epinephrine making the globe of the eye more prominent and enlarging the palpebral fissure.

UTERUS

The action of epinephrine on the uterus is most striking. Uterine anemia and violent contractions are elicited in animals which, if pregnant, may be made to abort. The uterus is also rendered more excitable to physiological or artificial stimuli.

The effect of epinephrine on the uterus varies with different animal species. The rabbit's uterus is stimulated whether pregnant or not. Inhibition is caused in the gravid or non-gravid uterus of the mouse, rat, or guinea pig. The uterus of the pregnant cat is contracted while the non-pregnant uterus is relaxed. In the dog, if pregnant, stimulation results; if non-pregnant, stimulation is followed by relaxation and inhibition. Similar results are obtained in the sow. The non-gravid uteri of the cow, ferret, and monkey are stimulated by epinephrine.

According to Bourne and Burn,⁷⁶ the uterine movements of the intact pregnant human uterus are inhibited by tolerable doses of epinephrine. Most authors, on the other hand, reported stimulation of the human uterus whether gravid or non-gravid, but these results were based on experiments in which large doses of epinephrine were applied to strips of muscle removed at operation.

As we have seen in Chapter VI, strips of uterine muscle are frequently used in the biological assay of epinephrine.

The above described reactions of the uterus under different

conditions are in every case identical with those observed after stimulation of the hypogastric nerves. These nerves carry both motor and inhibitory fibers to the uterus and hence the relative strength of these two sets of fibers determines the reaction in any given case.

EFFECTS OF EPINEPHRINE ON OTHER ABDOMINAL VISCERA

The spleen is very sensitive to epinephrine, contracting even with minute doses. This contraction involves arteries, muscular capsule, and trabeculae.

The gall bladder and sphincter of Oddi are relaxed by epinephrine; the bile ducts are contracted.

Epinephrine causes little effect on the bladder of most animals but in the cat and monkey relaxation (paralleling the effect of stimulating the hypogastric nerves) occurs, while contraction follows epinephrine injections in the goat and ferret.

Similar differences result from the action of epinephrine on the ureter. Contraction is observed in the cat, guinea pig, monkey, and man; contraction or relaxation in the dog; and no response in the ferret.

Epinephrine causes relaxation of the *tunica dartos*, contraction of the vas deferens, seminal vesicles and vagina, and increased tone of the prostate.

PILOMOTOR MUSCLES

Smooth muscle in general, if contracted by sympathetic stimulation, is readily made to contract by epinephrine. The pilomotor fibers, however, are an exception to this rule. Although readily affected by sympathetic stimulation their response to epinephrine in most animals is slight.

The apparently discordant effects of epinephrine on the skin muscles and *arrectores pilorum* are harmonised, according to Elliott,¹⁷⁷ when one considers the varying functional use of these muscles in different animals. Thus epinephrine has little effect on the hairs of a cat's back, but causes marked action on

the bristles on the scruff of the fox terrier's neck. The hair movement in the macaque is slight on the body but manifests itself well over the temples and forehead. The injection of 0.18 mgms. of epinephrine into the mongoose (*Herpestes mungo*), though insufficient to evoke a maximal dilation of the pupil, nevertheless, causes an extreme erection of the hairs on the animal's tail. The irritability of the *arrectores pilorum* thus varies directly with their normal functional use. According to Elliott¹⁷⁷ similar considerations are applicable to the varying reaction of other organs.

The rise of "goose flesh" in the human subject after an injection of epinephrine is due to the contraction of the *arrectores pilorum*.

MELANOPHORES

Epinephrine causes contraction of the melanophores of the frog as may be noted by microscopic observation of the effect of painting epinephrine on the web of the foot. The retinal pigment cells migrate distally even in the light, which normally causes their aggregation. In *Fundulus heteroclitus*, contraction of the skin melanophores has also been observed in doses as small as 1 to 50 million. This effect is replaced by expansion after a previous injection of ergotoxine.⁶³⁹

SECRECTIONS

Epinephrine stimulates the salivary, lachrymal, and other secretions. Since section of the *chorda tympani* and destruction of the superior cervical ganglion do not interfere with this reaction, the point of stimulation must be peripheral. Other secretions (gastric and mucous secretions of the mouth, esophagus, and trachea) are also stimulated. The response of these glands to epinephrine is not marked, however, for the ischaemia, simultaneously induced, reduces the secretory response to epinephrine.

Although sweat secretion in the cat's paw is under sympathet-

tic control, epinephrine injections fail to produce sweating.³⁷⁸ Likewise in man, Elliott¹⁷⁷ obtained a decrease in secretion from the skin of the hand, due probably to the accompanying vasoconstriction. However, injecting epinephrine directly into the pad of the cat's foot, after section of the sciatic nerve or under deep anesthesia, produces sweating.⁶³⁹ Also in the horse, a localized area of sweating is induced by the subcutaneous injection of epinephrine which lasts for several hours.^{262, 462} Intravenous injections cause generalized sweating.

Cow's¹³¹ demonstration of a vascular connection between the adrenals and kidneys led him to suggest a functional relation between these two organs. In the dog, Marshall and Kolls⁴³⁸ found no such connection; nor does any apparently occur in man. Addis, Barnett, and Shevsky²⁷⁰ found that subcutaneous injections of epinephrine produced an increase in urea secretion by the rabbit. Relatively large doses gave a decrease. Intravenously injected epinephrine inhibits the urine flow temporarily. The injection of 1 mgm. of epinephrine diminishes diuresis and salt excretion in man.⁶³⁹

In animals with vesical fistulae, Zunz and his collaborators⁶⁹⁹ also observed a diminution in the diuresis which follows the ingestion of urea and in the proportion of chlorides in the urine of fasting dogs after an injection of epinephrine. These effects are probably to be attributed to the vascular effects induced by epinephrine. Contraction of the blood vessels of the glomerular tuft would diminish the rate of urine flow while the subsequent contraction of the efferent vessels from the glomerulus would tend to increase the rate of filtration.

Variable results have been obtained in studies of the effect of epinephrine on gastric secretion. The secretion of milk is not affected. Due to the ischaemia produced by epinephrine the secretion of bile and pancreatic juice are inhibited.¹⁷⁰

STRIATED MUSCLE

Epinephrine (as does also stimulation of the sympathetic) decreases fatigability and increases the work capacity of an

isolated muscle. If a gastrocnemius muscle of a frog be stimulated to fatigue, injection of epinephrine will give a good degree of recovery. The irritability of frog's muscle stimulated through its nerve is so much increased by epinephrine that this drug may act as an antagonist of curare.²⁵²

The claimed effects of epinephrine on skeletal muscle can be explained by its action on the circulation, the rate of which is increased, and its stimulation of the metabolic processes, by increasing the oxygen consumption. The increased ability of fatigued striated muscle to perform work in the presence of epinephrine has also been ascribed to an acceleration in the rate of formation of lactic acid. The phosphagen content of the muscle is not at first affected but when most of it has been decomposed, the accelerated formation of lactic acid furnishes the energy necessary for the resynthesis of the phosphagen.⁴⁶³

BASAL METABOLISM

Subcutaneous injection of epinephrine causes a rise of the basal metabolic rate, as measured by the oxygen consumption, which lasts for several hours.⁵⁴⁴ In the rabbit, the injection of one milligram subcutaneously causes an elevation of 50 per cent or more in the basal metabolic rate which reaches its maximum in the third hour after injection. Injection of 0.1 mgm. per kilo of body weight in the dog causes a rise from 350 cc. to 490, after 20 minutes; 580, after an hour and a quarter; 480, after 3 hours.⁶³⁹

Since epinephrine does not affect the oxygen consumption of an animal paralyzed with curare or of a perfused limb, Bornstein⁶³⁹ concluded that epinephrine exerts its metabolic effects by central action. However, epinephrine raises the metabolism of a rabbit after section of the spinal cord. It does not raise the metabolism of an eviscerated animal. Epinephrine injections stimulate the metabolism of hibernating hedgehogs to such an extent as to raise their body temperature and rouse them from their winter sleep.⁶³⁹

Epinephrine still causes an increase in the oxygen consump-

tion in rabbits after thyroidectomy. The rise, however, is delayed somewhat in thyroidectomized animals and does not last as long as it does in normal animals.⁴³⁶

The injection of epinephrine does not affect the protein metabolism during exercise by a fasting man. The carbohydrate utilization, however, according to Dill and his collaborators,¹⁵⁸ is increased considerably. There is also a decreased excretion of acetone bodies indicative of the facilitating effect of epinephrine on the oxidation of carbohydrate. Epinephrine exerts the same effects on the blood sugar and lactic acid in work as it does during rest.

The above described results of Dill and his collaborators on man are opposed to the earlier experiments on anesthetized animals which purported to show that epinephrine inhibited the utilization of carbohydrate in the body. This conclusion which is based on an observed decline in the respiratory quotient is open to serious objections.⁴¹⁹

BODY TEMPERATURE

In man, therapeutic doses of epinephrine cause a rise of temperature of only a fraction of a degree.⁵⁴⁴ In experimental animals, however, marked fever can be induced. This hyperpyrexia is brought about by several factors. There is an increased heat production accompanying the increased metabolism, which is particularly striking in the liver. After removal of the liver, fever can not be induced. The increased work of the heart, under the influence of epinephrine, as well as the increased activity of other organs and tissues stimulated by epinephrine, all combine to give an increased heat production. This heat is not dissipated because of the marked cutaneous constriction which accompanies the reaction to epinephrine.

Whether epinephrine causes hyperpyrexia by interference with the heat regulating center is still debatable. Against the assumption of such an interference is the fact that it raises the temperature of the dog rendered poikilothermal by section

of the cervical cord and the hedgehog after removal of the heat regulating center.⁶³⁹

CARBOHYDRATE METABOLISM

Blum,⁶⁴ in 1901, first showed that an injection of epinephrine causes glycosuria. He suggested that the glycosuria observed by Claude Bernard after piqure of the floor of the fourth ventricle was due to liberation of epinephrine. The glycosuria is the result of hyperglycemia which in turn is brought about, in part at least, by glycogenolysis in the liver. After large doses of epinephrine, the liver may be depleted of its glycogen store. This glycogenolysis is not prevented by severing the nerves to the liver. The subcutaneous injection of 0.2 mgm. of epinephrine into a rabbit causes an hyperglycemia of several hours duration. Injected intravenously, on the other hand, the effect is very short due to the rapid destruction of the epinephrine.¹²⁸

Even in doses which do not elicit a rise in blood pressure, epinephrine still gives rise to hyperglycemia. Thus the continuous intravenous infusion of 0.0001 mgm. per kilo per minute to a rabbit causes hyperglycemia, but is without effect on the blood pressure. The effect of epinephrine on the blood sugar varies in different animals, being greater, for example, in rabbits than in cats, dogs, rats, or men. The hyperglycemia which follows the injection of epinephrine no longer occurs, as Mann and Magath⁴²⁵ have shown, after removal of the liver. However, the hyperglycemia may still be elicited in animals in which the glycogen store of the liver has been reduced to a minimum by prolonged fasting or by the injection of strychnine. In such animals obviously the liver can not be the source of the glucose which appears in the blood stream. In such fasting animals, indeed, not only does hyperglycemia occur but there is also a concurrent storage of glycogen in the liver. Further evidence against the view that the effect of epinephrine upon the carbohydrate metabolism is due solely to

a conversion of pre-existent glycogen into glucose has been advanced by Cori and Cori.¹²⁸ These authors showed that the mobilization of all the glycogen present in the liver could only account for part of the glucose which appears in the circulation after an injection of epinephrine. In fasting rats, these authors found that epinephrine caused a decrease in the glycogen content of the muscles with a concurrent increase in the glycogen store of the liver. Cori and Cori considered the changes induced by epinephrine as consisting of a breakdown of glycogen in the muscles to lactic acid, a resynthesis of this lactic acid in the liver to glycogen, and a breakdown of the liver glycogen to the glucose which appeared in the blood.

Corkill and Marks¹²⁹ demonstrated that in eviscerated cats, epinephrine causes a discharge of epinephrine from the resting muscles. None of this glycogen appears in the form of glucose and only a part of it as lactic acid. The remaining carbohydrate may be in the same form as the so-called "lost carbohydrate" which disappears after the injection of insulin. Its appearance may be due to the action of the epinephrine liberated by insulin. MacCleod,⁴¹⁹ however, has cast doubt on the existence of this "lost carbohydrate."

When the glycogen store of the liver is reduced by injections of phlorizin, hypoglycemia follows epinephrine injections. This depression of the blood sugar level is attributed by LaBarre and Houssa³⁷¹ to the stimulation of the secretion of insulin by the epinephrine.

The failure of piqure after section of the splanchnic nerves to induce hyperglycemia is explained by the lack of epinephrine secretion in the denervated glands. Stewart and Rogoff⁵⁸⁸ and MacLeod,⁴¹⁹ however, have concluded that the presence of the adrenals is not necessary for the development of the hyperglycemia which follows decerebration or piqure. The impulses transmitted along the sympathetic pathway from the diabetogenic center in the pons must therefore act independently of the adrenal glands and influence the glycogenic func-

tion of the liver directly. Epinephrine can thus not be held responsible for the hyperglycemia following decerebration, although it may play some part in bringing it about.

OTHER PHARMACOLOGICAL EFFECTS OF EPINEPHRINE

Epinephrine also causes other detectable changes in the animal economy but these are inconsiderable in their effects and for the most part are probably secondary to the influences described in preceding sections. Changes in acid-base balance, in salt metabolism, in blood concentration, *et cetera* have been described.⁶³⁹

As first noted by Vosburgh and Richards,⁶⁵¹ the injection of epinephrine shortens the coagulation time of the blood. Cannon and Gray¹⁰⁴ found the intravenous injection of 0.001 mgm. per kilo to shorten the time to one-half to one-third of the normal. This action is due to the liberation from the liver of substances concerned in the process of coagulation. Large doses inhibit coagulability. These effects presumably result from the action of epinephrine on the liver for epinephrine when added to shed blood does not affect coagulation.

The possible influence of epinephrine on protein metabolism is still debatable despite a number of investigations designed to determine this relationship.

TOXICOLOGY OF EPINEPHRINE

Large doses of epinephrine produce violent symptoms which may end in death. At first there is a period of excitement with rapid pulse and respiration. This reaction has occasionally been obtained in man where an intended subcutaneous injection has been accidentally introduced intravenously. Following the period of excitement, depression supervenes. The animal lies on its side, muscular movements are slowed, and paralysis of the limbs follows. The animal becomes dyspnoeic and finally dies in asphyxial convulsions. The paralysis is central, death being due to pulmonary edema.

The minimal lethal dose of epinephrine administered subcutaneously to man is from 10 to 20 mgms. per kilo of body weight; intravenously it is 0.1 to 0.25 mg. per kilo. Alarming symptoms have followed the intravenous injection of 0.3 mgm. As little as 4 mgms. subcutaneously has proven fatal.⁶³⁸

The lethal dose for the rabbit per kilo of body weight is 0.05 to 0.4 mgm. administered intravenously. For the dog or cat the lethal dose is 0.2 to 0.8 mgms. per kilo.⁶³⁸

The paralysis and convulsions which follow lethal doses of epinephrine are results of changes in the central nervous system presumably caused by vascular constriction. At autopsy, one finds multiple hemorrhages throughout the body. The heart is dilated. The lungs are edematous and filled with blood. Hemorrhage is also found in the other serous cavities and in the kidney. The pupils are dilated and the eyeballs project due to sympathetic stimulation.

The effects of sub-lethal doses of epinephrine is cumulative so that the injection of a lethal dose in two injections with an interval of some hours between the injections may still prove fatal. Atropine is a valuable antidote.⁶³⁹

THERAPEUTIC USES OF EPINEPHRINE

The versatile effects produced by the administration of epinephrine have given it wide usefulness in practical therapeutics.

Epinephrine receives wide application in conjunction with the use of local and spinal anesthetics. Here it acts by aiding the production of a bloodless field and, by preventing rapid absorption of the anesthetic, prolongs the period of anesthesia, maintains a high concentration of the anesthetic locally, and prevents toxicity due to its absorption.

The stimulating effect of epinephrine on the heart renders it useful as an analeptic. When injected directly into the cardiac muscle, the heart which has stopped may resume its beat, particularly with the aid of massage. Epinephrine has been

used as an analeptic particularly in cases of drowning and in *asphyxia neonatorum*.¹¹²

Epinephrine is an antagonist to most cardiac depressants particularly to chloroform and chloral. It is therefore used in cases of failing circulation during anesthesia.

In bronchial asthma, epinephrine relaxes the dilated bronchioles and the relief obtained by an asthmatic is often striking. The usefulness of epinephrine in asthma dates from before our knowledge of the sympathetic bronchodilators and before the experimental demonstration of the dilating action of epinephrine on bronchiolar constrictors. It is administered subcutaneously in doses of $\frac{1}{2}$ to 1 cc. of a 1:1000 solution.¹¹²

One of the most important therapeutic applications of epinephrine is due to its local effects in constricting blood vessels. Local ischaemia induced by epinephrine permits bloodless operations on the eye and on the nasal and oral mucosae. Epinephrine is also useful for stopping epistaxis. In the use of epinephrine surgically as a hemostatic, it is necessary to remember that small vessels subsequently bleed when the effect of the epinephrine has disappeared.⁵²⁰ The ischaemia due to epinephrine may also cause delayed healing and even necrosis. Although advocated for internal hemorrhage, it would seem to be contraindicated in this condition.

The application of the drug to a congested mucous membrane is followed by contraction of the swollen mucosa and constriction of the dilated blood vessels. The congestion of hay fever is temporarily relieved by such applications.

In urticaria, angioneurotic edema, serum sickness, and the nitroid crises following the injection of arsphenamine, epinephrine injections often give spectacular relief when administered subcutaneously in doses of $\frac{1}{2}$ to 1 cc. of the standard 1:1000 solution.¹¹²

In cases of acute traumatic shock in which there is a dilatation of the capillary bed with an increase in capillary permeability, it is doubtful if the transient cardiac stimulation and

vasoconstriction following epinephrine can be of any therapeutic effectiveness. On the other hand, where there exists, as a result of a weakened cardiac action, a low blood pressure, epinephrine acts as an efficient analeptic. This is the case, for example, in the collapse following excessive anesthesia²⁵⁷ or in the use of a spinal anesthetic.⁵²⁰ Due to the frequency with which epinephrine induces ventricular fibrillation in the presence of chloroform anesthesia, its use is contraindicated during or immediately after chloroform anesthesia.

Epinephrine may be used in averting the symptoms of hypoglycemia (*e.g.*, after an overdose of insulin) providing there is an adequate store of glycogen in the liver. If no such store exists, epinephrine obviously can only have a deleterious effect.

Epinephrine has also been advocated for use in poisoning (to prevent absorption), in diphtheria, heart block, Addison's disease, osteomalacia, rodent ulcer, tobacco amblyopia, and osseous repair. It is doubtful if it has any therapeutic value in these conditions.

Epinephrine injections have been advocated as a diagnostic aid in thyroid disease,¹¹⁷ hypertension, angina pectoris, irritable heart, and other conditions but its use for this purpose has not met with success. The use of epinephrine in angina pectoris is to be deprecated because of the strain it puts upon the heart.²⁴³

MODES OF ADMINISTRATION

Epinephrine is administered clinically by subcutaneous, intramuscular, intravenous, or intracardial injection, or by local application to mucous membranes. Its oral use has at times been advocated but it is doubtful, in view of the rapid destruction of epinephrine by the gastric and intestinal secretions and the extremely slow rate of its absorption by mucous linings, if such oral therapy can be of any significance. Such effects as have been claimed to result from oral therapy may in part be due to the oxidation products of epinephrine. These deriva-

tives of epinephrine are extremely toxic and in large doses may prove harmful even when administered orally. Severe gastric and intestinal cramps are the commonest symptoms following the oral administration of epinephrine. Doses of 4 cc. of a one per cent solution have been advocated⁸³ for the oral treatment of several conditions but in view of what we have stated above, the oral use of epinephrine must be deprecated.

That some epinephrine is absorbed after oral administration is manifested by the hyperglycemia resulting from the administration of 4 milligrams of epinephrine to man⁸³ and by the effects of an oral dose on the iris of the cat, after removal of the superior cervical ganglion. The epinephrine which is absorbed from the gastro-intestinal tract is carried first to the liver where it exerts its glycogenolytic action and is destroyed before it can exert any cardio-vascular effect. Rectal administration which results in absorption into the general circulation before reaching the liver is more effective than oral administration.

The pharmacological effects of epinephrine are rapidly elicited after injection but very transient if directly introduced into the blood stream.⁶⁶¹ When injected subcutaneously, epinephrine is absorbed along the lymph channels. The local vasoconstriction occurring at the point of injection retards the rate of absorption of the epinephrine and thus prolongs its action. The subcutaneous route of administration is always preferable except in emergency treatments where the circulation is too much retarded or the heart has stopped, in which cases intravenous and intracardiac injections, respectively, are required.³²⁸

As an analeptic, in cases where the heart has stopped, as in shock, anesthesia, electrocution, drowning, suffocation, *et cetera*, epinephrine must be injected intracardially to produce the desired effect. An injection into the heart muscle is much more effective than an injection into the cardiac cavity, for

absorption from the blood stream is relatively slow compared to the rapid distribution of the epinephrine by way of the lymphatics when injected into the myocardium. The intracardiac injection is best made through a fine needle, 8 cms. long, which is inserted above the fifth rib in the fourth, left, intercostal space. To avoid injury to the internal mammary artery, the injection should be made at a point about a finger's breadth from the left sternal margin.³²⁸

Hyman³²⁰ reports that of 250 intracardiac injections in moribund individuals, 25 per cent were followed by successful stimulation of the heart. Successful results have been reported in cases where the heart had stopped for over ten minutes.³²⁸

FATE OF EPINEPHRINE AFTER ITS ADMINISTRATION

The fate of epinephrine in the body is still unknown. It is not excreted in the urine but after large oral doses, catechol derivatives of epinephrine have been detected. The destruction of epinephrine does not occur in the blood stream for the blood has a protective action against the oxidation of epinephrine as do also the tissues in general. Nor do the lungs, capillaries, or arterial walls play a part in the destruction. It is most likely that the liver is the chief agent for the removal and destruction of epinephrine. An injection into the portal vein or absorption from the stomach gives only minimal effects since the epinephrine under these conditions reaches the liver before entering the general circulation. On the other hand, in animals with an Eck fistula, epinephrine has a very prolonged action.

PART III. THE CORTEX

With the failure of investigators to demonstrate the function of the adrenal medulla, interest was gradually shifted to the cortex as the important part of the adrenal. During the last two decades, numerous studies have been made on the function of the cortical tissue and the product of its secretion. The importance of the cortex for the maintenance of the functional integrity of the organism has been demonstrated most conclusively. There remains little doubt that the cortical cells of the adrenal elaborate a hormone of vital importance to the organism, and that death rapidly follows the exclusion of this hormone from the body. By what mechanism this hormone exerts its effects is still unknown. The various theories advocated thus far are, for the most part, unworthy of serious consideration since they are based on some specific manifestation of adrenal insufficiency and ignore all other aspects of the problem. It has been suggested that the cortical hormone is essential for the well-being of all the tissues of the body. If this be true one should be able to demonstrate striking effects of the hormone on *in vitro* preparations of tissue cultures.

A great advance in the study of the cortex has been made by the preparation of the cortical hormone in sufficiently pure and potent form to permit its application to clinical and experimental problems. The isolation of the hormone and its chemical study with a view toward its ultimate synthesis still remain as problems for the future, although some progress in these directions has already been made.

CHAPTER VIII

ADRENALECTOMY

Extirpation of the adrenals of experimental animals has been performed by a host of workers since Brown-Séquard's⁹⁶ pioneer researches in this field in 1856. Until recently, observation of adrenalectomized animals was the chief approach to an experimental study of adrenal function. The operation is essential for most studies on the glands and we shall, therefore, describe in some detail the general features of the operation as performed on various classes of vertebrate animals.

Inspired by Addison's⁷ description of a disease involving the adrenal glands, Brown-Séquard⁹⁷ undertook to reproduce the disease in the lower animals by extirpating the glands in rabbits, dogs, cats, guinea pigs, and rats. The fatal outcome of his operations (within a few hours to two days) led him to conclude that the adrenals were indispensable for life. Gratiolet²³⁸ repeating these experiments found that even unilateral adrenalectomy in the guinea pig was fatal due to post-operative peritonitis and he therefore concluded that the outcome was primarily induced by injury to the viscera and only secondarily by the deprivation of the adrenals. Philipeaux and Harley¹⁹⁸ also reported that adrenalectomy was not fatal to the rat if the operation were performed in stages.

In 1891 Abelous and Langlois³ verified Brown-Séquard's conclusion as to the vital importance of the adrenals for life. Hultgren and Anderson³²³ found that unilateral adrenalectomy was tolerated by the cat but that bilateral operation was fatal in 5 days when the glands were removed in two stages, but in only three days if removed at one operation. In rabbits, too, adrenalectomy was followed by death in 5 or 6 days, survival being prolonged by previous castration.

Strehl and Weiss⁶⁰⁰ carried out a series of operations on cats, guinea pigs, rabbits, rats, mice, and frogs and showed the fatal outcome in all of these animals.

To avoid the deleterious effects of trauma, Biedl⁵⁶ devised the following operative technique: He first dislocated the glands from their normal position and, with their blood vessels intact, attached them subcutaneously. Several days later, he removed the glands through a skin incision. His results confirmed his predecessors' views regarding the indispensability of the glands for life.

The earlier work cited above indicated that adrenalectomy was rapidly fatal in most animals. Subsequent work during the last two decades has shown, however, that, with a more refined technique and certain precautions, one can adrenalectomize cats, dogs, monkeys, and other mammals and have them survive for a few days to a few weeks in good condition. The rapidity with which death followed the operation in the hands of the earlier workers is attributable to the operative shock, hemorrhage, and general manhandling to which the animals were subjected. However, even in recent years expert surgeons have reported results little better, as regards the survival period of their animals, than those of the early part of the century. It is only with the development of some degree of skill and observation of certain precautions involved in the operative technique that the more prolonged survivals are attainable.

Until recent years, the rat, mouse, and rabbit were considered as exceptional in that adrenalectomy was supposed to be fatal in only a small fraction of these animals.¹⁹⁸ As shall be shown later, this view is erroneous being based on the results of an incomplete extirpation. With proper technique adrenalectomy in all the common laboratory animals can be carried out with recovery of the animals from the immediate effects of the operation and the ultimate development of a fatal adrenal insufficiency.

FACTORS DETERMINING THE SURVIVAL PERIOD AFTER
ADRENALECTOMY

Adrenalectomized animals are extremely sensitive to traumatic injury or hemorrhage and hence a finesse of surgical technique will be a factor in determining survival after adrenalectomy. The shock of trauma and hemorrhage involved in prolonged and clumsy handling of the viscera will obviously shorten the life of an already doomed animal. In addition to surgical technique, however, a number of other factors will determine the survival period, chief among which are the following:

- 1) the animal species
- 2) the age of the animal
- 3) the pre-operative treatment
- 4) the anesthetic and the duration of its administration
- 5) the completeness of the extirpation
- 6) the post operative treatment
- 7) the season of the year
- 8) the state of the reproductive system

Some animals (*e.g.*, the rat, goat, and frog) survive adrenalectomy much longer than others (*e.g.*, the dog or guinea pig) which is in accord with their comparative hardness and ability to withstand other forms of injury.

Age. Age is an important factor in determining the survival of a given animal species. Young animals survive for much shorter periods than adults. It is probable that the active metabolism and growth processes of the young individual induce a rapid depletion and more urgent demand for the cortical hormone than the relatively inactive tissues of the adult. In accord with this view is the fact that adrenalectomized young animals require relatively more of the cortical hormone than adults to maintain them in good condition.

The effect of age in determining the survival of animals after adrenalectomy has been demonstrated in rats by Firor and the

author¹⁹⁸ and by Sisson and March.⁵⁷⁷ The latter authors found the following survival periods:

Age in days.....	20	30	40	50	60	70
Average survival in days.....	5.6	6.2	8.3	14.9	14.4	17.0

Immature animals survive for a period of time which is only about one-half as long as the survival time of adult animals.

Pre-operative care. The pre-operative care is of utmost importance in determining the survival period after adrenalectomy. Animals obtained from sources in which temperature conditions, food, *etc.*, are not optimal succumb much more quickly than those maintained under good conditions and in a good state of health and nutrition. This is illustrated by the following experiment: A series of six cats were subjected to a room temperature of 10°C. for several days before the removal of the right adrenal. Such animals normally survive unilateral adrenalectomy without manifesting any signs of insufficiency. The cats of this series, however, all showed signs of adrenal insufficiency, and four of them died within ten days following the unilateral adrenalectomy.¹⁹⁸

Anesthetic. The anesthetic used and the duration of the anesthesia have an important bearing on the survival period of animals after adrenalectomy. Some of the long survivals reported by earlier workers are perhaps to be attributed to their avoidance of any anesthetic. That the duration of the period of anesthesia is of great importance is demonstrated by the following experiment: The average survival of five rats receiving ether for five minutes was fourteen days, while in five animals receiving ether for ten minutes the average survival period was only seven days or only half as long as in the first group.¹⁹⁸

In general, the use of ether as an anesthetic results in survival periods which are much shorter than those obtained when certain other anesthetics are used. Thus in dogs, Firor and

Grollman¹⁹⁸ found the survival period to be markedly prolonged by the use of spinal anesthesia instead of the usual anesthetization with ether. In rats survival is markedly prolonged by the use of intraperitoneally injected barbiturates as compared with the use of ether.

One may obtain a measure of the effect of the operative interference (including the action of the anesthetic) in reducing the survival period of an animal in the following manner: By efficacious therapeutic treatment the animal may be restored to normal and allowed to recover from the operative wound and resume its normal growth and activity. If treatment of such an animal now be suddenly stopped, it will be found that survival extends for a longer period than would have been observed had the animal not been treated. We may consider the difference between the observed survival period after cessation of therapy of a series of treated animals and a similar series allowed to die untreated as representing the period of life shortened by the operation.

The advantage of performing adrenalectomy in two stages in order to prolong life beyond the period observed in a one-stage operation, was first indicated by Langlois³⁸⁰ in 1893 and has been confirmed repeatedly by numerous workers. The explanation for this phenomenon is quite obvious from our preceding discussions. The extirpation of the more difficultly removable gland in a preliminary operation and the allowance of a sufficient time (a week or more) for recovery of the animal permits the extirpation of the remaining gland with a minimum of trauma and anesthesia. Dogs which survive adrenalectomy for an average of only 3 or 4 days by the one-stage operation will survive twice as long when the operation is performed in two stages with an interval of a week or more between operations.

Completeness of the extirpation. The completeness of the extirpation is obviously important in determining the period of survival. Fragments of the main gland or the microscopic

nests of accessory cortical tissue which lie in the vicinity of the main glands may hypertrophy to a sufficient degree to permit indefinite survival of the animal in good health after a preliminary period of mild insufficiency. In such cases macroscopic examination will reveal the presence of the hypertrophied glandular material. The occurrence of such hypertrophy has long been recognized. However, it has not been generally recognized that, although such remnants may be undetectable by the usual macroscopic necropsy, they may suffice to prolong life for some time.

Microscopic examination of the connective tissue of the space occupied by the adrenals will often reveal in animals that have survived an unusually long time after adrenalectomy the existence of exhausted cortical cells. These cells apparently functioned for a time but eventually, due perhaps to poor vascularization or other factors preventing their multiplication, were unable to maintain the life of the animal. The existence of such exhausted adrenal tissue must be borne in mind in testing the therapeutic efficacy of any preparation. Failure to examine microscopically the adrenal sites at autopsy may explain some of the claims for the therapeutic value of extracts which later work has shown to be utterly worthless. Mere macroscopic search for accessories as performed by most recent workers in proving the completeness of their adrenalectomies is no valid proof of a complete extirpation. Microscopic search must also be made for the exhausted nests of cells described above.

Post-operative care. The post-operative care of adrenalectomized animals is important in determining the length of their survival. They should be maintained at an equable temperature, free of all excitement. Excessive exercise should be avoided. A relished diet high in salts and carbohydrate is desirable. The ingestion of high protein diets reduces the survival period.^{31, 267}

Season. The survival of adrenalectomized animals is dependent upon their state of activity at the time of operation. Hibernating animals survive for extended periods while the same animals during their summer activity survive adrenalectomy for a much shorter time.⁸⁶

Pregnancy. H. A. Stewart⁵⁹³ first noted the prolongation of the survival of adrenalectomized pregnant cats as compared to non-gravid females. G. N. Stewart and Rogoff⁵²⁹ found a mean survival period of 7 days for male and $6\frac{1}{2}$ days for non-pregnant female dogs as compared to 22 days for pregnant animals. Similarly in rats, Firor and the author¹⁹⁸ observed a prolongation of life in pregnant as compared to non-pregnant animals. Other authors¹²⁵ have failed to note any prolongation in the survival of pregnant animals. Their results might be explained by the fact that during pregnancy (particularly when advanced) the glands are less easily accessible for operative removal. Since pregnant animals are more susceptible to injury by any operative manhandling, the survival after adrenalectomy may be unaffected or even shortened (as compared to non-pregnant animals) unless the operation is carried out with great technical skill and celerity.

The prolonged survival of pregnant animals may be explained by assuming that the cortical hormone produced by the embryo is transported through the placenta to the maternal tissues.

Estrus. As demonstrated by Rogoff and Stewart,⁵³³ dogs adrenalectomized during or shortly after estrus survive for a much longer period than animals in diestrus. The explanation for this phenomenon probably lies in the fact that the increased body activity during natural estrus stimulates the adrenals to the production of a greater store of the hormone in the body than is normally present. There is no reason to believe, as has been suggested, that the corpus luteum (whose cells resemble superficially those of the adrenal cortex) takes over the func-

tion of the adrenal. Nor has the author been able to find an appreciable supply of the cortical hormone in the corpora lutea of pigs or cattle which might act as a store for the hormone.

Firor and the author were unable to prolong the life of adrenalectomized dogs by inducing in them pre-operatively an artificial estrus by injection of theelin or the gonadotropic substances of pregnancy urine or of the hypophysis. Apparently such artificially induced estrus does not duplicate all the physiological changes occurring in natural estrus as is also indicated by other experimental work.

Castration. Castration according to some authors prolongs the survival of animals after adrenalectomy. One might anticipate that the reduced metabolic activity of the castrated animal would enable it to survive the extirpation of the adrenals for a longer period as compared to the survival of the more active normal animal. On the other hand, the increased adrenal activity of the female in estrus, described in the preceding paragraph, would tend to prolong its survival beyond that of a castrated female. The diversity of results obtained by different authors as to the effect of castration on survival after adrenalectomy may result from the extent to which the factors just outlined have affected their results.

Sex. Except for the effects of estrus and pregnancy, no appreciable difference has been demonstrated between the survival periods of male and female animals. In dogs Rogoff and Stewart⁵²⁸ found a mean survival of 7 days for 34 male dogs as compared to $6\frac{1}{2}$ days for 39 non-pregnant females. The observed difference is of no significance in view of the marked variation of the individual results from the mean. Sisson and March⁵⁷⁷ report that female rats survive longer than males, but their conclusion is based on the results obtained on only 11 animals. In a series of 100 consecutive operations on immature rats selected at random from the data of Firor and the author the average survival for male animals was 6.0 days as compared to 6.1 days for the females. It is possible that the

survival of rats adrenalectomized during estrus is longer than those operated during diestrus but this has not been proven.

ADRENALECTOMY IN VARIOUS ANIMAL SPECIES

Fishes. Adrenalectomy in fishes has a particular interest because of the separation of the interrenal and chromaphil tissues as distinct organs. They thus offer a natural separation of the compound organ of the higher animals.

Pettit⁴⁹³ performed the first operations on eels but his extirpations were only partial and were intended to demonstrate hypertrophy in the remaining fragments. Biedl⁵⁶ extirpated the interrenal bodies of the torpedo. He observed expansion of the melanophores, a gradually increasing muscular weakness which progressed until the animals could no longer turn, muscular convulsions, opisthotonus, a decrease in the respiratory rate, and death in 3 to 7 days. These experiments demonstrated the indispensability for life of the interrenal bodies in this species. In the eel, on the other hand, Vincent⁶⁴⁷ found that the extirpation of the interrenal bodies did not result in death but Giacomini's²¹⁸ demonstration of accessory interrenal tissue in this species explains Vincent's results.

Recent observations³⁵³ have confirmed the earlier work of Biedl. The difficulties involved in performing interrenalec-tomy in fishes render the observed results open to the objection that they are to be attributed to operative shock. Maintenance of the adrenalectomized fish with a supply of the cortical hormone until it has recovered from the operation would remove the basis for this objection.

Frog. Adrenalectomy in frogs is difficult to perform due to the close attachment of the adrenals to the kidneys (Figure 1). Complete removal of the glands, which is best accomplished by the thermo-cautery, necessarily involves renal injury. The operation, first performed by Abelous and Langlois³ in 1891, has been carried out by many subsequent observers. The

animals do not survive the operation well during the summer, when they die within one to three days, but during the winter months the animals survive for one to three weeks if maintained at a low temperature.³⁰⁰ This seasonal variation is also observed in other cold blooded animals. One might expect that the depressed metabolism of hibernating periods would lead to less rapid deterioration of the organism than during the period of bodily activity. The survival period is markedly shortened if the poikilothermic animal is subjected to an elevated external temperature.

Adrenalectomy in frogs leads to a train of symptoms similar to those observed in the mammal. The animals in insufficiency are easily fatigued. There is incoördination and weakness of the hind legs and paresis involving first the flexor and adductor and finally the extensor muscles. Eventually the fore limbs are also affected. Death is preceded by marked edema, which is to be attributed probably to renal injury since the close apposition of the adrenals to the kidneys renders adrenalectomy without serious renal injury virtually impossible.

Abelous and Langlois³ noted that the length of survival of their frogs was inversely proportional to the intensity of their metabolism. Exercise, for example, hastened the onset of the final stages of insufficiency. These authors also experimented with some success on the prolongation of life by the use of grafts⁴ and by the use of saline extracts of adrenal glands. They found the blood of their animals dying of insufficiency to manifest toxicity when injected into normal animals.⁵

Adrenalectomy in frogs is best performed through a lumbo-dorsal incision. A fine cautery is used to destroy the glands. Since no capsule separates the adrenal from the kidney one can not avoid injuring the latter organ.

Birds. Adrenalectomy in birds was first performed by Gourfein²³⁵ in 1896. He found the operation in pigeons to be fatal within 4 to 24 hours. If one-eighth to one-tenth of one

of the organs was allowed to remain, the birds survived for 15 days. The adrenals in birds are so situated as to make the operative approach exceedingly difficult. Subsequent workers have consequently avoided the use of birds although much of interest might be learned by studying the effects of adrenalectomy in this species.

Gourfein extirpated the glands through an incision extending obliquely from the caudal tip of the sternum to the ischium. His birds recovered from the operation but soon began to manifest muscular weakness and died usually within a few hours.

Rat and mouse. The advantages and ready availability of the rat (*Mus norvegicus*) as an experimental animal have made it the subject of many studies on the effects of adrenalectomy. The older view that the rat differs from other mammals in being able to withstand the effects of adrenalectomy due to the presence of widespread accessory tissue has been thoroughly disproven in recent years. Jaffe³³² showed that of 90 adrenalectomized rats, 35 per cent died within 30 days of the operation, the majority dying before the 13th day, with the typical symptoms of acute adrenal insufficiency. Forty-six per cent showed chronic insufficiency and died within seven months without the presence of gross accessories being manifested at autopsy. In the remaining 19 per cent, which were unaffected by the operation, large accessory cortical bodies were found at autopsy. Pencharz, Olmsted, and Giragosintz⁴⁹¹ proceeded further in their analysis and concluded that the rat is no exception to the rule that insufficiency and death invariably follow a complete adrenalectomy. In 62 rats death occurred in from two to eighteen days with no survivals. If only 5 per cent of the gland were left at operation, rapid regeneration occurred and the animal survived. Subsequent workers¹⁹⁸ have confirmed these results and demonstrated that the rat is no exception to the general rule that adrenalectomy is fatal. Survival is due to incomplete extirpation, which

consists either in allowing cortical remnants of the main glands to be left or in failing to remove the scattered accessory nests of cells present in the peritoneal tissue in the neighborhood of the glands. In every case of indefinite or prolonged survival, microscopic examination has revealed the presence of hypertrophied cortical tissue in this region. The assumption of the existence of widespread accessory tissue in other parts of the body is entirely unnecessary (*cf.* Chapter II).

The description of the operative procedure for adrenalectomy in the rat shall be deferred to Chapter XVII where it shall be described in connection with the assay of the cortical hormone.

Adrenalectomy in the mouse and its consequences do not differ from those observed in the rat and require no special description.¹⁹⁸ The mouse is a useful experimental animal involving studies on the androgenic zone which it possesses (Chapter IV).

Rabbit. The rabbit, like the rat, has been the subject of much experimentation and controversy regarding the outcome of adrenalectomy. Until recently it has been the general opinion that a large percentage of rabbits survive the operation due to the widespread occurrence of accessory bodies. This view, however, as in the case of the rat, is erroneous and explicable by the great difficulty of performing a complete extirpation without so much manipulation and hemorrhage as to kill the animal by operative shock.

The rabbit's adrenals are very delicate and may be easily torn. Such detached fragments may hypertrophy and explain some of the survivals by "accessory bodies" reported in the literature.¹⁹⁸

The left gland lies about a half of a centimeter above the upper pole and about one centimeter medial to the inner border of the kidney. It lies in a hollow between the *psaos* and *quadratus lumborum* muscles, in the angle formed by the vena cava and the large vein which drains the lumbar muscles. The right gland lies two to three centimeters cephalward of the

left in an angle between the vena cava and the right renal vein. It is partially covered by the vena cava.

The above description makes it evident why adrenalectomy is so unsatisfactory in the rabbit. Many of the animals die within 24 hours due undoubtedly to the shock of the operation. Many never recover from the anesthetic.

In a series of rabbits which recovered from the operation the following survival periods were noted by Firor and Grollman¹⁹⁸ after a one-stage operation under ether:

Number of rabbits.....	13	8	2	1	1	2	3	1	1	1	1	1	1
Survival period in days....	Less than 1	1	2	3	4	6	7	9	11	18	65	68	Over 120

Ninety per cent of the animals survived 11 days or less. In these, there were the usual manifestations of adrenal insufficiency,—progressive loss of weight, subnormal temperature, anorexia, and a liability to suffer death from apparently minor causes. The act of catching the rabbit would often throw it into convulsions similar to those which follow an overdose of insulin. All the rabbits were autopsied and serial sections made of suspicious residues in the vicinity of the normal site of the adrenal glands. In the rabbits surviving 9 days or less no adrenal tissue was demonstrable. In the rabbit surviving 11 days no adrenal tissue was seen at autopsy, but microscopic examination revealed a small piece of typical adrenal cortex. The rabbit surviving 18 days had a small bit of adrenal tissue in the peritoneal fascia. In the rabbits surviving 65 and 68 days a glandular mass the size of a normal adrenal gland was found. These had not been present at the first operation and represented, therefore, an active regeneration of tissue left at operation. In the rabbit surviving 120 days a well formed cylindrical mass of cortical tissue about 3 mm. in diameter and 6 mm. long was found at autopsy in the

interior of the abdominal vena cava. Whether this was its original site or whether it developed from cellular masses accidentally implanted during resection of the vessel is debatable.¹⁹⁸

The above described results indicate that the generally accepted view regarding the frequency of the existence of scattered cortical tissue in the rabbit is erroneous. Complete removal is difficult; but it is unnecessary to assume that tissue capable of supporting life exists along the vena cava and is diffusely scattered throughout the dorsal peritoneal spaces or in the reproductive organs, as claimed by a number of investigators.

Guinea pig. The guinea pig is ill-suited for adrenalectomy. It is extremely sensitive to any surgical manipulation and hemorrhage, and hence in the hands of most experimenters death has followed adrenalectomy in this species within a few hours to a day following operation. To add to the difficulty, the right gland is intimately connected to the vena cava. As in the rabbit, one risks a fatal hemorrhage or the possibility of leaving glandular tissue behind, which, in surviving animals, hypertrophies and allows extended survival. The guinea pig like the rabbit should be avoided for experiments involving a complete adrenalectomy.

Dog. The dog, because of the many advantages it offers as an experimental animal, has been the subject of numerous studies on the effects of adrenalectomy. It is comparatively easy to perform the operation so as to remove all the gland. Accessory tissues are rarely present or, at least, are removed with the connective tissue surrounding the main glands, for rarely do the animals survive for extended periods as in the case of the rabbit or rat.

Adrenalectomy in dogs is best performed by the lumbar approach. The smaller species are more easily adrenalectomized than large fat animals in which one has to penetrate

a thick layer of muscle and fat before reaching the glands. As already pointed out, the use of spinal anesthesia leads to a more prolonged survival than the use of ether. The objection to the use of spinal anesthesia is the fact that pain impulses are still elicited on manipulation of the splanchnics and their associated ganglia. Some authors have recommended the use of nembutal as an anesthetic.²⁶⁷ Judging from the experimental data on rats the barbiturates should be preferred to ether in adrenalectomy.

Strict aseptic technique is necessary in adrenalectomy on dogs, cats, and other animals susceptible to infection. The increased susceptibility of the adrenalectomized animal to infection renders strict asepsis more imperative in this operation than in other experimental surgical work.

The adrenals are extirpated through an incision, 2 to 3 inches long, extending slightly ventrally and distally from the angle of the last rib. The lumbar muscle is retracted and the ventral layer of the dorsal fascia exposed and slit, exposing the retroperitoneal tissue behind the kidney. The large vein which runs outward from the midline over the adrenal is dissected free, clamped, and followed to the gland. Since the adrenals are fragile, care must be exercised not to grasp the gland. After the gland has been dissected free, the pedicle is ligated and the adrenal removed. Any point of hemorrhage must be carefully ligatured. The wound is closed in the usual manner.

The most complete study of the survival period of adrenalectomized dogs is that of Rogoff and Stewart.⁵²⁸ In a two-stage operation under ether anesthesia, they found that about two-thirds of their 74 animals lived from 4 to 9 days after the second adrenalectomy. About 10 per cent of the animals lived 10 to 12 days while 2 animals survived until the fifteenth day. About one-sixth of the dogs survived less than 5 days. The average survival of all their animals was about 7 days.

The survival of dogs after a single-stage adrenalectomy under spinal anesthesia is indicated in the following table of Firor and his collaborators:²⁵¹

Days following operation.....	1	2	3	4	5	6	7	8
Number of animals dying on day indicated.....	4	6	5	4	3	5	2	1

Cat. The ready availability of the cat and the relative ease with which its adrenals can be extirpated have made it a favorite animal for this type of experiment. The classic study of adrenalectomy and its effects in the cat is that of Elliott.¹⁸⁰ He removed the glands of 25 animals in stages with an interval of 3 to 9 months between the two operations and observed a survival which ranged from 6 to 23 days. Elliott avoided the splanchnic nerves in his operation.

Cats withstand the effects of adrenalectomy well and their survival period is usually much longer than it is in dogs. The glands are situated in less intimate contact with the large vessels than they are in the dog and hence are more easily removed. Extraperitoneal excision through a lumbar incision as in the dog is to be preferred, for much less trauma is inflicted by this approach than when the glands are removed through an abdominal incision.

Even after extirpation under ether anesthesia and by a one-stage operation, most cats survive 3 to 7 days when the operation is properly performed. The use of a more satisfactory anesthetic and extirpation in two stages should give much longer survivals. The average survival of cats after a two-stage operation was found to be 11 days by Rogoff and Stewart.⁵³⁴ Swingle and Pfiffner⁶¹³ found an average survival of 7.7 days while Britton in the single stage operation reports an average survival of 7.5 days.⁸⁵

Other mammals. Adrenalectomy has been performed in a few cases in monkeys. In these animals, the adrenals are

easily accessible through a posterior extra-peritoneal approach and may be readily extirpated. Kahn³⁴⁵ first performed this operation in a monkey and found that the course of the resulting insufficiency was similar to that observed in other mammals. Recent investigators have confirmed Kahn's results.¹⁹⁸ The reported development of pigmentation in a monkey maintained on an apparently inadequate dose of the cortical hormone²⁶⁷ would indicate that this species may be useful in studying this phenomenon which is so striking a symptom in the adrenal insufficiency of man.

Moore and Purinton⁴⁵⁹ performed adrenalectomy in several goats. Their results indicate an exceptionally long survival in this animal. Since the goat is unusually well adapted for studies of the metabolism, blood chemistry, circulation, *etc.*, it should prove superior to the more common laboratory animals for many experimental studies on adrenal insufficiency.

The results of adrenalectomy in the opossum (*Didelphys virginiana*), the squirrel (*Sciurus carolinensis*) and the marmot (*Arctomys monax*) do not differ materially from those observed in the cat or dog.⁸⁶

THE RÔLE OF ACCESSORY BODIES

Death may often occur following adrenal insufficiency even in the presence of accessory tissue. Examination of such tissue at autopsy will show interstitial fibrosis, vacuolization, infiltration of giant cells, and, in general, a picture indicative of "exhaustion atrophy."³³⁵ Apparently under certain conditions the accessory tissue or remnants of the main gland are unable, due to inadequate blood supply, perhaps, to undergo hypertrophy. Consequently they function for a short while but eventually become exhausted. Such exhausted tissue is often encountered in rats dying of chronic insufficiency several months after adrenalectomy. Accessory tissue in cats or dogs is often incapable of maintaining life after adrenalectomy. However, maintenance of such animals for a week or more on the

cortical hormone will permit the hypertrophy of these accessories or fragments of the main gland left at operation and subsequent survival of the animal. Failure to appreciate this fact has undoubtedly led to many of the erroneous claims of successful replacement therapy. Only by careful microscopic observation of the tissues at the adrenal sites can one determine the completeness of a given operation.

The oft-quoted claims of Wiesel,⁶⁷⁶ regarding the occurrence of accessory bodies in the rat in the region of the epididymis, has never been confirmed by subsequent investigators.^{198, 335} Wiesel claimed to have proven functional capacity of the epididymal accessory bodies by performing left-sided adrenalectomy in 10 young male rats which were killed 3 to 12 weeks later. The normally microscopic accessories in the region of the epididymus were found to be hypertrophied to the huge size of 5 mms. As pointed out by Jaffe,³³⁵ it is doubtful if such hypertrophy ever occurs in accessories after unilateral adrenalectomy because compensation is rapidly effected by enlargement of the remaining gland. Moreover, in several hundred rats of different ages, Jaffe³³² was unable to demonstrate by careful microscopic section any accessories in the region of the testes, vas, or epididymis, in either normal or adrenalectomized rats. Firor and the author have confirmed Jaffe's findings.¹⁹⁸

According to Jaffe gross accessory bodies are found in about 8 per cent of normal rats and in 20 to 25 per cent of doubly adrenalectomized rats, but these are always situated near the main glands. By adopting the technique described in Chapter XVII, one can ensure the extirpation of these accessories together with the main glands.

RELATIVE IMPORTANCE OF CORTEX AND MEDULLA

That it is the cortex and not the medulla which is indispensable for life has been demonstrated by a number of investigators. Biedl,⁵⁶ Wheeler and Vincent,⁶⁷⁰ and Crowe and

Wislocki¹⁴¹ excised the left adrenal of cats, rabbits, and dogs, removed half of the right gland, and cauterized the medullary substance in the remaining half of the gland. Subsequent histological examination of the adrenal tissue revealed in some cases complete absence of any medullary tissue. Nevertheless the animals survived for long periods in good health. Pende⁴⁹³ and Stewart and Rogoff⁵⁸⁷ excised one adrenal and denervated the remaining gland. Although no epinephrine could be detected in the blood draining the remaining gland, the animals remained in good condition indicating that lack of epinephrine was not the cause of the deficiencies observed after adrenalectomy. Houssay and Lewis³¹¹ split the left adrenals of dogs, scooped out the medulla, and ten days later excised the right gland. Their animals remained entirely normal. At autopsy, histological examination of the adrenals revealed complete absence of the medullary tissue.

The extirpation of the interrenal bodies of the elasmobranch fishes leads, as we have seen, to an insufficiency resembling that which follows adrenalectomy in mammals. The fact that extracts prepared from this purely cortical tissue serves as a replacement therapy in the insufficiency of mammals which follows adrenalectomy is further evidence that it is the absence of the cortex which is responsible for these manifestations.²⁵⁰

It requires only a small remnant of the adrenal cortex to support life. As was first shown by Langlois,³⁸⁰ one-sixth to one-eleventh of the total cortex suffices to preserve life. According to Biedl,⁵⁶ about one-eighth is necessary; according to Wislocki and Crowe,⁶⁸⁵ about one-fifth; and according to Bornstein and Gremels,⁷⁰ one-fourth. From our previous considerations it is evident that the fraction necessary will depend upon the conditions observed in performing the operation. In fact in rats, as we have seen, even an invisible group of cortical cells may suffice after their hypertrophy to maintain normal life.

That it is the cortical tissue and not the medullary which is

responsible for the fatal outcome which follows adrenalectomy is now generally accepted as an incontrovertible fact. Many lines of evidence might be cited to support this conclusion. The fact that cortical extracts free of epinephrine even when administered orally maintain adrenalectomized animals in normal physiological condition is the most direct proof that absence of the cortical secretion alone is responsible for all of the manifestations which follow adrenalectomy. The possible importance of the medullary secretion has already been discussed in Chapter VI.

CHAPTER IX

PATHOLOGICAL PHYSIOLOGY OF ADRENAL INSUFFICIENCY

The normal physiological functions of animals in adrenal insufficiency are disturbed in many ways. Some of these pathological manifestations are the results of the anatomical and blood chemistry changes described in Chapters X and XI while the basis for others is still a matter of uncertainty.

In general, the physiological changes observed in experimental adrenal insufficiency are rather characteristic but certain minor differences occur depending upon the animal species investigated and upon the rapidity with which the insufficiency develops. Many of the phenomena to be described are not observed in animals surviving adrenalectomy for only a brief time. One must avoid confusion with the manifestations due to the surgical shock attendant upon adrenalectomy. The dysfunctions due specifically to absence of the adrenal cortex are best brought out by withdrawing the administration of the cortical hormone from adrenalectomized animals maintained on adequate doses of the hormone until the original operative wounds have healed. In this way the onset of insufficiency is delayed and one has the opportunity of observing the symptomatology and physiological variations occurring in uncomplicated cortical insufficiency.

In dogs surviving less than fifty hours, Banting and Gairns³¹ observed the following symptoms before the onset of the coma which precedes death:

Rapid pulse	Loss of appetite
Elevated temperature	Terminal fall in blood sugar
Thirst	Anuria
Restlessness	Vomiting
Salivation	Diarrhea
Weakness	Convulsions

Some of these symptoms (*e.g.*, the elevated temperature, thirst, and restlessness) are probably due to operative shock and are not observed in animals surviving for longer periods except for the first day following operation.

In completely adrenalectomized animals, after recovery from the anesthetic, one notes no obvious abnormal manifestations. The animals are alert, eat and drink, and are apparently in perfect condition. The blood pressure is normal; the constituents of the blood undergo no noteworthy change. Gradually, however, symptoms of insufficiency supervene. The animal becomes apathetic, refuses food, and may vomit. Muscular movements become slow and uncertain. Weakness of the hind legs develops which may cause the animal's gait to become unsteady. Eventually the animal lies prostrate. As insufficiency progresses, the body temperature falls in warm-blooded animals. The skin becomes cold; the mucosae turn pale. Pus may appear in the eyes and the pupils become dilated. Muscular twitches and convulsions may occur. The respiration is first rapid, then slow. There may be anuria. Death occurs in coma, with respiratory paralysis while the heart is still beating.

The delay in the appearance of the symptoms of insufficiency following adrenalectomy may be attributed to two factors: 1, A store of the vital cortical hormone may be present in the body and it is only after the exhaustion of this store that disability ensues; 2, The tissues are able to function normally for a time without the hormone. Extraction of various tissues, such as liver, pituitary, corpora lutea, *etc.* demonstrates the presence of small amounts of a substance capable of maintaining life in the adrenalectomized animal. It is likely therefore that a store of the hormone is left in the organism after adrenalectomy. The effect of adverse physical conditions, toxins, trauma, *etc.* in precipitating an adrenalectomized animal into insufficiency indicates, however, that the second of the above listed possibilities is also a factor in explaining the survival of animals in good health for some time after adrenalectomy.

GASTRO-INTESTINAL DISTURBANCES

Anorexia is one of the earliest clinical symptoms of adrenal insufficiency in dogs, cats, rabbits, and man. In the rodents some food may be ingested shortly before death but the food and fluid intake is markedly reduced with the advent of insufficiency. Usually at the first sign of disinclination to take food, an animal may be tempted by offering it some delicacy. As the insufficiency progresses there is finally a complete aversion to all food. If the animal survives for some time, it may become very thin due to inanition.

The immediate cause of the anorexia of adrenal insufficiency is still undecided. It may be due perhaps to the inflammation, congestion, or ulceration of the gastro-intestinal tract or it may be secondary to the disturbances in the composition of the blood and the accumulation of the nitrogenous waste products of metabolism. Harrop and his co-workers²⁶⁸ suggest that the anorexia may be due to the absence of free hydrochloric acid from the stomach.

Vomiting is a very common symptom in adrenal insufficiency, particularly in dogs. The animal may be unable to retain any food and the introduction of small amounts of fluid through a stomach tube may be followed in a few minutes by its ejection. The vomitus usually contains bile and may be blood stained.

Watery or bloody diarrhea is a frequent symptom of adrenal insufficiency, particularly in dogs and rabbits, less commonly in cats or rats.

ASTHENIA AND MUSCULAR WEAKNESS

As insufficiency develops animals become more and more apathetic. They ultimately refuse to move or take an interest in the surroundings. Animals which preoperatively were active or ferocious may appear gentle after adrenalectomy due to their disinclination to indulge in any activity.

A characteristic gait results from the weakness of the hind legs when the insufficiency has progressed to a moderate degree.

At first there is a spasticity of the legs. The animal walks unsteadily, lurches from side to side, and may fall. Weakness of the hind legs may progress until they can no longer be moved and the animal drags itself on the floor. It finally lies feebly on its side with limp legs and its head hanging low.

The muscular weakness of adrenal insufficiency is not due to lack of intrinsic muscular power, for even in moribund animals the muscles can be stimulated to normal activity.¹⁸⁰ However, after incomplete adrenalectomy in rats, one observes a marked asthenia and diminution in the body activity.¹⁶⁷ The isolated muscles of animals in insufficiency have been shown to be more easily fatigued than those of normal animals. This may possibly be due to an abnormal accumulation of lactic acid in the stimulated muscle of adrenalectomized animals or to their carbohydrate impoverishment.³⁷⁷ Intravenous injection of epinephrine, by raising the blood pressure in moribund animals, will often cause a disappearance of the extensor rigidity which characterises the late stage of insufficiency. There is thus no interference with the control of the skeletal muscles by the central nervous system in adrenal insufficiency and the observed prostration and muscular weakness are not attributable to a paralysis of the skeletal muscles or their nerves.¹⁸⁰

BODY TEMPERATURE

In adrenal insufficiency the body temperature gradually drops⁴⁴⁰ reaching values which may be lower than 32°C. at death (Figure 10). During the first hours after adrenalectomy or after a sub-lethal injury to the adrenals there may be an elevation of the body temperature (*cf.* Chapter XIII). These changes in the body temperature are reflections of corresponding alterations in the basal metabolic rate which shall be discussed in greater detail in Chapter XIII.

RESPIRATION

Hyperpnoea is a common symptom in the early stages of adrenal insufficiency. Some authors⁷¹ have attributed to this

hyperpnoea a particular importance in producing the manifestations of insufficiency. However, this view is not tenable for it is only in animals dying soon after adrenalectomy that one observes any unusual hyperpnoea. Thus in one of Bornstein and Holm's⁷¹ experiments the basal respiratory minute volume of a dog rose from 2.53 liters per minute to 7.19 liters, with a drop in the CO₂ tension of the alveolar air from 4.27 per cent

FIG. 10. CHANGES IN VARIOUS PHYSIOLOGICAL FUNCTIONS IN ADRENAL INSUFFICIENCY

Composite records of results obtained on dogs adrenalectomized in stages. The animals received no therapy during the period of observation and died seven days following the removal of the second gland.

to 1.18 per cent; the body temperature fell from 38.2° to 35.7°; the oxygen consumption fell from 109.6 cc. to 82.5 cc.; and death occurred 7 $\frac{3}{4}$ hours after the operation. The changes observed in this case must be attributed in great part to operative shock.

In animals which have survived for some time and then gone into insufficiency, the respiration is usually slow and not easily

stimulated by factors such as an increase in room temperature or an injection of epinephrine. The respiratory rate in dogs may drop to 5 or 6 per minute. Respiratory paralysis is the immediate cause of death, for the heart continues to beat after the cessation of respiration.

CIRCULATION

The circulatory system manifests no signs of dysfunction in the early stages of adrenal insufficiency. The blood pressure remains normal and there is no sign of cardiac decompensation.

When the first clinical signs of insufficiency are evident, the pulse rate becomes rapid and may be irregular. Later, when the clinical symptoms of insufficiency become pronounced, the pulse rate becomes slowed and often assumes an idioventricular regular rhythm or an irregular rhythm with the manifestations of some degree of heart block.

Electrocardiographic studies by Nicholson and Soffer⁴⁷¹ demonstrated the existence in adrenalectomized dogs of a slow auricular fibrillation. This would be expected to lead first to a partial heart block and eventually to a complete block with the assumption by the ventricle of its idiopathic rhythm. This disturbance in cardiac function may be due to the elevation of the potassium content of the blood and the disturbance in the normal *potassium-calcium* ionic ratio. Such a disturbance would explain also the earlier observations of Loewi and Gettewert⁴⁰¹ on the hearts of frogs dying of adrenal insufficiency.

The blood pressure does not fall appreciably until a short time before death. The cardiac output, on the other hand, according to Harrop *et alii*,²⁶⁸ diminishes progressively after the initiation of the first symptoms of insufficiency. This drop in cardiac output may be looked upon as a reflection of the decreased metabolic activity of the organism and not necessarily indicative of cardiac insufficiency.²⁴³

Two chief factors are probably responsible for the circula-

tory disturbances in adrenal insufficiency. The abnormal composition of the blood will affect the nutrition and rhythmicity of the heart and thus lead to its inefficiency, while the decrease in the blood volume will vitiate the hemodynamic relationships which make possible normal circulatory activity. The latter factor is undoubtedly of greatest importance and is probably responsible for the ultimate drop in blood pressure.

THE BLOOD

Changes in the blood are an important and frequent observation in animals in adrenal insufficiency. There is a loss of fluid from the blood stream which results in a concentration of the formed elements so that the relative volume of the red blood corpuscles as determined by the hematocrite may rise considerably. That this represents an actual loss of fluid from the blood stream and not merely its transferral from plasma to corpuscles is shown by the concomitant increase in the number of red blood corpuscles, which is approximately proportional to the observed increase in their volume as determined by the hematocrite and by the increase in the total solids of the plasma (Figure 10).

The differential leucocyte count in adrenal insufficiency is also altered from the normal.¹²⁶ Thus in cats, Zwemer and Lyons⁷⁰³ found a decrease in the relative number of polymorphonuclear neutrophils with a corresponding increase in the number of small mononuclear lymphocytes, similar to that observed clinically in chronic wasting diseases. The above described blood picture may be obscured by shock or infection.

Several investigators have attempted to measure the decrease in blood volume during adrenal insufficiency. Unfortunately the dye methods utilized for this purpose give entirely unreliable results, as might be anticipated from a simple consideration of the basis for the application of these methods. In normal animals one allows a given length of time (empirically determined) to elapse before drawing the blood sample in

which the concentration of the injected dye is determined. If the period of time is too long, the dye will be lost from the circulation; if too short, it will not mix properly with the blood. In adrenal insufficiency, the circulation is slowed and hence one is not justified in using the same period of time as one does in normal animals. We must therefore disregard the extant data on the blood volume in adrenal insufficiency and rely on the other findings which indicate a decrease in this function.

ALTERED SENSITIVITY

Animals in adrenal insufficiency are hypersensitive to many extraneous influences. This abnormal sensitivity to toxins and infections will be considered in detail in Chapter XVII. Insufficiency also renders animals hypersensitive to such influences as heat or cold, muscular exercise, emotional disturbances, and the like.

Adrenalectomized animals are unable to maintain their body temperature when cooled. Thus de Marval⁴⁴² found that on subjecting rats to an external temperature of 10°C. their rectal temperature dropped 5.9° as compared to 2.5° for normal animals. The metabolic rise, which normally follows subjection to cold, does not occur in adrenal insufficiency.²¹⁹

One can markedly reduce the survival period of adrenalectomized animals by subjecting them to extremes of temperatures. Animals which normally survive for a week can be thrown into an acute and fatal insufficiency by subjecting them for a few hours to temperatures which the normal animal can endure with impunity.²⁷⁹

Adrenalectomized animals are unable to withstand severe muscular exercise and are easily precipitated into insufficiency by exertion. A brief period of struggling may bring on the final stages of insufficiency.

The adrenal cortical hormone is essential for the normal activities of the organism and hence an excessive manifesta-

tion of these activities probably depletes the residual supply of hormone still present in the body of the adrenalectomized animal. The fact that animals survive for sometime after adrenalectomy indicates either that their life is maintained during this period by the supply of hormone left in the tissues at operation, or that these tissues can carry on their activities for some time without the hormone. In either case, excessive activity of any kind will either deplete the store of available hormone or hasten the ultimate collapse of the organism. We should expect therefore that all influences tending to stimulate the activity of the organism should hasten the onset and aggravate the insufficiency of the adrenalectomized animal.

Although hypersensitive to many extraneous and toxic agencies, animals in adrenal insufficiency react sluggishly or not at all to many stimulants. In the moribund cat, as Elliott¹⁷⁷ showed, there is an apparent paralysis of the vaso-motor and cardio-accelerator nerves. Other nerves react in an approximately normal manner. Thus nicotine has no pressor action in cats dying of cortical insufficiency even after preliminary atropinization.¹⁸⁰ This apparent paralysis of the vaso-motor nerves is actually due to a change in the non-striated muscle for pituitary extract and barium chloride have little or no effect on the blood pressure. Epinephrine, however, still causes pressor and cardio-accelerator effects. The vaso-motor nerves thus appear to be paralysed because the muscle cannot respond to their excitatory impulses. Hence stimulation of the splanchnics or injection of nicotine cause little rise in blood pressure. As Elliott pointed out, the paralysis of the vaso-motor nerves is not due to shock for in moderate shock there is little paralysis.

It is not surprising that the physiological response of animals in adrenal insufficiency should be less pronounced than it is in normal animals. In insufficiency we are dealing with an animal, the vital processes of whose organs and tissues are at a

low ebb. Severe infectious processes, malnutrition, avitaminosis, and similar debilitating agencies also render an animal less reactive to many forms of stimulation.

REPRODUCTIVE SYSTEM

The reproductive function of animals in adrenal insufficiency is in abeyance.^{439, 689} Although manifesting normal sexual activity soon after adrenalectomy, this ceases when the symptoms of insufficiency are manifest. Male animals become impotent and females remain in a state of permanent diestrus. These changes have been studied in detail in rats but similar changes occur in the larger mammals. This dysfunction of the reproductive system shall be described in greater detail in Chapter XIV.

LIVER AND KIDNEY

Aside from the pathological manifestations described in the preceding sections, functional tests will reveal dysfunction of other visceral organs. Thus the liver is probably affected judging from the abnormalities in the carbohydrate metabolism described in Chapter XII. Whipple and Christman²⁶⁹ found the excretory power of the liver for the excretion of phthalein dyes to be reduced by the removal of three-fourths or more of the adrenal tissue.

The kidney⁵⁴⁹ likewise manifests an inability to excrete injected urea or creatinine⁴³⁸ and, as we shall see in Chapter XI, the changes in the inorganic constituents of the blood in adrenal insufficiency are most likely due to renal dysfunction.

It must be emphasized that all the physiological dysfunctions described in the preceding sections do not manifest themselves immediately following adrenalectomy. For some time the animal may be entirely normal. It is only when the supply of cortical hormone present in the body has been exhausted that insufficiency sets in with its attendant manifestations.

It may rightfully be argued that many of these manifestations are merely terminal events which are encountered before death from many causes. Thus a *pre-mortem* drop in blood pressure is commonly seen in many conditions. The same may be true of some of the other symptoms which develop late in insufficiency and which may be secondary to the primary disturbance caused by removal of the vital hormone of the adrenal cortex. Until we know definitely how the hormone acts in the body it is impossible to form a clear concept of the sequence of events which in their totality give the picture of adrenal insufficiency. This picture will vary in individual cases depending upon the particular organs most affected in any given animal. Thus, whereas the immediate cause of death in a given dog may be due to circulatory failure attributable to a diminished blood volume, hypoglycemia due to hepatic injury may be the chief factor terminating life in a given rat. In both cases other deficiencies contribute to the final fatality.

CHAPTER X

ANATOMICAL PATHOLOGY OF ADRENAL CORTICAL INSUFFICIENCY

Considering the marked and diverse symptoms manifested during life in adrenal cortical insufficiency, one is surprised at autopsy to find relatively little in the nature of gross pathological changes. A radical dysfunction of an organ may apparently be undetected anatomically, particularly when the disability is acute in its onset. In acute adrenal insufficiency, too short a time elapses for the development of gross changes which are evident by the methods of anatomical pathology.

Pigmentation of the skin which is a striking (although not an essential) symptom of adrenal insufficiency in man, has not been reproduced with certainty in the experimental animals. Some of the earlier investigators (Brown-Séquard,⁹⁷ Nothnagel,⁴⁷⁴ Tizzoni,⁶²⁸ and others) described the deposition of pigment in the skin or blood of animals the adrenals of which had been injured, but subsequent workers have failed to substantiate these claims. Harrop and Weinstein²⁶⁷ have recently reported some slight pigmentation in an adrenalectomized monkey maintained for some weeks with the cortical hormone. To attain conditions comparable to those obtaining in Addison's disease it is necessary to maintain animals in a state of chronic insufficiency for a long period of time and subject their shaved skin to the sunlight. Thus far such attempts have been futile (*cf.* Chapter XXI). It is possible that man (and possibly the ape) is unique in that some property of his epidermis permits the deposition of pigment.

The blood in animals dead of adrenal insufficiency is often thick and dark due to loss of fluid from the blood stream and incomplete oxygenation during the last moments of life. The

hemoconcentration, as we shall see in Chapter XI, is probably due to loss of fluid through the kidneys due to tubular injury which prevents reabsorption of water and threshold substances. The tissues also appear to be dehydrated showing that there has also been a loss of fluid from the tissue spaces. Obviously the loss of fluid from the circulation can not have been due to damage of the capillary endothelia with a consequent inability to retain fluid, as has been claimed; for if such were the case one would observe an edematous swelling rather than dehydration of the tissues in adrenal insufficiency.

In animals which have been maintained for sometime in a state of cortical insufficiency, one notes at autopsy an almost complete disappearance of the body fat. The anorexia seen in insufficiency leads to starvation with the depletion of the reserve stores of fat and carbohydrate of the body.

GASTRO-INTESTINAL TRACT

The outstanding pathological finding at autopsy of animals dying of acute adrenal insufficiency is a marked hyperaemia of the visceral organs—stomach, intestines, liver, spleen, kidney, pancreas, thymus, and hypophysis. Frequently, hemorrhages into the gastro-intestinal canal are prominent and ulceration may be visible. There may be submucosal ecchymoses in the intestinal tract. The gum margins are often congested and bluish and ulcers may be found under the tongue.³¹

Lesions of the gastric mucosa are found in a large percentage of rats, mice, cats, and dogs dying of adrenal insufficiency. There may be an injection of the mucosa, widespread superficial erosions, or true punched-out ulcers.¹⁹⁷ The ulcers develop at the site of local hemorrhages in the gastric mucosa and penetrate to the muscular coat of the stomach with complete destruction of the mucosa. The ulcers as observed in the dog⁴²³ are round or oval, 2 to 20 mms. in diameter, and are usually multiple although at times a single ulcer may be encountered. They are cone-shaped with the base at the sur-

face. The gastric lesions develop, as Mann⁴²³ showed, in the absence of pancreatic secretion and bile and hence are not due to regurgitation of these fluids.

Although at times the entire gastro-intestinal may be congested, the chief sites of congestion are the stomach, duodenum, and rectum. Hemorrhages from the inflamed rectal mucosa give rise to the bloody diarrhea observed in insufficiency, particularly in the dog.

At autopsy one often finds bile in the stomach. The intestines are sometimes filled with a brownish watery fluid which may contain blood.⁵²⁸

LIVER

Banting and Gairns³¹ found much degeneration in the liver cords of dogs dying of adrenal insufficiency. This degeneration varied in degree from slight changes to actual necrosis of the cells. There was also great vascular congestion and hemorrhage which was more marked in areas about the central vein with pressure changes in the liver cords and fatty degeneration. This degeneration had proceeded in the longer surviving animals to a point where little normal tissue remained.

The above described changes in the liver would explain the results of the liver function tests observed in adrenal insufficiency and account in part perhaps for the observed abnormalities in carbohydrate metabolism (Chapter XII).

KIDNEY

Although many authors have failed to note any obvious pathology of the kidneys in animals dying of acute adrenal insufficiency, some have described definite changes—particularly an increased lipid content of the renal cortex. Such an increase in the lipoidal content of the cortex of the kidney with congestion of the pyramids was found by Hartman and his collaborators.²³⁰ There was a high-grade, general hyperemia involving chiefly the cortex with swelling and vacuolization of the epithelium of the *tubuli cortorti*. In frozen sections stained

with Sudan III large quantities of lipoidal substances were found. These lipids were in the form of droplets in the tubular epithelium and corresponded to the vacuoles which were observed in paraffin sections. Banting and Gairns³¹ also described hyperemia of the kidney, swelling of the tubules, and albuminous casts containing blood cells in the lumina of the tubules in dogs which had died of adrenal insufficiency.*

LYMPHATIC SYSTEM

Hypertrophic changes in the lymphatic system associated with loss of adrenal cortical function due to disease or experimental ablation have been frequently observed.³⁵ These changes in the lymphatic tissues were first noted in Addison's disease. During life one may observe enlargement of the lymphatic nodules at the base of the tongue and of the tonsils and other palpable lymphatic tissues. At autopsy there is marked enlargement of Peyer's patches and isolated follicles in the gastro-intestinal tract and mesentery. Star⁵⁵ first noted the persistence of the thymus in patients dying of Addison's disease. The spleen often shows a moderate enlargement with hyperplasia of the follicles. Indeed, the general lymphatic enlargement sometimes seen in Addison's patients resembles that of the so-called "*status lymphaticus*."³⁵

Animals dying of chronic adrenal insufficiency also show a striking hypertrophy and regeneration of the thymus and lymphatic tissues generally. The changes in the thymus of the rat after adrenalectomy have been studied in detail by Jaffe.³¹ In young animals adrenalectomy stimulates the growth of the thymus while in mature animals active regeneration of the thymic tissue occurs. At autopsy, huge thymic glands are encountered. In larger mammals the short period of survival which follows adrenalectomy does not allow time for any regeneration of the thymus. In cats and dogs maintained for long periods on minimal doses of the cortical hormone, this hypertrophy is, however, very prominent.

The lymph nodes of animals in adrenal insufficiency are also

* Cf. also Jour. Path. and Bact. vol. 40, p. 483.

markedly enlarged. The immediate cause of this hyperplasia, like that of the thymus, is unknown. Banting and Gairns³¹ described an enlargement of the lymph nodes in dogs accompanied by a proliferation of plasma cells, endothelial leucocytes, enlarged macrocytes, and a great increase in the reticular tissue, with obliteration of the germinal centers. McMahon and Zwemer,⁴²⁰ on the other hand, describe the change in cats as consisting of an enlargement of the germinal centers which are filled with young lymphocytes and lymphoblasts showing active mitosis.

As to the significance of the *status lymphaticus* observed in adrenal insufficiency several possible explanations may be presented. Kahn³⁴³ suggested that it is a compensatory mechanism to counteract a primary injury to the myelo-erythroblastic system. Marine and Jaffe³³⁵ suggested that the hyperplasia of the thymus is a response to hyperthyroidism induced by adrenal insufficiency. It is true that in hyperthyroidism (as well as in hypothyroidism) one observes hyperplasia of the lymphatic tissues. However, as shall be shown in Chapter XIII, adrenalectomized animals maintained on an inadequate dose of cortical hormone show no signs of hyperthyroidism. In fact, they manifest the symptoms of a depressed thyroid function. It is consequently difficult to accept the hypothesis that the hyperplasia of the thymus is a response to hyperthyroidism induced by adrenal insufficiency. It would be more logical to ascribe the observed hyperplasia to the thyroid insufficiency which is secondary to chronic cortical insufficiency (Chapter XIII). However, until our knowledge of the exact function of the thymus and other lymphatic tissues is elucidated, it is futile to speculate concerning the significance of their hyperplasia in adrenal insufficiency.

PANCREAS

Congestion of the pancreas is one of the commonest findings at autopsy of animals dead of adrenal cortical insufficiency.

It is practically always found in dogs and rats. Its significance, however, has not been determined. It is questionable if the observed changes are of the nature of a true pancreatitis to which one might attribute some of the symptoms of adrenal insufficiency. Rogoff and Stewart⁵²⁸ describe the pancreatic changes as consisting of dilatation of the small vessels in the islets with marked congestion of the veins in the trabeculae.

CIRCULATORY SYSTEM

The heart shows no abnormality in most cases. In frogs dying of adrenal insufficiency Loewi and Gettwert⁴⁰¹ found the heart markedly dilated. In cats, Moore and Purinton⁴⁵⁹ observed the heart to be filled with clots formed during life. The increased coagulability of the blood in adrenal insufficiency may account for their occurrence, although in dogs or cats dead of insufficiency one often observes that the blood does not clot when shed.³¹

The blood vessels also manifest no obvious pathological changes. During life they are so markedly collapsed in the agonal stages of insufficiency that it is difficult to draw blood from them. This collapse is probably secondary to a combination of effects due to loss of blood volume, the weakened cardiac action, the hypotension, and possibly the stagnation of blood in the congested visceral organs.

CENTRAL NERVOUS SYSTEM

Although earlier observers described vacuolization of nerve cells particularly in the *medulla oblongata* and cerebral cortex, their animals died several hours after adrenalectomy and hence their observations can not be ascribed to uncomplicated adrenal insufficiency. There has been no recent work on the nervous system in insufficiency, and further studies in this field are indicated. It is questionable if the mental symptoms observed in adrenal insufficiency in animals or in man (Chapter XXI) are due to anatomical changes in the cerebrum or whether these

symptoms result from functional changes induced by the deranged blood chemistry which accompanies cortical insufficiency.

The changes found in the endocrine organs of animals dead of adrenal insufficiency shall be considered in Chapter XIV.

Despite the relative paucity of detailed studies of the anatomical changes occurring in adrenal insufficiency, it is evident that a number of tissues and organs manifest definite injury. The manifold nature of the symptomatology of adrenal insufficiency is indicative of a widespread involvement of many organs. The available experimental data is still too meager, however, to permit one to define accurately the relation of the various clinical symptoms to the different organs responsible for them and further work along these lines is indicated. It is desirable, however, in making such studies to maintain the experimental animals on an adequate dose of the cortical hormone after adrenalectomy until all effects of the operation have disappeared. In this way one avoids the effects of trauma and anesthesia and allows the animal to survive for a longer period during which the changes due to insufficiency can develop.

CHAPTER XI

THE BLOOD CHEMISTRY IN ADRENAL CORTICAL INSUFFICIENCY

The composition of the blood is characteristically altered in adrenal cortical insufficiency as experimentally produced in animals or as it occurs in Addison's disease. The chief alterations occur in the water, sodium, potassium, magnesium, glucose, chloride, non-protein nitrogen, and cholesterol content. There is thus a profound upset in the normal composition of the blood which will be reflected in the tissues and will produce marked physiological effects in the organism. A consideration of the changes in the glucose concentration of the blood will be deferred to the next chapter.

CHOLESTEROL

The normally high cholesterol content of the adrenals and the fact that changes in the cholesterol content of the adrenals are often associated with corresponding changes in the blood led earlier observers to consider the adrenals as regulators of the cholesterol metabolism. Feeding cholesterol results in an enlargement of the adrenals with an increase in their cholesterol content. Adrenalectomy, as Grigaut, Rothschild, Chauffard, and Landau³⁷⁶ first demonstrated, is followed by an increase in the cholesterol content of the blood. Abnormal conditions not involving the adrenals primarily are however, also, attended by changes in the level of the blood cholesterol. For example, in infections, uremia, hypothyroidism, and cachexia, the blood cholesterol is reduced while in biliary obstruction, diabetes, nephrosis, and hyperthyroidism it is elevated.

The disturbances in cholesterol metabolism which occur in adrenal insufficiency are not caused by the absence of the

adrenal tissue *per se*, for by treatment of an adrenalectomized animal with an adequate supply of the cortical hormone, the blood cholesterol may be maintained at its normal level. The most probable explanation of the observed hypercholesteremia observed in adrenal insufficiency is that it is a result of hepatic dysfunction induced by the absence of the adrenal cortical hormone. This hypercholesteremia is usually not manifested until after other changes due to cortical insufficiency (such as the increased non-protein nitrogen and the changes in the inorganic constituents of the blood) have already occurred. As we shall see later, the earliest changes evident in the blood chemistry in insufficiency are probably due to renal dysfunction. The hypercholesteremia and hypoglycemia, both of which are of later occurrence, are attributable, in the author's opinion, to liver injury. The liver normally secretes cholesterol and its esters into the bile and any interference with its normal activity would tend to result in an elevation of the cholesterol content of the blood.

The claim, first made by Stewart⁵⁹³ and by Landau,³⁷⁶ that the injection of cholesterol prolongs the life of adrenalectomized animals, is based on too inadequate data to merit its acceptance. In rats, Firor and the author were unable to demonstrate a prolongation of life after the injection or oral administration of either cholesterol or lecithin.

NON-PROTEIN NITROGEN

Marshall and Davis⁴³⁸ demonstrated the rise in the non-protein-nitrogen (particularly the urea) concentration of the blood which follows adrenalectomy in cats. After the injection of urea or creatinine, much less of these substances was excreted than in normal animals. These authors also observed a diminution in the excretion of phenolsulphonephthalein and concluded that normal kidney function is dependent upon the integrity of the adrenals or upon the product of their secretion. Subsequent writers have amply confirmed these results.^{264, 404, 530, 693, 704}

The rise in the non-protein-nitrogen of the blood is one of the earliest manifestations of adrenal insufficiency and is evident before many of the other characteristics of adrenal insufficiency such as weakness, anorexia, *etc.* become prominent. The non-protein-nitrogen content of the blood may reach

FIG. 11. CHANGES IN THE BLOOD CHEMISTRY IN ADRENAL INSUFFICIENCY

Composite records of results obtained on dogs adrenalectomized in stages. The animals received no therapy during the period of observation and died seven days following the removal of the second gland. With the exception of the pH, the values are expressed in terms of milligrams per 100 cc.

values as high as five to six times the normal (*cf.* Figure 11) but often animals die of typical insufficiency with a non-protein nitrogen which is only twice the normal. Death can not be due to the accumulation of this catabolite for experimentally one can produce much higher values than occur in adrenal insufficiency (*e.g.*, by ligation of the ureters) without fatal result.

The accumulation of the waste products of metabolism must, however, exert deleterious effects which add to the other deficiencies of the organism.

The increased non-protein-nitrogen observed in adrenal cortical insufficiency consists for the most part of urea, the chief end-product of protein catabolism. Other non-protein-nitrogen constituents such as creatinine and uric acid are also increased, the former attaining values several times those observed in normal animals.

WATER

The water content of the blood and tissues is characteristically altered in adrenal insufficiency. During the early stages of insufficiency a diuresis may occur. This loss of water from the body together with that normally lost through the lungs and skin is not replaced by the ingestion of additional quantities of water. In fact, the animal in adrenal insufficiency manifests no thirst despite the abnormal loss of fluid from its body. Similarly, patients suffering from Addison's disease will not manifest a degree of thirst consonant with the state of dehydration of their tissues.

The existence of an actual negative water balance in adrenal insufficiency has been experimentally demonstrated^{265, 398} in dogs by the excess volume of the water present in the excreta over the total water intake. The actual loss of water from the body is greater than is indicated in these studies which do not take into account the appreciable loss of water through the lungs. There can be no doubt, therefore, that adrenal insufficiency is accompanied by a loss of water from the body and that there is no attempt to replace this loss by an increased ingestion of fluids.

Gradinescu²³⁶ in 1913 showed that the concentration of the blood in adrenalectomized animals was accompanied by no change in the electrical conductivity, the freezing point, the viscosity, or the refractive index. He, therefore, concluded

that the hemoconcentration was due to loss of fluid through the capillary endothelium. This view which has recently been revived by Swingle and his collaborators⁶¹⁴ is entirely untenable, for such endothelial leakage would necessarily result in an obvious anasarca and edema of the tissues. The reverse of these conditions is actually found. The tissues of animals dying of insufficiency manifest a dehydration but never an abnormal hydration as was claimed by Gradinescu.

In adrenalectomized cats, Baumann and Kurland⁴⁴ found the plasma to constitute 57 per cent of the total blood volume as compared to 65 per cent in normal animals. This decrease in the plasma volume was attended by an increase of 8 to 10 per cent in the plasma solids. Similar changes have been found in dogs²⁶⁸ and other experimental animals. There is thus a decrease in the relative water content of the blood plasma which may be brought about in one of three ways: by a loss of an excessive amount of water through the kidneys, by a shift of the water from the plasma into the blood corpuscles and tissue cells, or by an increase in the content of solids of the blood. From the obvious dehydration and negative water balance which occur in adrenal insufficiency, it may be concluded that the observed hemoconcentration is a result primarily of the loss of water from the body. The state of dehydration of the tissues speaks against the view that any considerable amount of water could have been lost from the blood to the tissues. Nor is there any reason for supposing that the observed anhydremia is only relative and the result of an increase in the absolute solid content of the blood. That there is an actual loss of fluid from the circulation and not merely a transfer from the plasma to the red corpuscles is indicated by the increase in the number of red corpuscles which roughly parallels the hematocrite readings. Thus in an experiment of Harrop *et alii*,²⁶⁸ in which the plasma volume decreased from 72 to 57.5 per cent, the red blood corpuscles increased from 4.2 to 6.1 millions per cu. mm. There is, however, no in-

creased concentration of the erythrocytes in the peripheral blood as contrasted with the venous blood such as occurs in surgical or traumatic shock in which the loss of fluid presumably occurs by leakage through the capillary endothelium.

Before attempting to explain the changes in the water metabolism observed during adrenal insufficiency, it is necessary to consider the changes in the concentration of the osmotically active constituents (sodium, potassium, chloride, *etc.*) which accompany the changes occurring in the water metabolism.

SODIUM

The inorganic constituents of the blood undergo marked changes in their concentration during adrenal insufficiency. These changes are most pronounced in the case of sodium, chloride, and potassium, as shown in Figure 11, in which are reproduced typical curves of the blood chemistry changes occurring in the dog following adrenalectomy. The sodium content of the blood plasma is usually decreased about 15 per cent below its normal level in experimental cortical insufficiency while the chloride content is diminished to a somewhat lesser extent.

The drop in the sodium and chloride contents of the blood in adrenal insufficiency is due to a loss of these substances in the urine. During the early stages of adrenal insufficiency, as Loeb,³⁹⁸ Harrop,²⁶⁵ and their coworkers have shown, there is a negative balance of sodium and chloride due to an excretion of these substances in the urine in an amount which exceeds their intake by the organism.

As shown in Chapter XI, anatomical evidence indicates injury of the renal tubules in adrenal insufficiency, and one can adequately explain all the observed changes in the blood chemistry which occur in adrenal insufficiency on the basis of this tubular injury. According to our present theories of renal function, the blood plasma is filtered through the glomeruli to give a protein-free ultrafiltrate in which the filterable

constituents of the blood are present in the same concentration as they are in the plasma. From this protein-free ultrafiltrate, the renal tubules reabsorb water and the so-called threshold bodies including sodium and chloride, and return these constituents to the blood stream. On the other hand, the non-threshold substances, such as urea, are not reabsorbed but appear in the urine in relatively concentrated solution due to the reabsorption of water and other threshold substances. Injury to the tubules might therefore be expected to result in an increased excretion of water, sodium, and chloride due to the inability of the kidney to reabsorb these substances. The injury to the tubules may also result in the back diffusion of non-threshold substances, such as urea, from the tubular lumina to the blood and thus result in a faulty excretion of the non-protein-nitrogen catabolites and their accumulation in the body. As we have seen, these changes in the sodium, chloride, and non-protein-nitrogen constituents of the blood are precisely those which occur in adrenal insufficiency.

POTASSIUM AND OTHER CATIONS

Simultaneously with the decrease in the sodium and chloride contents of the blood plasma, one observes in adrenal insufficiency an increase in the concentration of potassium and magnesium. Baumann and Kurland⁴⁴ found an increase of 42 and 23 per cent of these ions, respectively, above their normal values in adrenalectomized cats. Hastings and Compere²⁸⁵ observed potassium values as high as 20 millimoles per liter, compared to 3 for normal animals. Changes in the blood calcium are not noteworthy and rather irregular in their occurrence.⁶²⁵

The large amounts of potassium which appear in the blood in adrenal insufficiency may originate either from the ingested food or from the tissues. Normally the potassium ingested in the food is excreted in the urine. In adrenal insufficiency where the loss of sodium and chloride through the kidney

threatens to disturb the normal osmotic pressure of the organism, one might expect the ingested potassium to be retained to compensate for the loss of other electrolytes. Should the amount of potassium ingested not suffice, this ion might also diffuse from the muscles and other tissues into the blood in order to maintain an osmotic balance. Whether or not the loss of potassium from the muscles is responsible for the muscular asthenia and weakness observed in adrenal insufficiency is problematical. It is also possible, of course, that a loss of the normal impermeability of the muscle cells results in the diffusion of potassium into the blood. The evidence available, at present, which purports to show that the permeability of the muscles is abnormal in adrenal insufficiency is, however, not valid. The fact that the potassium content of the blood rises during the late stages of insufficiency when little or no food is being ingested would indicate that it, in part, originates from the tissues. In any case, it is plausible to assume that the same tubular injury which makes it impossible for the kidney to reabsorb sodium and chloride efficiently also fails to prevent the diffusion of potassium from the tubules back into the blood. The presence of an abnormal concentration of potassium in the blood will, as we have seen, help to maintain the normal osmotic pressure of the blood.

The ratio of the sodium to the potassium content of the blood plasma drops to about half of its normal value in adrenal insufficiency. Similarly the ratio of potassium to calcium is increased about one-third. The maintenance of a proper ratio between the above-mentioned electrolytes is of fundamental importance to the organism for any disturbance in these ratios alters appreciably the physicochemical properties of protoplasmic systems. The alteration of these ratios in adrenal insufficiency is probably secondary to the loss of sodium and chloride through the kidney and a consequence of the maintenance of an approximately normal osmotic pressure. The resulting disturbed ratios will undoubtedly act deleteriously

on many organs and may be responsible for their dysfunction in adrenal insufficiency. For example, the cardiac arrhythmias often observed in terminal stages of adrenal insufficiency may be attributed to the high potassium content of the blood and the low potassium-calcium ratio.

ANIONS

Of the blood anions, the concentration of chloride is most markedly affected as might be anticipated from the changes occurring in the sodium concentration already described. However, the loss of blood chloride is not quantitatively so great as that of the sodium loss (*cf.* Figure 11). The relative preponderance of the loss of sodium over that of the chloride results in a decrease in the bicarbonate concentration of the blood and a disturbance in the normal acid-base equilibrium. The inorganic phosphate and sulphate are also increased but this increase is usually not prominent except in the later stages of insufficiency.

The kidney normally maintains the hydrogen ion concentration of the blood at a constant value. When its function is disturbed in adrenal insufficiency, this control is inadequate and we find a marked disturbance of the acid-base equilibrium of the blood. This disturbance is marked by a fall in the pH of the blood (*cf.* Figure 11) accompanied by a decrease in the bicarbonate content and carbon-dioxide combining power of the blood. There is thus an acidosis in adrenal insufficiency which is probably responsible in part for the hyperpnoea observed in the late stages of insufficiency.

THE MECHANISM OF THE OBSERVED CHANGES IN THE BLOOD CHEMISTRY

The osmotic pressure of the blood and tissues of mammals is maintained with a high degree of constancy thus affording the organism a "*milieu intérieure*" of constant colligative composition. The osmotic pressure readily adjusts itself, by rapid

shift of salt and water, to any change threatening its deviation from the normal. To speak of an abeyance of the osmotic forces, as assumed by some authors in discussing adrenal insufficiency, is obviously as absurd as it would be to speak of an abeyance of the law of gravity in explaining some terrestrial phenomenon.

Gradinescu²³⁶ first demonstrated that the osmotic pressure of the blood was normal in adrenal insufficiency. Howard and the author have also found that blood removed from dogs dying from adrenal insufficiency has a normal freezing point thus indicating that despite the profound changes occurring in the composition of the blood, this physico-chemical property remains constant.

Stewart and Rogoff⁵⁸⁸ found a decrease in the conductivity of the blood and serum which would indicate an absolute decrease in the total ionic concentrations. This decrease in the concentration of the ionic constituents of the blood is compensated for by the elevated non-protein-nitrogen of the blood in maintaining the osmotic pressure at its normal value.

We can picture the chain of events which bring about the changes in the composition of the blood and tissues in adrenal insufficiency as consisting essentially of the following events: Renal injury causes in the early stages of insufficiency an abnormal loss of sodium chloride, water, and certain other threshold substances. This loss reduces the blood volume which imperils the efficiency of the circulation. To overcome this loss of blood volume, there is a passage of water from the tissues into the blood stream, accompanied by a passage of salts, to maintain a constant osmotic pressure.

The changes in the inorganic constituents of the blood plasma observed in adrenal insufficiency are reflected in corresponding changes in the whole blood so that there is a shift of these electrolytes between plasma and corpuscles to maintain the normal equilibria existing across the corpuscular membrane.

Attempts have recently been made to simulate the loss of

sodium in adrenal insufficiency by injecting isotonic glucose into the peritoneal cavity and later withdrawing the fluid after ionic equilibration has occurred. In this way one obtains a marked fall in the concentration of the serum sodium and a slight drop in the osmotic pressure of the serum. A shift of water into the blood cells depletes the extracellular fluid. This procedure, as utilized by Gilman,²²¹ does not reproduce exactly the conditions obtaining in adrenal insufficiency for it does not involve a loss of fluid from the circulation. The rapidity with which the depletion of sodium occurs is not comparable to the more slowly progressive changes occurring in adrenalectomized animals. In the latter, the accumulation of non-protein nitrogen constituents in the blood will counteract the development of any drop in osmotic pressure such as follows the intraperitoneal injection of glucose. Gilman demonstrated an analogy between the sensitivity of his animals to hemorrhage with that observed in adrenal insufficiency. His conclusion that a shift of water into the cells occurs in adrenal insufficiency as it does in his procedure does not, however, follow. In adrenal insufficiency the loss of fluid through the kidneys with a consequently diminished blood volume accounts for the sensitivity to hemorrhage, without necessitating the assumption of a passage of water into the cells and tissues such as occurs after the intraperitoneal injection of glucose.

Harrop,²⁶⁶ Loeb,³⁹⁸ and their coworkers have reported studies on the water, sodium and chloride balances in animals during cortical insufficiency as well as during periods of recovery following the injection of the cortical hormone. They have demonstrated that the loss of sodium and chloride through the urine in excess of the amounts ingested is greater than can be accounted for by the loss of these constituents from the plasma. Part of the excreted sodium and chloride must be derived, therefore, from the tissues.

The injection of cortical extract into animals in adrenal in-

sufficiency results in a period of diuresis in which the accumulated nitrogenous and other catabolites are excreted. This diuresis differs, however, from the initial diuresis which ushers in cortical insufficiency by a diminished excretion of sodium. The restored kidney is now able to excrete the waste products of the blood while retaining the essential constituents. As the kidney resumes its normal activity, the excessive concentrations of potassium and magnesium in the blood are excreted or restored to the tissues; the water, sodium, and chloride of the blood are replenished from ingested food and fluid; and the normal distribution of the various inorganic constituents in the body is restored.

The argument has been advanced that the loss of sodium chloride in adrenal insufficiency can not be attributed to damaged renal function because the loss of this substance is notably greater than the loss of water. This view ignores our modern concept of the rôle of filtration and reabsorption in the elaboration of urine. The assumption of an interference with reabsorption as outlined above not only explains the facts but is in accord with modern views of renal function. The view that the adrenal cortical hormone in some way controls "osmotic pressure" is too fantastic to require comment. The assumption that the hormone controls the electrolyte level of the blood by some mysterious hormonal regulation is equally unnecessary if we accept the view (for which there exists both anatomical and physiological evidence) that the renal tubules are injured and are unable to perform their normal reabsorptive and secretory functions.

The view that the accumulation of non-protein nitrogen is secondary to the fall in blood pressure is contrary to the available facts. The blood pressure is still normal at a time when the non-protein-nitrogen blood level is markedly elevated. The increased non-protein-nitrogen of the blood must be looked upon either as a further consequence of the renal injury or as a mechanism to maintain a normal osmotic pressure of

the blood. In the elasmobranch fishes, urea serves this function of maintaining the osmotic pressure at the level of the sea-water. An injury to the tubules which would prevent reabsorption of sodium chloride might also be expected to allow the diffusion back into the blood stream of the non-protein-nitrogen from the glomerular filtrate. The osmotic pressure of the blood would be a factor in determining the extent of this reabsorption and thus incidentally serve to maintain the osmotic pressure of the blood at its normal level.

THE RÔLE OF THE ELECTROLYTE DISTURBANCE IN THE CAUSATION OF THE SYMPTOMS OF ADRENAL INSUFFICIENCY

The question arises as to what part the disturbance in the electrolyte balance plays in causing the symptoms and ultimate death in adrenal insufficiency. Other pathological conditions are also attended by a loss of inorganic base from the body; *e.g.*, diabetic acidosis, severe diarrhea, high intestinal obstruction, cholera, *etc.* These conditions are accompanied by profound weakness, prostration, anorexia, nausea, vomiting, a fall in blood pressure, retention of non-protein nitrogen, acidosis, and anuria—conditions which resemble those of the “shock” observed in the terminal stages of cortical insufficiency. In some instances, particularly as observed in man, the acute onset of the symptoms of adrenal insufficiency are attributable to the disturbed electrolyte balance of the organism, for the administration of sodium chloride will bring about in these cases a striking relief and recovery from imminent death.³⁹⁹ However, adrenal insufficiency can not be considered as the result solely of a disordered metabolism of the body electrolytes. Animals can not be kept alive indefinitely by the administration of salts in amounts which maintain an approximately normal blood composition. Such animals die of the typical symptoms of insufficiency even when the sodium, chloride, and other blood constituents are sufficiently close to normal to preclude the possibility that death is due to a de-

iciency of these ions. According to Britton and Silvette,⁹¹ adrenal insufficiency in the opossum (*Didelphys virginiana*) and marmot (*Arctomys monax*) is accompanied by blood serum sodium and chloride levels which are greater than normal. Apparently, in these animals, the renal injury is not a predominant factor in producing the effects of adrenal insufficiency. If one assumes that the adrenal cortical hormone is essential for the normal function of several organs, or perhaps even for all of the tissues, one would expect different species or different animals of the same species to manifest symptoms which are attributable to different deficiencies. Thus, whereas a given dog may die of an acute disturbance of the electrolyte and water balance due to renal injury, a marmot might die of carbohydrate deficiency due to hepatic injury. Remedying either of these deficiencies may temporarily postpone death, but ultimately some more fundamental disturbance, the nature of which we are still ignorant, will result in death from adrenal cortical insufficiency.

THE ELECTROLYTE DISTURBANCE IN MAN

In Addison's disease one also finds the same disturbances in blood chemistry as are observed in the experimental animals. Hypoglycemia may reach convulsive levels in moribund patients. Rowntree⁵³⁸ observed an increase in the blood urea in patients which was in proportion to the degree of adrenal insufficiency.

Marañón and Collazo⁴²⁸ in seven patients dying of Addison's disease found the water content of muscle (obtained by biopsy) to be 75 per cent as compared to 80 per cent in normal individuals. The water content of the blood of 24 patients with this disease averaged 76.8 per cent compared to 82 per cent for normal individuals. The potassium content of the blood serum was 31.1 mgms. per cent as compared to 20 for the normal. On the other hand the sodium and chloride concentrations were not markedly deranged.

Other observers have, however, demonstrated marked abnormalities of the sodium and chloride concentrations of the blood of patients in adrenal insufficiency. Thus Loeb³⁹⁹ found the sodium content of two patients to be 108 and 109 millimoles per liter as compared to 138 for normal individuals. The serum potassium in these patients was 8.7 and 8.1 millimoles per liter compared to 4.8 for the normal. The chloride was reduced from its normal value of 105 to 70 and 73 millimoles, respectively. These observations led Loeb to suggest the use of sodium chloride in the treatment of Addison's disease, with dramatic improvement in some cases.

SALT THERAPY IN ADRENAL INSUFFICIENCY

The derangement of the salt metabolism in adrenal insufficiency will obviously exert a deleterious effect on the organism and any method tending to restore the normal balance will have a beneficent effect in mitigating such symptoms as are attributable to this deficiency. The fact that injection of saline or of Ringer's solution is capable of extending the life of adrenalectomized animals was first demonstrated by Soddu⁵⁸⁰ and independently proven by Marine and Baumann,⁴³⁵ Stewart and Rogoff,⁵³¹ and many recent observers.

In figure 16 is shown the beneficent effects of administering 1.0 gram of sodium chloride plus 0.5 gram of sodium bicarbonate daily (mixed in their regular food) to six adrenalectomized rats. Such adjuvant therapy, as seen in figure 16, results in a material prolongation of life, but it does not permit normal growth in young animals nor does it avert ultimate death from adrenal insufficiency. The claim⁵⁴¹ that administration of salts or Ringer's solution, admixed with the food or in lieu of the animals' supply of drinking water, permits the indefinite survival of adrenalectomized rats has not been confirmed by the author nor by other observers. The apparent indefinite survival and normal growth of animals on a replacement therapy consisting solely of inorganic salts must be

attributed to incomplete adrenalectomy. Animals which would normally succumb to the effects of an adrenalectomy may in the presence of microscopic nests of interrenal tissue manifest no signs of adrenal insufficiency when supplied with adequate doses of inorganic salts. The withdrawal of the salt may even throw the animals into adrenal insufficiency and lead to death. However, in these cases, some adrenal cortical tissue is present and it is only by microscopic section of the adrenal sites that this tissue is demonstrable. It is preposterous to claim that gross *post-mortem* examination revealed no accessory cortical tissue, for in the rat, at least, the small nests necessary for maintaining life in the presence of salts are not detectable by the naked eye among the connective tissue which abounds at the site of the adrenals.

The deficiencies in the organism brought about by a loss of sodium chloride from the body are pronounced in their effects, but are readily remedied by the administration of an excess of salt. This fact has led investigators to misinterpret certain of their observations, and conclude that the administration of salts to an adrenalectomized animal^{266, 541} or to patients suffering from Addison's disease³⁵¹ offers a complete replacement therapy. This view is entirely misleading. Although useful in mitigating the symptoms due to loss of electrolyte from the body, the administration of salts does not serve as a replacement therapy for the cortical hormone. Neither adrenalectomized animals nor man can be maintained on such therapy beyond a reasonable period of time.

Another current misconception is based on the view that the administration of both an excess of salt and of the cortical hormone is necessary to supply a complete replacement therapy in adrenal insufficiency. Adrenalectomized animals or patients with Addison's disease maintained on inadequate doses of the cortical hormone will react much more favorably when an excess of salt is simultaneously administered with the hormone, than when they receive an insufficient amount of

the hormone alone. However, with an adequate dose of the hormone, the addition of this extra salt is unnecessary, and one can relieve all the symptoms of adrenal insufficiency by the use of the cortical hormone alone plus the normal salt requirement of the individual.

It is quite conceivable that in certain cases the loss of electrolyte has proceeded so far that a resumption of the normal salt balance is impossible without an adequate exogenous source of electrolyte. In any case, the administration of salt will aid in mitigating the serious symptoms due to electrolyte loss, and thus aid in the recovery of the organism. Consequently, the use of salt in adrenal insufficiency is to be highly recommended in all acute cases and in chronic conditions where the cost of the adrenal cortical hormone makes it necessary to economize on its use.

The beneficent effects of salt therapy as well as its limitations are well illustrated by experiments on adrenalectomized month-old rats. If one maintains such animals on the cortical hormone for about a week and then replaces the hormone with 0.2 gram sodium chloride plus 0.1 gram sodium bicarbonate per rat per day, one finds that the animals will grow and appear normal for a period of a week or ten days. Their subsequent reactions to salt therapy will depend upon the completeness of the adrenalectomy. If completely adrenalectomized, growth ceases on the salt therapy and death of the animal follows in the course of about a week. If rests of cortical tissue are present, due to an incomplete operation (*cf.* Chapter XVI), the animals will continue to live when maintained on the adjuvant salt treatment. If these rests hypertrophy, the salt therapy may be discontinued and the animal which would have died had it remained untreated with salt or hormone following operation, now survives and grows normally on its regular diet. If the cortical rests fail to hypertrophy, the animal may be maintained alive for several weeks, but it will grow at a subnormal rate, and eventually die of adrenal in-

sufficiency. Salt therapy in the experiments just cited prevents the impoverishment of the body of its vitally important electrolyte, and aids in overcoming adrenal insufficiency, but the salt does not replace the cortical hormone nor does it prevent the fatal outcome of the adrenalectomy unless sufficient cortical tissue remains to furnish, after its hypertrophy, an adequate supply of the vital hormone.

Results, similar to those described in the preceding paragraph are obtained in experiments on adult dogs or other experimental animals. Failure to appreciate the effects of administering large amounts of salt on the survival of adrenalectomized animals, has undoubtedly led to many of the fallacious conclusions regarding the alleged potency of various cortical extracts reported by earlier workers. On the other hand, failure to appreciate the effect of the presence of microscopic nests of cortical tissue on the survival of adrenalectomized animals has led to the false conclusion that salt is able to serve as a complete replacement therapy for the cortical hormone.

CHAPTER XII

THE RELATION OF THE ADRENALS TO CARBOHYDRATE METABOLISM

Considerable confusion exists in the earlier literature concerning the rôle of the adrenals in carbohydrate metabolism. Following Blum's⁶⁴ demonstration that epinephrine injections elicit glycosuria, numerous workers attributed importance to epinephrine secretion as a regulator of glucose metabolism. Aside from numerous technical sources of error and misinterpretation of observed findings, confusion resulted from a failure to dissociate the hypoglycemic effects of adrenal cortical insufficiency from the hyperglycemic effects of epinephrine.

In the normal organism the glucose content of the blood is maintained at a relatively constant level. The utilization of glucose by the muscles and tissues is accompanied by glycogenolysis (the breakdown of glycogen) in the liver and muscles which process furnishes a supply of glucose to replace that consumed in metabolic processes. The mechanism of this carbohydrate control involves a number of physiological reactions. Stimulation of the splanchnic nerves or the injection of epinephrine, as we have seen (Chapter VII), cause a liberation of glucose from the liver. Stimulation of the brain stem in the region of the pons (the piquê of Claude Bernard) causes a reflex stimulation of glycogenolysis which is mediated through the splanchnic nerves but fails to occur (if these nerves are cut or the adrenals are removed) only when the glycogen store of the liver is reduced. If large stores of glycogen are present in the liver, adrenalectomy or severance of the splanchnics does not prevent the appearance of hyperglycemia. Moreover, atropine, which paralyzes the parasympathetic nerves, prevents the development of hyperglycemia following pontine

decerebration. Hence the adrenals are not the only organs involved in the changes in carbohydrate metabolism which follow the operations described above. The parasympathetic nerves by influencing the secretion of insulin from the pancreas also play a part. The liver is also affected directly for if the hepatic nerves are cut, stimulation of the splanchnics produces a lesser degree of hyperglycemia than normally.

In considering the relation of the adrenal glands to carbohydrate metabolism, it is necessary to dissociate the effects due to epinephrine and those due to the cortical hormone. As we have seen in Chapter VII, epinephrine exerts a striking hyperglycemic activity. The hyperglycemia which follows the injection of many drugs and accompanies various physiological procedures is due to the liberation of epinephrine from the medulla. Hyperglycemia can be elicited, however, in the absence of the adrenals, as Stewart and Rogoff⁵⁸⁸ and others have shown, so that epinephrine is not the sole agency responsible for a rise in the blood sugar. For example, Sundberg⁶⁰⁶ has shown that operative interference, pain, *etc.* give rise to an hyperglycemia in adrenalectomized animals, but the hyperglycemic response is less marked than it is in animals with intact glands.

In the adrenalectomized animal, not only is there an absence of the medullary secretion, but, unless the cortical hormone is administered, the results are complicated by the partial cortical insufficiency which may be simultaneously produced. The variation in the degree of this cortical insufficiency accounts for the divergence in the results obtained by different authors on the reaction of the blood sugar to stimuli after excision of the adrenals.

EFFECTS OF CORTICAL INSUFFICIENCY

Bierry and Malloizel⁵⁸ in 1908 first demonstrated the existence of hypoglycemia in dogs after adrenalectomy. This result was confirmed by Porges⁵¹¹ who also showed that the hypogly-

cemia was accompanied by a reduction of the glycogen content of the liver, the glycogen content of the gastrocnemius muscle remaining normal. Porges also demonstrated the occurrence of hypoglycemia in some patients suffering from Addison's disease. Schwartz⁵⁵⁹ showed that after adrenalectomy the glycogen of the liver of rats might vanish. Other authors have repeatedly confirmed the above results which indicate a tendency to hypoglycemia and a reduction in the glycogen store of the liver and muscles in animals in adrenal cortical insufficiency. The results of Stewart and Rogoff,⁵⁸⁸ who found normal values in adrenalectomized rabbits, are obviously due to the absence of any cortical insufficiency in their animals. The completeness of the operation and the time allowed to elapse before making the observations will determine the result obtained. As hypertrophy of accessory bodies and unextirpated remnants of the main gland proceeds, the degree of cortical insufficiency diminishes and the signs of an abnormal carbohydrate metabolism disappear. There always remains, however, any abnormal sensitivity which may be due to absence of the medullary tissue.

The hypoglycemia of adrenal insufficiency is in no way the result of failure of the medullary secretion but is entirely the result of absence of the hormone elaborated by the cortex. This is shown by the experiments of Houssay and Lewis³¹¹ who extirpated the medulla from dogs leaving the cortical tissue intact. In such animals in which no medullary tissue was demonstrable, the blood sugar was normal. Bøggild⁶⁵ has also demonstrated that it is the cortex which is responsible for the hypoglycemia which follows adrenalectomy. In depancreatized animals the blood sugar level was not affected by removal of the medulla in severe diabetes, but a slight reduction followed in mild cases. Nor as Leloir³⁸⁶ has shown does unilateral adrenalectomy with denervation of the remaining adrenal, modify the intensity of pancreatic diabetes. This operation does not affect the blood sugar level of normal animals. Extir-

pation of one adrenal and denervation or removal of the medulla of the remaining gland might be expected to cause a temporary slight degree of cortical insufficiency. Hence the demonstration of hypoglycemia soon after such operations is no evidence that the medullary deficiency is responsible for the observed effects. It is only after sufficient time has elapsed for the normal secretion of the cortical hormone to adjust itself by hypertrophy of the remaining cortex that one is justified in interpreting the observed effects as due to medullary deficiency. Under these conditions it has been repeatedly demonstrated that no abnormalities of the carbohydrate content of the blood, liver or muscles occur under normal conditions. We can, therefore, conclude that the hypoglycemia and reduced glycogen content of the tissues observed in adrenalectomized animals is due to cortical insufficiency. In Addison's disease the hypoglycemia which occurs in some cases is likewise attributable to the cortical insufficiency.

There is some evidence that the mobilization of glycogen from glucose is interfered with in the absence of an adequate supply of the cortical hormone. Thus in dogs, 48 hours after adrenalectomy, injected glucose disappears slowly; and muscular glycogen is not increased unless one injects the adrenal cortical hormone or insulin.³⁸⁶ Although the muscle glycogen may be normal a day or two following adrenalectomy in the dog, it rapidly disappears after muscular fatigue and its resynthesis proceeds very slowly. Normally the muscle glycogen is rapidly restored after its diminution by severe exercise. After adrenalectomy, however, this restoration is greatly delayed. Intravenous injection of glucose does not appreciably hasten the resynthesis of glycogen, while the administration of the cortical hormone results in a rapid restoration of the normal glycogen content.¹⁵⁰ The cortical hormone is thus essential either for the normal synthesis of glycogen by the muscle or for maintaining the normal balance between the blood sugar and the glycogen of the various tissues.³⁸⁶

RÔLE OF THE MEDULLA

Although the adrenal cortex is responsible for the hypoglycemia which accompanies adrenal insufficiency, the medulla may play a part in counteracting the hypoglycemic reaction which follows the injection of certain drugs, as for example, insulin. Thus cats in which the adrenal medullae are excised or in which the right gland is removed while the left is denervated are said to be hypersensitive to insulin.⁸⁸ This hypersensitivity is not due to deficiency of the hepatic store of glycogen which may be normal. Since insulin (*cf.* Chapter VII) stimulates the secretion of epinephrine, one might anticipate a more pronounced hyperglycemia to follow the injection of insulin in animals in which the medulla has been inactivated. This stimulation of epinephrine secretion by insulin was demonstrated in the anastomosis experiments of Houssay, Lewis, and Molinelli.³¹² In rats and in rabbits, on the other hand, in which an incomplete adrenalectomy has been performed, the increased sensitivity to insulin which follows the excision of the glands disappears with time according to some authors.⁵⁸⁸ It is possible that in these animals the discharge of epinephrine which follows insulin injection is of minor importance (as compared to the cat or dog) in compensating for the hypoglycemic action of insulin. Sundberg,⁶⁰⁶ however, observed a greater sensitivity in rabbits in which cortical tissue only was present and in which there was no evidence of cortical insufficiency. Takáts and Cathbert⁶¹⁹ have also found that denervation of the adrenals raises the sugar tolerance of normal dogs, increases their responsiveness to insulin, and decreases their responsiveness to the hyperglycemic action of epinephrine.

The theory, that the hyperglycemia following epinephrine injections is caused by an inhibition of insulin secretion from the pancreatic islet cells, is refuted by the occurrence of this hyperglycemia in depancreatized animals. The hypoglycemia which follows the primary hyperglycemia induced by epinephrine has been attributed to stimulation of the vagus which causes an increased secretion of insulin.

Insulin and epinephrine are antagonists. The mobilization of sugar induced by epinephrine is counteracted by the stimulation of sugar utilization and the inhibition of glycogenolysis in the liver which insulin induces. However, this antagonism between epinephrine and insulin is limited to their effects on carbohydrate metabolism. Epinephrine is capable of causing glycogenolysis after insulin poisoning and hence may be used as an antidote to overdosage of insulin, when the liver store of glycogen is not depleted. That hyperglycemia, induced by insulin injections and probably also by other methods, causes an increased epinephrine secretion, is shown by the depletion of the epinephrine stores of the adrenals after insulin poisoning and by the reaction of the denervated heart and iris of the cat. These reactions are absent in adrenalectomized animals and may be prevented by injections of glucose.

The injection of large doses of insulin causes a depletion of the liver glycogen and a considerable diminution of the muscle glycogen. After removal of the adrenals, however, there is little effect on the muscle glycogen.¹⁵¹ Since epinephrine also depletes the liver of its glycogen and diminishes the glycogen content of the muscles, the latter effect when following insulin injections has been attributed to epinephrine.

In hypophysectomized rabbits, insulin hypoglycemia still liberates epinephrine into the blood stream, but this epinephrine fails to restore the blood sugar to the normal level in spite of the presence of ample reserves of liver glycogen. There is also a diminution in the hyperglycemic response to injected epinephrine.¹²⁴

The question of whether or not emotional hyperglycemia is due to increased epinephrine secretion should be settled by noting the blood sugar changes occurring in adrenalectomized animals, maintained on adequate quantities of cortical hormone following emotional disturbances.

EFFECTS OF THE CORTICAL HORMONE

The claim of earlier workers that the adrenal cortical hormone causes a rise in the blood sugar of normal animals has not been confirmed by subsequent workers.²⁶⁷ Hyperglycemia is caused by a number of substances and the presence of impurities in the extracts accounts most likely for the earlier results. Silvette's⁵⁶⁹ claims of an *in vitro* effect of the cortical hormone on the blood sugar are not borne out by an examination of the probable errors of his observations as calculated from his data.

There is ample evidence accumulated by numerous authors since 1908 that adrenal cortical insufficiency is accompanied by abnormalities in the carbohydrate metabolism. The claim, however, that this breakdown in normal carbohydrate metabolism constitutes "the first critical contingency in adrenal insufficiency" or is the pre-eminently "potent" factor in adrenal insufficiency, as claimed by Britton and his co-workers,⁸⁹ is untenable. Many animals (dogs in particular) may show marked symptoms of adrenal insufficiency without an appreciable hypoglycemia or reduction in the glycogen stores of the body below their normal fasting levels.

It is very true that in a few cases (both in experimental animals and in man) hyperglycemia is a predominant symptom of adrenal insufficiency.^{17,473} In animals, particularly those which have been subjected to a chronic insufficiency of several months duration, the convulsions characteristic of hypoglycemia may develop and are relieved by an injection of glucose. In the vast majority of cases, however, this is not the case and injection of glucose does not cause any striking alleviation of the symptoms of insufficiency. As Banting and Gairns,³¹ Stewart and Rogoff,⁵⁸⁸ and others have shown, the hypoglycemia of dogs may be only a terminal finding and hence not to be seriously considered as involved in the primary manifestations of adrenal insufficiency. Moreover, the blood sugar of dogs dying of adrenal insufficiency may often be

normal or even slightly above normal. It is thus unjustifiable to lay too much stress on the relation of the adrenal cortex to carbohydrate metabolism. We must consider the observed changes in the carbohydrate metabolism as only one of the many abnormalities associated with cortical insufficiency.

The exact mechanism responsible for the changes in the carbohydrate metabolism observed in adrenal insufficiency are still unknown. Part of the deficiency may be due to the starvation which results from the anorexia of adrenal insufficiency. The chief factor is most probably the inability of the liver and muscles to function properly without an adequate supply of the cortical hormone. The synthesis of glycogen in the liver and muscles may be deficient in the absence of this vital hormone. The exact rôle which other glands—the pancreas, pituitary, *etc.*—play is also unknown. Pancreatectomy does not prevent the usual fatal outcome or mitigate the symptoms of cortical insufficiency.⁴⁰² Further work is indicated before we can authoritatively discuss the exact nature of the relationship between the adrenal cortex and carbohydrate metabolism.

CHAPTER XIII

THE ADRENALS AND THE RESPIRATORY METABOLISM

The basal metabolic rate of an organism, measured usually by the rate of oxygen consumption in the fasting, resting condition, is constant and predictable from a knowledge of the animal's size. This metabolism representing as it does the intensity of the metabolic processes is easily altered by any factor which modifies the activity of one or more organs. The thyroid gland is preëminently important in regulating the metabolic rate, but other organs, including the adrenals, may also alter the metabolism either through their effects on the thyroid or on the anterior lobe of the hypophysis (which in turn affects the thyroid) or by their direct effects on the metabolic processes of the tissues. The exclusion from the animal economy of a hormone necessary for the normal activities of a tissue or organ will result in a change in the respiratory metabolism. The observation of such a change does not imply, however, that the hormone in question controls the metabolism in the same sense as one is justified in speaking of the control of the metabolism by the thyroid.

Epinephrine, as we have already seen (Chapter VII), increases the metabolic rate by direct action on the tissues. It is very doubtful, however, if the medullary secretion normally controls or affects to any great extent the metabolic rate, although, to be sure, some effect will secondarily result from any stimulus which affects the secretion of epinephrine. Adrenalectomized animals maintained on adequate doses of cortical hormone, or rats, in which the medullary tissue has been removed leaving only cortical tissue, have a normal metabolic rate. These experiments indicate the dispensability

of the medullary tissue for the maintenance of the normal metabolic processes as reflected in the oxygen consumption.

The adrenal cortical hormone, on the other hand, is either itself necessary for the maintenance of the normal oxidative processes in the tissues, or at least its absence makes impossible the normal utilization of oxygen by the tissues. This dependence of the metabolism on the adrenal cortical hormone has been repeatedly demonstrated by the fall in the basal metabolic rate which follows adrenalectomy. This fall in the metabolic rate is proportional to, and is the primary cause of the fall in body temperature which accompanies adrenal insufficiency.²⁶⁸ The observed diminution in the oxygen consumption and body temperature are directly proportional to the degree of adrenal insufficiency.^{23, 568} Extremely low values may be obtained in the terminal stages of insufficiency (*cf.* Figure 10).

A severe but non-fatal injury to the adrenals, on the other hand, causes a significant and prolonged increase in the metabolism. This has been demonstrated by Marine and Baumann⁴³⁴ in rabbits and confirmed by Scott⁵⁶¹ in cats. This temporary increase in basal metabolism is also reflected in an increased body temperature and has been observed by the author many times in rats, cats, and dogs immediately following double adrenalectomy.

Marine and Baumann found that the rise in the metabolic rate after adrenalectomy is followed by a fall to or below the normal. This occurred whether the rabbits died of adrenal insufficiency or lived indefinitely due to the presence of accessory or hypertrophied remnants of tissue left at operation. The average maximum rise of metabolism following the operation was 23 per cent of the basal value.

The rise in metabolism just described is produced by thyroid activity for it is absent if a preliminary thyroidectomy has been performed, and is reflected in a thyroid hyperplasia noted after partial adrenal destruction in the cat or rabbit. This hyperplasia and hyperfunction of the thyroid are probably

secondary effects of trauma to the nerve plexuses near the adrenal, for adrenal insufficiency tends to cause hypo- rather than hyper-plasia of the thyroid. The rats in Tsuji's⁶⁴¹ experiments, in which he found thyroid hyperplasia after adrenalectomy, died within a few days following operation. The short period of survival of his animals indicates the degree of traumatization to which they must have been subjected and render his conclusions regarding an *adrenal-thyroid* relationship of no significance.

Marine and Baumann were⁴³⁴ impressed by the similarity in the clinical course of acute adrenal insufficiency and the unusual form of Graves' disease which runs a rapidly fatal course with severe asthenia, prostration, and coma. They also considered the increased appetite, sleekness of the fur, rapid healing of the operative wound, and the diarrhea which followed sublethal injury to the adrenals to be suggestive of hyperthyroidism. These experiments led Marine⁴³³ to suggest that the adrenals were involved in the etiology of exophthalmic goitre, a view which shall be discussed in Chapter XIV.

A satisfactory explanation of the rise in metabolic activity following sub-lethal injury to the adrenals has not been presented. This rise is also frequently seen for some hours following a complete bilateral adrenalectomy and hence might be attributed to a moderate degree of adrenal insufficiency as suggested by Marine and Baumann. However, this explanation is improbable if we consider the fact that slight or moderate degrees of insufficiency induced by methods other than those involving direct physical injury to the adrenals and its contiguous structures do not cause a rise in the metabolic rate. Such an insufficiency when produced, for example, by withdrawing from an adrenalectomized animal the adequate supply of the cortical hormone upon which it had previously been maintained does not cause any temporary hyperpyrexia. It would seem, therefore, most logical to attribute the rise in metabolism observed after sub-lethal injuries of the adrenal

to an effect other than the temporary reduction in the secretion of the cortical hormone. Were the increased metabolism attributable to cortical insufficiency, one would expect it to occur some time after adrenalectomy as one of the first symptoms of failure of the hormone. The adrenals are supplied with such a rich network of nerves that injury to these structures may perhaps reflexly cause the observed increase in metabolic rate.

Webster and his collaborators⁶⁶³ found that the injection of the adrenal cortical hormone into adrenalectomized cats restored the respiratory metabolism to its normal value in from 24 to 48 hours. This restoration was also obtained in thyroidectomized animals. The injection of the adrenal cortical hormone did not affect the oxygen consumption of normal cats or rabbits but did cause an increase in metabolism in thyroidectomized cats in most animals. Webster *et alii* concluded that the adrenal cortical hormone can exert an influence on the mechanism controlling the respiratory metabolism and that this effect can occur independently of the thyroid gland.

As regards the diminished respiratory metabolism in adrenal insufficiency, it is unnecessary to assume that this implies any direct control by the cortical hormone. So many of the organs and tissues of the body in adrenal insufficiency are manifesting a dysfunction that a decrease in their oxygen consumption would be anticipated. Until further evidence to the contrary is available, it suffices to attribute the reduced oxygen consumption and heat production to the general morbid state of the animal in adrenal insufficiency. It is unnecessary to assume any more fundamental control of the metabolism by the adrenal cortex.

As stated in Chapter VII the requirements of the organism for the cortical hormone are determined by the general metabolism. This does not, however, indicate any direct control of the metabolic rate by the hormone, for with increased activity one might expect the tissues to require a proportionately larger amount of any hormone vital to their normal function.

CHAPTER XIV

THE RELATION OF THE ADRENALS TO THE OTHER ENDOCRINE ORGANS

The various endocrine organs are intimately related to one another and mutually interdependent for their normal activities. Ablation of one endocrine gland will usually be reflected by obvious alterations in the structure and functional activity of the others. The alterations in activity of one gland will affect the general activity of many parts of the organism and these changes in turn will be reflected in the activities of the endocrine organs. It is, therefore, erroneous to suppose, as many endocrinologists do, that changes occurring in any endocrine gland as a result of a modification in the activity of another gland indicate that the latter "controls" or produces a "hormone" which activates the former. Such loose reasoning is responsible for the prolific multiplication of "hormones" which are in many cases only hypothetical entities invented to cover ignorance of the nature of some change occurring in the body.

The adrenal has often been pictured as controlling many other glands and being responsible for their dysfunctions or suffering from their "miscontrol." It cannot be denied that the other endocrine glands reflect disturbances in adrenal activity and *vice versa*, and we shall, in the present chapter, describe these changes and attempt to evaluate their significance.

HYPOPHYSIS

The adrenal glands and the hypophysis are mutually dependent upon the integrity of one another, for extirpation of either of these organs results in anatomically demonstrable changes

in the other. Hypophysectomy causes a very marked atrophy of the adrenal cortex which may reduce the cortex to one-fifth of its normal size, the medulla remaining unaffected. Implants of the pituitary into hypophysectomized rats prevents this adrenal atrophy. Evans³⁰⁹ first demonstrated the hypertrophy of the cortex which follows the administration of alkaline extracts of the anterior pituitary. Houssay *et alii*³¹⁰ showed that such extracts caused hypertrophy of the adrenal cortex in the absence of the hypophysis, thyroid, or sex glands and after section of the splanchnic nerves.

Clinically, likewise, one observes changes in the pituitary in diseases involving the adrenal cortex and changes in the adrenal in acromegaly, hypophyseal cachexia, and other pathological involvements of the hypophysis. In acromegaly, the cortex is hypertrophied whereas the hypophyseal atrophy of Simmonds' disease is accompanied by atrophy of the adrenals. In Addison's disease Kraus³⁶⁶ found a marked diminution in the number of normal basophilic cells of the pituitary in some cases. In extreme cases normal basophilic cells are entirely absent from the gland. The eosinophiles are also diminished in number and may be abnormal.

Experimentally, changes in the hypophysis may be induced in animals maintained for long periods in a state of chronic adrenal insufficiency. Thus in dogs maintained on minimal doses of adrenal cortical hormone for periods of 100 days and then allowed to die of adrenal insufficiency, Grollman and Firor²⁴⁸ found changes in the pituitary which resembled those reported in patients dying of Addison's disease. There was an increase in vascularity of the hypophysis, dilatation of the capillaries, and a marked diminution in the number of basophilic cells which, in one dog, had completely disappeared. In the rat, the increased vascularity was less striking than in the dog. Nor was there as marked a diminution or disappearance of the basophilic cells. However, the staining of these basophilic cells was very abnormal. As in Addison's disease, the

changes in the eosinophilic and chromophobic cells were not as striking as the changes observed in the basophilic cells.

In determining the relative rôles played by the adrenal cortex and the pituitary in the symptomatology of a primary adrenal or pituitary dysfunction, it is necessary to differentiate between the effects of acute and chronic insufficiency. The stunted growth, subnormal body temperature, and reduced reproductive and body activity of hypophysectomized animals cannot be remedied by treatment with large amounts of the adrenal cortical hormone. This would indicate that the symptoms of hypophyseal insufficiency are not due to a secondarily induced adrenal cortical insufficiency. Nor can the stunted growth, sub-normal body temperature, and reduced body activity of adrenalectomized animals be remedied by the injection of the anterior pituitary hormone. Hence we must conclude that these symptoms of acute adrenal insufficiency are not attributable to a secondarily induced hypophyseal insufficiency. It is true that in animals in diestrus as a result of adrenal insufficiency, estrus can be induced by the injection of large doses of estrogenic or gonadotropic hormones.⁵⁶⁷ This is not, however, an indisputable proof that the initial reproductive dysfunction resulting from adrenal insufficiency is hypophyseal in origin. The pituitary gland of hypophysectomized rats has, however, been shown to be deficient in gonad-stimulating power, which is presumptive evidence for the view that pituitary dysfunction may be responsible for the abeyance of the estrus cycle in adrenalectomized rats. The general debility and cachexia of animals in adrenal insufficiency, however, can also be held responsible for the abeyance of reproductive activity.

Although no good evidence exists for attributing the symptoms observed in acute adrenal insufficiency to pituitary dysfunction and *vice versa*, the anatomical findings already cited would indicate that chronic adrenal insufficiency does result in pituitary injury. Such injury, as one would anticipate, will

manifest itself clinically by causing changes in physiological functions normally under hypophyseal control. As demonstrated by Grollman and Firor,²⁴⁸ animals maintained for some time in a state of chronic adrenal insufficiency assume a clinical picture which is essentially that of hypophyseal cachexia. The physiological deficiencies manifested by such animals are not remedied by treatment with the adrenal cortical hormone but respond to treatment with extracts derived from the pituitary. Grollman and Firor concluded, therefore, that the hypophyseal insufficiency induced by interference with normal adrenal activity is primarily responsible for producing an essential part of the syndrome observed in animals suffering from chronic adrenal insufficiency.

Several methods were utilized in the production of a chronic adrenal insufficiency. Completely adrenalectomized animals (rats, cats, or dogs) were treated for extended periods with a minimal amount of the adrenal cortical hormone sufficient to maintain life but insufficient to maintain growth in young animals or to maintain a normal body temperature, respiratory metabolism, and reproductive and general activities in adult animals. It is extremely difficult to maintain such a state of insufficiency in young animals for an extended period of time but in full-grown animals it may be done with ease. A second method of inducing a chronic adrenal insufficiency consisted in performing an incomplete adrenalectomy. The operation being incomplete, cellular residues remained which eventually gave rise to sufficient gland to maintain life after treatment of these animals for a week to ten days with an active preparation of the adrenal cortical hormone. A third procedure for producing a chronic insufficiency consisted in ligating the blood supply to the adrenals. Although a large percentage of the animals on which this operation is performed succumb to the effects of an ensuing acute adrenal insufficiency, some survive and develop the symptoms of chronic insufficiency.

The general symptomatology of animals in a state of chronic

adrenal insufficiency induced by the methods just described differs from that observed in acute adrenal insufficiency in several important respects. Acute adrenal insufficiency responds readily to treatment with the adrenal cortical hormone, is rapidly fatal in untreated animals, and is accompanied by a rapid loss of weight and other symptoms characteristic of adrenal insufficiency. Animals brought into the state of what we have called a chronic adrenal insufficiency live for long periods of time without treatment, do not respond to adrenal cortical therapy, maintain a constant body weight, and are free of many of the symptoms characteristic of acute adrenal insufficiency. Such animals show a slight degree of asthenia, fail to gain weight under a *luxus* diet, maintain a slightly lowered body temperature, and show a diminished capacity for reproduction. At autopsy evidence of pathological changes are noted chiefly in the degree of inanition and loss of general body fat, marked atrophy of the reproductive system, atrophy of the thyroid gland, and a generalized hyperplasia of the lymphatic system with regenerative enlargement of the thymus.

The growth of rats may be stunted by interference with normal hypophyseal or adrenal function. Adrenalectomy results in a permanent cessation of growth which can be entirely prevented by adequate replacement therapy with the adrenal cortical hormone. However, should the adrenal cortical hormone be administered in small doses one is able to prolong the life of adrenalectomized young animals without supplying sufficient hormone to permit growth. Incomplete removal of the adrenals, leaving only a minute portion of the glomerulosa of the gland, suffices for regeneration of the gland and prolonged survival. In many cases such animals assume their normal adult size and manifest no symptoms of adrenal insufficiency. In some cases, however, one observes that such animals after a preliminary period of growth ultimately cease to grow and maintain a constant weight for long periods of time. The preliminary normal growth observed in these

FIG. 12. THE EFFECT OF ADRENAL CORTICAL AND PITUITARY EXTRACTS ON GROWTH

Adrenal cortical and anterior pituitary growth hormone were administered to female rats after the following experimental procedures: *Curve 1*, normal unoperated control; *2*, ligation of the adrenal pedicles; *3*, incomplete adrenalectomy; *4*, hypophysectomy. The animals were given no treatment during the twenty-day periods indicated on the abscissae as 0 to 20, 40 to 60, 80 to 100, 120 to 140, and 160 to 180 days. Each animal received 3 rat units of the adrenal cortical hormone daily during the periods of 20 to 40, and 100 to 120 days. They received 1 cc. of anterior pituitary growth hormone extract daily during the periods of 60 to 80 and 140 to 160 days. (Reproduced through the courtesy of the American Journal of Physiology.²⁴⁸)

animals is similar to that noted in hypophysectomized young rats in which growth does not cease until sometime after operation.

In Figure 12 are reproduced typical curves which show the effects of administering the adrenal cortical hormone or the growth hormone of the anterior pituitary body to rats whose growth had been stunted by various procedures. The periods of treatment extended for 20 successive days, each period of treatment alternating with periods of an equal length of time during which no treatment was administered.

As shown in Figure 12, administration of the adrenal cortical hormone is without the least effect on the growth of rats stunted by chronic adrenal insufficiency or by hypophysectomy. On the other hand, the administration of extracts of the anterior lobe of the pituitary is accompanied by a remarkable growth approximating or exceeding that observed in normal animals. It may be concluded, therefore, that the cessation of growth in animals maintained for long periods in chronic adrenal insufficiency is not due to lack of the adrenal secretion but is due to pituitary insufficiency induced secondarily by the adrenal insufficiency.

The reproductive function of animals in adrenal insufficiency is in abeyance, as described in Chapter IX. In the rat, the periods of diestrus may extend for months during chronic adrenal insufficiency. However, in animals in which growth has ceased for several months, spontaneous oestrus may still occur at intervals of several months, compared to 4 to 7 days for normal rats. The animals conceive and may rear a normal litter. About half the animals die during either pregnancy or lactation. In such animals there is no evidence of failure of mammary function and the young are normally nourished. Undoubtedly, the strain of pregnancy like other strains upon the organism (excessive heat, cold, drugs such as histamine, *etc.*) which are innocuous to normal animals cannot be borne by animals in chronic insufficiency. In animals surviving the

period of lactation, the body weight is found to be the same as before pregnancy. Any growth hormone in the fetuses is apparently not transmitted to the mother.²⁴⁸

Male animals in chronic adrenal insufficiency show impotence with atrophy of the reproductive system similar to that observed in hypophysectomized animals. Repair of the reproductive system in neither male nor female animals occurs when adrenal cortical hormone is administered in doses of two to three rat units daily. On the other hand, a ready response, similar to that obtained in hypophysectomized animals, is elicited by injections of extracts of the pituitary. These observations indicate that the hypophysis is probably responsible for the observed failure of the reproductive system in long continued chronic adrenal insufficiency.

Certain metabolic changes are common to both hypophysectomized animals and those maintained in a state of chronic adrenal insufficiency. Thus, the body temperature is reduced about equally in both cases. In neither hypophysectomized animals nor those maintained in a state of chronic adrenal insufficiency for long periods can the body temperature be elevated to normal by administration of the adrenal cortical hormone. On the other hand, administration of either desiccated thyroid, orally, or the injection of thyroxine or anterior pituitary extracts containing the thyrotropic principle results in an elevation of the body temperature to normal. In Table 1 are summarized the results obtained on a series of rats and dogs after hypophysectomy or in chronic adrenal insufficiency. The results of Table 1 may be interpreted as due to successive changes in the *adrenal-pituitary-thyroid* complex. The primary adrenal insufficiency causes an irreversible injury of the anterior pituitary. This pituitary dysfunction, in turn, results in a thyroid insufficiency which gives rise to the observed symptoms.

The results cited in the above paragraphs point strongly to an intimate relation between the adrenal cortex and the ante-

rior lobe of the hypophysis. Besides the pathological findings in acromegaly, Addison's disease, *etc.*, other clinical conditions present even more striking indications of a close pituitary-adrenal relation. Thus Cushing's syndrome, in which one finds basophilic adenomata of the hypophysis, and the adreno-genital syndrome (to be described in Chapter XXIII) which accompanies certain adrenal tumors, are often clinically indistinguishable due to their almost identical symptomatology.

TABLE 1

The body temperature of animals in a state of chronic adrenal insufficiency

The data in the table represent averages of daily temperatures obtained during the course of one week. The average deviation of the individual readings from the mean values recorded in the table was less than 0.3° (Grollman and Firor²⁴⁸).

NUMBER OF ANIMALS IN SERIES	ANIMAL SPECIES	OPERATIVE PROCEDURE	RECTAL TEMPERATURES		
			Untreated	During thyro- tropic therapy	During thyroid medica- tion
			°C.	°C.	°C.
4	Dogs	Normal controls	39.6		
2	Dogs	Hypophysectomized	38.7	39.8	39.5
2	Dogs	Chronic adrenal insufficiency	38.6	39.7	39.8
8	Rats	Normal controls	39.1		
4	Rats	Hypophysectomized	36.5	39.2	38.7
4	Rats	Chronic adrenal insufficiency	36.6	39.3	39.0

Similarly the differential diagnosis of Simmond's disease (pituitary cachexia) from Addison's disease is sometimes extremely difficult. Both diseases share the common symptoms of asthenia, anorexia, loss of weight, dizziness, and hypotension. The question arises as to the extent to which adrenal insufficiency is responsible for these manifestations of a primary pituitary disease and whether some of these symptoms as observed in Addison's disease are not due to secondary effects on the pituitary. The answer to these questions must be sought in the effects of therapy with the adrenal cortical and

anterior pituitary hormones in the two diseases. The few preliminary reports which have been made on the results of such studies may be ignored since they were based on the use of extracts the hormone content of which was negligible and involved only a short period of clinical observation with no subsequent verification of the diagnosis or the effect of withdrawing the extracts.

It should be emphasized, however, that although secondary pituitary insufficiency may be expected to occur in Addison's disease (as is borne out by anatomical findings) the chief symptoms are certainly not attributable to hypophyseal dysfunction but resemble those observed in acute experimental adrenal insufficiency. The infantilism observed in Addison's disease when it occurs in children and the progeria which has been found to accompany sclerosis of the adrenals are probably due to pituitary insufficiency.

As stated above, the injection of alkaline extracts of the pituitary causes hypertrophy of the adrenals and prevents the atrophy which follows hypophysectomy. The tendency to ascribe all effects which follow injections of pituitary extracts to an hypothetical new "hormone" has resulted in the assumption that the above described effects on the adrenal cortex are due to an "adrenotropic hormone"¹⁸⁵ which normally regulates the adrenal. Scrutiny of the experimental data, upon which the assumed existence of this hormone is based, does not, however, inspire confidence. The available extracts derived from the hypophysis are at their best relatively crude concoctions. The adrenals, moreover, are extremely sensitive and readily respond by hypertrophy to the injection of foreign materials into the organism. Hence one might justifiably doubt that the adrenal hypertrophy observed after injection of the so-called adrenotropic hormone is the result of specific stimulation by a hormonal entity. The so-called thyrotropic hormone of the pituitary also causes hypertrophy of the adrenals. This action is mediated by the thyroids for it fails to occur in thyroidecto-

mized animals.⁴⁰⁰ However, preparations free of the thyrotropic hormone have been prepared which still cause hypertrophy of the adrenals.¹²¹ One can explain all the available facts without assuming the existence of a specific pituitary hormone which controls the adrenal. Nor need one assume that the adrenal elaborates a separate hormone which is necessary for normal hypophyseal activity; for the most highly purified cortical extracts, which fail to remedy certain symptoms of chronic adrenal insufficiency, are still capable of maintaining in adrenalectomized animals all the physiological functions attributed to pituitary activity.

The above considerations have dealt exclusively with the relation of the anterior lobe of the pituitary to the adrenal cortex. Theories have also been elaborated involving an assumed physiological relation between epinephrine and the posterior lobe of the pituitary as well as between the latter and the adrenal cortex. No valid evidence exists to substantiate these assumed interrelationships and hence we need not discuss them here.

THE THYROID

The existence of an antagonistic relationship between the thyroid gland and the adrenal cortex is suggested by a number of well-established observations and was clearly stated over a decade ago by Marine and his collaborators.⁴³³ Their views were based chiefly on the observation that sublethal injury to the suprarenal glands caused marked chronic hyperthermia provided the thyroid were intact. As we have already seen in Chapter XIII, this hyperactivity of the thyroid resulting from adrenal injury may more reasonably be attributed to operative effects, for true adrenal insufficiency is characterized by a reduction in the metabolism.

Thyroidectomy does not result in any constant change in the adrenal cortex⁶⁰⁵ nor does it always greatly affect the survival period of adrenalectomized animals. The administration of

thyroid substance causes an enlargement of the adrenals⁵¹³ which may be looked upon as secondary to the increased metabolic activity. Reactions in general which cause an increased metabolic activity are accompanied by hypertrophy of the adrenal cortex. Feeding thyroid extracts to adrenalectomized animals markedly reduces their survival period which is also a specific example of the effect caused generally by an increase in metabolism.

A number of experiments have been carried out on the effects of feeding or implanting adrenal tissue on the thyroid or upon the metamorphosis of amphibia.¹⁸⁶ Such experiments have no significance in determining the relation of the thyroid and adrenal glands for it is doubtful if any cortical hormone was being administered by the methods utilized in these investigations. Any observed changes are to be attributed to the toxic effects of the administered tissue.

The etiology of hyperthyroidism has been attributed to some unbalance between the adrenal and thyroid glands. Marine's⁴³³ demonstration of an increased thyroid activity in sublethal injury to the adrenals led him to hypothecate that hyperthyroidism is due to dysfunction of the adrenals. He attempted, therefore, to alleviate the symptoms of Grave's disease by administration of fresh and dried adrenal substance. The reputed beneficent effect of this therapy is open to criticism and more recent work has shown that administration of neither cortical extracts nor fresh raw cortical tissue results in any clinical improvement⁶⁶⁴ in patients suffering from hyperthyroidism.

Normal metabolic activity in the higher organism is dependent upon both the thyroid and the adrenal gland. The extirpation of either gland leads to a reduced metabolic activity. The effects of thyroidectomy are chronic and are not incompatible with life while the decreased metabolism which follows adrenalectomy is perhaps only a reflection of the general dysfunction of the tissues generally. Too great emphasis should

not, therefore, be placed on the superficially analogous effects of extirpating the two glands on the metabolic rate. Administration of thyroxin increases the metabolism far above its normal, while administration of excessive amounts of the adrenal cortical hormone does not influence the metabolic rate. This speaks against the view that the adrenal directly controls the metabolism, in the sense that the thyroid does, or that it exerts a control over the thyroid as claimed by Marine's theory. The metabolic activity of thyroxin is not dependent on the integrity of the adrenals for a rise in metabolism can still be elicited in the adrenalectomized animal by injection of thyroxin. It may justifiably be argued that in such cases a sufficient amount of the cortical hormone is still present in the body, for in the late stages of adrenal insufficiency, thyroxin no longer causes an elevation in the metabolism.

It would seem logical to conclude, therefore, that no intimate *thyroid-adrenal* relation exists. Injury to the adrenals or their extirpation may lead to a temporary overactivity of the thyroid. However, this need not be due to dysfunction of the adrenals but is probably a reflex effect of trauma. Overactivity of the thyroid by increasing the metabolism of the organism incidentally increases the demand for the adrenal cortical hormone. There is no evidence, however, that either hypo- or hyperactivity of the adrenals affects thyroid activity. The results of Tsuji⁶⁴¹ indicating hyperplastic changes in the thyroid of adrenalectomized rats are of no significance since his animals died so soon after operation. Mahorner⁴²¹ failed to confirm Tsuji's results, in dogs.

The relation between the adrenal medulla and its secretion product, epinephrine, and the thyroid has been the subject of numerous researches. Attempts have been made to ascribe a close interrelation between these two organs but the results are inconclusive. The increase in oxygen consumption which follows epinephrine injections (Chapter VII) is elicited and, in fact, accentuated after thyroidectomy.⁴³⁶ The effects of

thyroidectomy and feeding of desiccated thyroid substance on the epinephrine content of the adrenals has given conflicting results.²⁹⁴ The results following injections of thyroid extracts have been equally inconclusive. The older experiments in which it was claimed that such injections sensitized the organs innervated by the sympathetic nervous system to a subsequent injection of epinephrine have been shown to be non-specific reactions to foreign protein. Thyroxin induces no such sensitization.⁶³⁹

Koehlsche and Kendall³⁵⁴ have recently studied the effect of administering adrenal cortical extracts on the nitrogen excretion of dogs after injection of thyroxin. They consider their results as indicating the existence of a *thyroid-adrenal* interrelation.

PARATHYROIDS

Injection of epinephrine modifies the calcium content of the blood and attempts have, therefore been made to link parathyroid and adrenal medullary activities. The assumption of such a relationship has not, however, been substantiated. With the transferral of emphasis from the medulla to the cortex as the important part of the adrenals, attempts have been made to demonstrate an intimate relation between the adrenal cortex and the parathyroid glands. Thus the fact that both the administration of parathyroid extracts and adrenalectomy increase the cholesterol content of the blood suggested that parathyroid hyperactivity depresses the activity of the cortex. The hyperplasia of the adrenals noted in a case of *osteitis fibrosa* suggested, on the other hand, that over-activity of the parathyroids stimulates the adrenals. The relative ease with which the cholesterol content of the blood can be affected and the many factors which cause an hypertrophy of the adrenals renders the evidence just cited for the existence of an *adrenal-parathyroid* relationship exceedingly flimsy.

Rogoff⁵²⁴ has suggested that some of the symptoms of adrenal

insufficiency are due to parathyroid overactivity which causes a disturbance in the calcium metabolism, because changes in calcification of the dentin of rat's teeth after adrenalectomy resembled that caused by administration of parathyroid hormone. The muscular twitchings, spasms, and tetanic convulsions often occurring in animals in insufficiency may be due to a disturbance in calcium metabolism. Although Rogoff and Stewart⁵³⁰ reported a decided hypercalcemia in most of their animals, other observers have noted no great change in the blood calcium to indicate any significant alteration in the calcium metabolism. However, it must be remembered that changes in other ions particularly phosphate, which is also increased, will act to modify the state and hence the physiological effectiveness of the calcium. Hence too great emphasis should not be placed upon the total calcium concentration as determined by analysis. There is, however, no good evidence to show that the parathyroid glands are stimulated to over-activity by adrenal insufficiency and one cannot accept the suggestion that the changes in the alimentary tract observed in adrenalectomized animals are due to an excessive secretion of the parathyroid hormone.

In the adreno-genital and Cushing's syndrome (*cf.* Chapter XXIII) due to tumors of the adrenals and pituitary, one observes a softening of the bones due to decalcification. This evidence of a possible parathyroid disturbance may be ascribed to changes in the ovaries or pituitary, which are known to exert an influence on the calcium metabolism. Both the pituitary and the ovaries are affected in these conditions and hence it would seem more logical to attribute the observed effects to the action of these glands on parathyroid activity rather than to assume any control by the adrenals over the parathyroid. As in the case of the thyroid, therefore, we must conclude that there exists no valid evidence to indicate any intimate adrenal-parathyroid interrelationship.

THE GONADS

The idea of an intimate relation between the gonads and the adrenals has recurred for several centuries (*cf.* Chapter I). The earlier bases for such a view have long been shown to be invalid and need not be reiterated here. The more recent theories which have been elaborated to explain an adreno-gonadal interrelationship have, however, also been unsatisfactory. On the one hand, there exists the indisputable evidence to be presented in Chapter XXIII of a clinical syndrome associated with certain tumors of the adrenal. This has led to the obvious conclusion that the adrenals are related to the reproductive organs and that their hyperactivity leads to hyperfunction of the gonads. On the other hand, attempts to demonstrate experimentally such a simple interrelationship have failed. This apparent paradox is due to the failure of previous investigators to distinguish properly between the androgenic tissue and the remainder of the cortex. In the author's opinion, the adrenal gland is comprised of three functionally distinct tissues,—the chromaphil, the interrenal (or true cortical), and the androgenic. The last named is limited (*cf.* Chapter IV) to man and certain animals, is only temporary in its existence, and differs anatomically and histochemically from the true cortical tissue. It is this androgenic tissue which, in the author's opinion, is responsible for the pathological adreno-genital syndrome and probably for certain physiological changes in the reproductive system of the lower animals.

In the present section we shall consider the less important relation between the gonads and the medullary and cortical tissues. In Chapters IV and XXIII are discussed the experimental and clinical relations of the androgenic tissue to the gonads.

There is no good evidence for assuming any important relation between the medulla and the gonads although insignificant differences in medullary activity of the male and female or-

ganism have been noted. Epinephrine injections do not affect the estrus cycle of mice.⁶³⁹ Attempts have been made to find changes in the epinephrine content of the adrenals occurring during pregnancy in order to attribute certain pathological manifestations occurring in this condition to an hypothetical hypo- or hyperadrenalinemia. These attempts have yielded only conflictory and contestable results.

Sex differences in the size of the adrenals have been repeatedly demonstrated, the gland in the female being somewhat larger than in the male. In the rat, for example, Hatai and Jackson²⁸⁶ found the adrenals to be about 20 per cent larger per unit of body weight in the newborn female as compared to the male. This difference gradually disappears so that in a 20 to 40 gram rat the adrenal weights are the same in the two sexes. Thereafter, however, the sex differentiation again appears and the adrenals of a 300 gram female rat are almost twice as large as those of an equal sized male.

Cyclic changes in the size of the adrenal cortex coincident with the oestrus cycle were first pointed out by Stilling.⁵⁹⁵ Similar changes have been described by Anderson and Kennedy¹⁴ in the mouse. In doves, Riddle⁵²¹ described an adrenal hypertrophy coincident with ovulation. In the male also adrenal hypertrophy during rut has been described in rabbits, squirrels, and amphibia.⁶⁹⁵

The reproductive cycle is accompanied by such profound changes in the general activity of the body as a whole that one need not attribute any specific importance to the changes occurring in the adrenal cortex. Such changes may be looked upon merely as a reflection of increased body activity requiring among other things an hyperfunction of the adrenal cortex. It is unnecessary to assume any more direct adreno-gonadal relationship and hypothecate the changes in the adrenal to be the prime instigators of the sexual activity. The same criticism is applicable to the changes in the adrenal during pregnancy or after castration. It must be remembered that all

organs and tissues of the body are to some extent interrelated and any change in one occurring concomitantly with that in another need not imply a fundamental interrelation between the two. This is particularly true where the interrelation is only minor, as in the cases to be described below.

An increase in weight of the adrenals during pregnancy has been described by numerous observers in many animal species (Guieysse,²⁵⁶ and Watrin⁶⁶⁰ in the rabbit; Tamura⁶²² in the mouse; Verdozzi,⁶⁴³ Kolde, and Kolmer³⁶¹ in the guinea pig; *etc.*). Donaldson¹⁶¹ failed to find any enlargement in the rat during gestation, a result which has been confirmed by subsequent workers.¹⁵ Where such hypertrophy occurred it was due to a coexisting infection. As we have seen in Chapter IV, the degeneration of the androgenic tissue at the inception of pregnancy is of more significance than the above described minor enlargement of the gland. Moreover, it is questionable if the increase in weight of the gland during gestation really represents an hypertrophy. It may merely be the result of an increase in the lipid store of the gland coincident with the increased lipid content of the blood which occurs during pregnancy.

Experiments on the effects of castration on the size of the adrenals have given conflicting results.¹⁶ In rabbits Livingston³⁹⁷ found a tendency to slight enlargement after castration in the male, but none after spaying in the female. Tsubura,¹⁶ on the other hand, found the average weight of the adrenals of 14 castrated rabbits to be 0.5424 grams as compared to 0.4176 grams in the controls. In the guinea pig, the respective weights were 0.2516 and 0.1759 grams. It is questionable how much of the adrenal hypertrophy observed after castration is to be attributed to infection, the effects of anesthesia, shock, *etc.* Failure to consider the effects of these variables (particularly infection) on the adrenal may account for the enlargements observed by some authors.¹²

The failure of the reproductive system which follows adrenal-

ectomy has been repeatedly demonstrated by many observers since attention was first called to this phenomenon by Novak⁴⁷⁶ in 1914. Adrenal cortical insufficiency induced by disease (as in Addison's disease) or experimentally leads to cessation of the reproductive activity. In the male there is atrophy of the testis with overgrowth of the interstitial tissue and impotence. In the female there is a cessation of oestrus and atrophy of the ovaries and other reproductive organs. This interference with the normal reproductive processes has been observed clinically in Addison's disease and may be produced experimentally with ease by maintaining animals in a degree of insufficiency compatible with life. Thus, rats in which accessory cortical tissue is left at the time of a bilateralec-tomy may survive with stunted growth but exhibit an oestrus cycle only rarely and remain sterile. At autopsy one finds in such animals marked atrophy of the genital tract. In male rats dying of adrenal insufficiency, one finds at autopsy pale soft and often edematous testes. Histologically the sper-matic tubules are disorganized and degenerated.⁴⁷⁶ The sper-matocytes are affected and spermatogenesis is abated.²⁰⁵ There is an overgrowth of interstitial tissue. The secondary sex organs (prostate, seminal vesicals, *etc.*) are atrophic. In the female there is marked degeneration of the ova and an increase in the interstitial cells of the ovary. Corpora lutea formation does not occur. The uterus atrophies, both the musculature and mucosa assuming an immature appearance. Similar observations have been made in mice by Masui.⁴⁴³

The above described failure of the reproductive system is in part possibly secondary to changes in the pituitary, as already indicated in a preceding section. Implantation of normal rat pituitary bodies into adrenalectomized rats in diestrus results in a re-establishment of the oestrus cycle. This indicates that a pituitary insufficiency secondary to the adrenal insufficiency may be the direct cause of the failure of the reproductive system. At least the oestrus cycle is still elicitable by anterior

pituitary implants even in the absence of adrenal activity. In males implantation of the anterior pituitary gland or injection of urine of pregnancy still stimulates the accessory sex organs. However, neither procedure prevents atrophy of the testis.⁵⁶⁷

Attempts to affect the adrenals by injection of various sexual hormones have been negative. Thus menformon, theelin, and androthin produce no observable changes in the adrenals. Contrariwise, the injection of the adrenal cortical hormone which supports life is without demonstrable effect on the reproductive system. Experimenters who claimed to demonstrate precocious puberty in rats by injections of the hormone and other changes in the reproductive tract were misled by the crudity of the extracts which they had employed. Many workers in endocrinology are unaware of the ease with which one can produce changes in certain organs, particularly the thyroid, adrenals, and reproductive system, by injection of any one of a number of non-specific substances. Thus lecithin, as Jaffé and Ransweiler³²¹ showed, may produce hypertrophic or atrophic changes in the sex organs after long injection. Cholesterol produces marked hypertrophy of the uterus in rats. Thus the average weight of the uterus in six rats injected on alternate days for a month with a one per cent solution of cholesterol was 0.300 grams as compared to 0.170 grams in the control animals. The ovarian weights were not influenced by the injections. Precocious maturity in rats (as evidenced by opening of the vagina) and luteinization of immature ovaries may be obtained by injecting extracts of a variety of tissues.³²¹ Great caution is, therefore, necessary before drawing any conclusions as to the existence of fundamental endocrinological relationships between organs. It is necessary to exclude the possibility that impurities such as lecithin, cholesterol, choline, histamine, *etc.*, which contaminate extracts of glandular products, are producing the observed effects rather than some hormone which one is perhaps more desirous of crediting with the observed effects. As already stated many effects attrib-

uted to the adrenal cortical hormone were in reality due to impurities in the extracts utilized and this is true of the results of those who claimed to have elicited changes in the reproductive system by the use of cortical extracts. These extracts potent as regards their ability to maintain normal growth and activity in adrenalectomized animals exert no demonstrable effect on the gonads when injected into normal animals.⁵⁷⁵ Even after unilateral ovariectomy performed to reduce the potential ovarian activity, Howard and Grollman³²¹ were unable to induce any observable change in the remaining ovary or in reproductive activity by the injection of concentrated adrenal cortical extracts.

The above described considerations demonstrate the absence of any intimate relation between the adrenal cortex proper and the gonads. In Chapter XXIII, however, we shall consider an interesting group of clinical cases in which sexual and developmental abnormalities occur coincident with or as a result of an abnormal growth of the androgenic zone of the adrenal. It was because of these abnormalities that earlier workers suspected and sought so assiduously an *adreno-gonadal* relationship, which gave rise to the numerous researches cited above. Their failure to appreciate the true significance of the androgenic zone prevented their teaching the true significance of the *adreno-gonadal* relationship. It is a specialized tissue of the adrenal only (which we have denoted as the androgenic tissue and described in Chapter IV) which is intimately related to the reproductive system. The cortex proper, although essential for the well-being of the sex glands, neither controls them, nor does it by hyperactivity produce any demonstrable changes in the reproductive system.

PANCREAS

As we have seen in Chapter XII, dysfunction of the adrenal cortex is accompanied by an impoverishment of the carbohydrate supplies of the body. Since insulin, the product of the

islet tissue of the pancreas, is a hormone of such great importance for normal carbohydrate metabolism, one would logically be inclined to seek an explanation of the abnormalities observed in adrenal insufficiency in a disturbed pancreatic function. Most of the work has been confined to the relation of the action of insulin to epinephrine, which we have already considered in previous chapters. The possible existence of a relation between the cortex and the activity of the islet tissue has not been adequately studied. Since the hypophysis is also intimately related to carbohydrate metabolism, some of the changes observed in adrenal cortical insufficiency may be secondary to hypophyseal dysfunction. Further work in this field is necessary, however, before one can elucidate the relation, if any, which exists between the internal secretion of the pancreas and that of the adrenals.^{20, 386, 402, 424}

CHAPTER XV

THE PREPARATION OF EXTRACTS OF THE ADRENAL CORTICAL HORMONE

Attempts to prepare extracts of the adrenal cortex containing its vitally important hormone have been made by many workers.⁴⁵⁵ Abelous and Langlois³ in 1891 prepared extracts which they claimed prolonged the survival period of adrenalectomized frogs. Brown-Séquard⁹⁸ in the following year also prepared extracts which, he claimed, gave temporary relief when administered to dying adrenalectomized animals. Numerous other workers reported the preparation of extracts which they believed to contain the active principle of the adrenal cortex, but these claims like those of their predecessors have received little credence, for subsequent work showed the extracts to be incapable of maintaining life in adrenalectomized animals for any appreciable length of time.

It would seem quite obvious from our present knowledge that the extracts of earlier workers could have contained only an infinitesimal amount of the hormone, contaminated by a mass of extraneous impurities. It is not surprising, therefore, that with time all these preparations have been well-nigh forgotten, and it is not necessary to describe or criticize them here.

The preparation of extracts containing the cortical hormone is rendered difficult for several reasons. In the first place, the amount of hormone present in the glands is relatively small. It might be argued that our available methods remove only a small fraction of the hormone present in the glands, but this assumption is very unlikely, as shall subsequently be shown. The small amount of hormone present in the gland is demonstrated by the large amounts of the glandular material which must be fed to elicit any therapeutic effects. Rapid heating

of fresh cortical tissue to 100°C. preserves the hormone content of the adrenals, but it requires several hundred grams of this tissue per day to maintain the life of an adrenalectomized dog.²⁴⁶ Injection of fresh saline suspensions of the cortex into cats or dogs desensitized to beef protein also fails to elicit any therapeutic effects because of the relatively large amount of material necessary to supply an appreciable amount of the hormone. The adrenal thus differs from the thyroid which may be effectively administered by feeding the dried gland, or the pituitary which when implanted elicits the effects of its growth hormone in hypophysectomized animals.

Another important difficulty in the preparation of extracts of the adrenal cortical hormone results from the fact that unless the medulla is thoroughly removed immediately after slaughter of the animal considerable amounts of epinephrine and its disintegration products will be present in the extracts. Both epinephrine and its oxidation products are exceedingly toxic even when ingested orally in large quantities and their removal from the final extracts is therefore essential. Any beneficent effect of the hormone present in a given extract will be masked by the toxic effects of epinephrine or its derivatives unless these be thoroughly removed.

The adrenal glands are noteworthy for the rapidity with which they undergo autolysis. The adrenal cortical hormone is very rapidly destroyed after death and hence proper preservation of the fresh glands is essential for the preparation of an active extract.²⁴⁵

The failure to appreciate the importance of the factors just enumerated accounts undoubtedly for the results of the early workers who attempted to prepare extracts containing the hormone. They failed to effect a sufficient concentration of the hormone, did not thoroughly remove the noxious impurities present in their extracts, and improperly preserved the glands so that only traces of the hormone were present in their final extracts. It is not unexpected therefore that their products

were ineffective at best, and were highly toxic when administered in large doses.

The commercially available preparations consisting of dried preparations of the fresh glands or glycerine extracts are worthless insofar as their content of the cortical hormone is concerned for the reasons already cited. Some of the effects that have been reported as following their use are undoubtedly attributable to the toxic action of the large amounts of epinephrine and its derivatives which they contain.^{41, 42} None of these preparations can conceivably exert any therapeutic effect which depends upon the presence of the cortical hormone as is demonstrated by their inability to prolong the life of adrenalectomized animals.²⁴⁹

The extracts which have attracted the greatest attention in recent years are those of Swingle and Pfiffner,⁶¹² and Hartman and his collaborators.²⁷⁵ The latter first described a preparation (cortin) in which the hormone was supposedly precipitated by salting it out of aqueous solution. It is very unlikely that this preparation contained any appreciable amount of the hormone. Subsequently Hartman and Brownell²⁷⁴ described a second method based upon the successive use of various lipoidal solvents.

Swingle and Pfiffner's method consisted essentially in an application of the procedures which had been successful in isolating the estrogenic substances. Their claims of the great potency of their extract and its ability to maintain adrenalectomized cats and dogs alive for over one hundred days were most striking and numerous workers immediately began to apply the new extract to experimental problems as well as to Addison's disease.⁶¹³ Although the immediate acclaim which heralded the new extract seemed to substantiate the claims of its originators, subsequent work proved disappointing. Many of the patients reported in the literature as "saved" from imminent death by the "hormone" ultimately died within or in many cases before the average time of their survival when

left untreated. The reports of immediate remedial effects in a disease characterized by spontaneous remissions must be accepted with caution and are of no value in determining the potency of a given preparation. It is only when patients have been maintained in health for a period surpassing that which they would have survived had they remained untreated (*cf.* Chapter XXI) that one can boast of any therapeutic effect from a given treatment.

The results on experimental animals also failed to confirm the claims of Swingle and Pfiffner as to the high potency of their preparations, for relatively large doses of extract prepared by their method often fail to serve as a complete replacement therapy in adrenalectomized animals.^{245, 523} Aside, however, from the question as to the potency of extracts, as prepared by the methods of Swingle and Pfiffner or Hartman and Brownell, both methods are unnecessarily complex and expensive, and sometimes yield products of marked toxicity. They need not, therefore, be considered in further detail here.

The effectiveness with which the adrenal cortical hormone is adsorbed from neutral aqueous solution by charcoal permits the preparation of extracts in a relatively simple manner. The *charcoal-hormone* combination thus prepared can be administered directly by mouth without necessitating the elution of the hormone. The hormone can, however, be eluted from its charcoal combination, freed of contaminating impurities, and thus prepared in a form suitable for parenteral administration. In the following sections shall be described the details of the methods utilized for obtaining these preparations.

THE PREPARATION OF THE CHARCOAL-HORMONE COMBINATION

Beef glands being most readily available are generally used as the source of the hormone. Pig glands are more difficult to obtain but yield approximately three times as much hormone per unit weight as do beef glands. Glands derived from sheep or calves are also probably rich in the hormone, but the

difficulty of properly collecting and preserving them has heretofore rendered their use impracticable.

The adrenals rapidly undergo autolytic changes and the cortical hormone is rapidly destroyed after death. To illustrate the course of this destruction various portions of a batch of glands obtained from one source were extracted and assayed. One portion of the glands was removed from the carcasses of freshly slaughtered animals and dropped into liquid air. These glands were then extracted and found to contain 500 rat units (as defined in Chapter XVI) per kilo of the original whole glands. A second portion of the glands was frozen with "dry ice" at the abattoir and packed in this refrigerant for two days before extraction. From each kilogram of these glands, an extract containing 100 rat units was obtained. A third portion of the glands was placed in the refrigerator of the abattoir and sent frozen to the laboratory on the following day. The extract prepared from these glands contained only 10 rat units per kilo of glandular material. A fourth portion of the glands was sent unfrozen to the laboratory. The extract prepared from this material was found to contain only a trace of the hormone.

The deleterious effects of poor preservation of adrenal glands on the yield of the hormone are obvious from the experiment just described. In order to ensure preservation of the hormone, the glands should be ground into two and a half to three times their volume of acetone immediately on removal from the animal.

The adrenal cortical hormone is firmly attached to the lipids of the adrenal cells and is removed only when these lipids are simultaneously removed. Hence attempts to extract the hormone from the glands by the use of aqueous acid or alkaline solutions have been unsuccessful. Dehydrating the freshly ground glands *in vacuo* at a low temperature destroys the cellular structure and permits extraction of the hormone by water. Unfortunately, this method of extraction is not practical. It

is, therefore, necessary to use a lipid solvent (acetone, alcohol, or ether) which extracts the adrenal lipids and incidentally the cortical hormone. Acetone is by far the most practical solvent, for it not only extracts the hormone efficiently but removes less extraneous matter, and hence leads to the easier production of a relatively pure product.

The fresh glands are thoroughly macerated and shaken at intervals for some hours with the acetone into which they were originally ground. This operation is carried out at room temperature for the acetone apparently inhibits the enzymic activity which acts so deleteriously in the intact unfrozen glands.

The supernatant solvent is now poured off from the glandular material. This glandular residue is refluxed three times with approximately one-third of the acetone which was poured off, the mixture being pressed after each refluxing. In this way one carries out the three refluxings with the 2.5 to 3 volumes of acetone originally used. The refluxing of the glands is essential in order to extract the hormone which is tenaciously held by the lipids of the adrenal tissue. Three successive refluxings for about one-half hour, at the boiling point of acetone, extracts about 95 per cent of the hormone present in the glands. A fourth and fifth reextraction and refluxing give only small amounts of the hormone. Since the purified preparations of the hormone are soluble in acetone, it would be improbable that this solvent fails, in subsequent extractions, to dissolve the hormone. Moreover, extraction of the glandular residue with ethyl alcohol, ether, ethylene dichloride, acid, or alkali fails to yield any appreciable amount of the hormone.

Since the adrenal cortical hormone is not completely adsorbed from aqueous acetone solution by charcoal, the acetone must be removed before proceeding to adsorb the hormone on charcoal. After being chilled in the ice chest, the acetone extract, obtained as described in the preceding paragraph, is

filtered to free it from the separated lipids. The clear filtrate is distilled *in vacuo* at 35 to 40° until all the acetone is removed. The completeness of this removal may be determined from the specific gravity of the residual aqueous fluid, which should be over 0.99 at room temperature. After being chilled in the ice chest, the solution is again filtered to remove the separated lipids. The filtrate is carefully neutralized with an aqueous solution of NaOH until neutral (pH 7.0) and shaken at intervals with activated charcoal (norit or decolorizing carbon). One gram of charcoal is used for every 200 grams of glandular material originally extracted. After some hours (6 or more, depending on the efficiency of the shaking) the carbon is collected on a Buchner funnel. The filtrate contains only a trace of hormone, but this can be recovered by treatment again with charcoal.

Before utilizing the charcoal-hormone combination prepared, as described above, it is necessary to remove most of the epinephrine and other impurities which contaminate it. To remove these impurities the charcoal-hormone combination is suspended in about 3 times its volume of 10 per cent hydrochloric acid (1 part of 33 per cent HCl to 10 parts of water). After thorough agitation, the suspension is filtered on a Buchner funnel. The charcoal is then suspended in about 3 times its volume of a 0.1 N sodium hydroxide solution, agitated, and again filtered. It is then again washed with 3 volumes of 2 per cent hydrochloric acid and finally washed with a little distilled water and filtered. It is now ready for use. The charcoal-hormone combination may also be effectively freed of adsorbed impurities by washing on the Buchner funnel with dilute ammonia, water, and ethyl alcohol, respectively. These reagents elute an inappreciable amount of the hormone.

The amounts of acid and alkali used for washing the charcoal are relatively small, and hence do not remove an appreciable quantity of the hormone. The trace removed can be reclaimed by neutralizing the washings and readsorbing on a

FIG. 13. THE GROWTH CURVES OF ADRENALECTOMIZED RATS TREATED WITH THE CHARCOAL-HORMONE PREPARATION

Four rats, adrenalectomized at the age of 30 days were treated daily with 0.1 gram each of the charcoal-hormone combination (equivalent to 80 grams of whole pig adrenal glands) which was mixed with their food. Lettuce was added twice weekly. At the age of 90 days, when the animals had reached adult size, treatment ceased and the animals gradually developed the symptoms of adrenal insufficiency and died within the course of the 3 weeks following cessation of treatment. At autopsy no regenerated adrenal cortical tissue or accessory glands were demonstrable. The lungs were normal and there were no evidences of any disease other than the typical findings of adrenal insufficiency. (Reproduced through the courtesy of the *Journal of Biological Chemistry*.²⁵¹)

little charcoal. The charcoal-hormone compound when administered therapeutically comes into contact with many times its own volume of gastro-intestinal juices which succeed in

eluting the hormone from its adsorbant. Since the charcoal remains in contact with the alkali for only a few minutes during the washing, no appreciable amount of the hormone is destroyed by this procedure.

For administration to rodents, dogs, or other experimental animals, the desired amount of charcoal is thoroughly admixed with the food. For clinical application it may be administered in capsules or compressed into coated tablets to avoid access of air and possible oxidation of the hormone.

Although the adrenal cortical hormone is rapidly destroyed (probably by autolytic processes) in the adrenal glands after death, the hormone is quite stable in the form of its charcoal adsorbate. Thus a preparation assayed at monthly intervals was found to undergo no detectable deterioration for a period of one year when left exposed at room temperature and in contact with the air.

The charcoal adsorbate of the hormone, as shown in Figures 13 and 15, is capable of maintaining life in adrenalectomized animals, the hormone being eluted in the gastro-intestinal tract from its combination with the charcoal. The ability of the charcoal-hormone preparation to maintain life in adrenalectomized dogs has also been demonstrated by the author and his collaborators.²⁵¹

In preparing or purifying extracts of the adrenal, it must be remembered that the cortical hormone attaches itself readily to lipids and is carried down to some extent whenever lipids are precipitated. The hormone thus precipitated may be recovered by dissolving the lipid in alcohol or acetone, diluting with water to precipitate the lipid, distilling off the alcohol or acetone, and adsorbing the hormone on charcoal as described above. When glands are not freshly ground into the acetone used for their extraction, one finds a great accumulation of the extraneous lipid present in the aqueous residue left after removing the solvent. This lipid will contain an appreciable amount of the cortical hormone, which can be recovered by the method

just outlined. Failure to remove this lipid thoroughly from the final aqueous solution will also interfere with the efficient adsorption of the hormone by charcoal.

ELUTION OF THE HORMONE FROM ITS CHARCOAL COMBINATION

When large amounts of the charcoal adsorbate are administered, the efficiency of the utilization of the hormone is reduced, probably because of the incomplete elution of the hormone in its passage through the gastro-intestinal tract. To avoid this loss of activity the hormone must be eluted from the charcoal and administered uncombined. This elution may be satisfactorily accomplished by the following procedure: The charcoal-hormone combination is suspended in three to five times its volume of chloroform. After some hours the chloroform is removed through a Buchner funnel and the process repeated six times. The chloroform is removed by distillation *in vacuo* leaving a brownish resin soluble in aqueous ethyl alcohol. This alcoholic solution may be administered as such, or the resin may be dissolved in a little ethyl alcohol and incorporated with sodium chloride, lactose, starch, or other suitable carrier and administered in capsules or compressed tablets.

The use of the product just described avoids the necessity of administering large amounts of inert charcoal. When relatively small amounts of the hormone are to be given, the charcoal combination proves adequate. When large doses of hormone are necessary, however, the use of excessive amounts of charcoal would be inefficient and undesirable, and in these cases the oral administration of the eluted hormone is preferable. For clinical use, an alcoholic solution (prepared as described above) would offer an ideal vehicle for the administration of the hormone orally. Alcohol (or glycerine) would incidentally serve as a preservative and prevent destruction of the hormone.

EXTRACTS FOR PARENTERAL ADMINISTRATION

In cases of patients in crisis and in other conditions in which oral therapy is impossible, the use of a purified aqueous extract

suitable for parenteral administration is desirable. The following procedure has been found by the author to give extracts suitable for parenteral (subcutaneous, intraperitoneal, intramuscular, or intravenous) administration.

The brownish resin obtained after eluting the charcoal hormone combination with chloroform and removing the chloroform is dissolved in the smallest possible amount of ethyl alcohol. About 10 volumes of ethyl ether (free of peroxides) is now slowly added and any precipitate which may form is discarded. To the alcohol-ether solution is now added distilled water (1 cc. for each 50 grams of the original glands) and the ether and alcohol removed by distillation *in vacuo*. The resulting aqueous solution is filtered and the water-insoluble precipitate again treated in the same manner as was the original resin. This repetition is necessary in order to remove the hormone which is adsorbed on the lipoidal precipitate. If an abundant precipitate is present repeated extraction and precipitation may be necessary for the complete transferral of the hormone to aqueous solution. The combined aqueous solutions are now evaporated *in vacuo* at 45°C. The resinous material which remains in the flask is again dissolved in the least amount of ethyl alcohol and precipitated with an excess of ether as described above. The hormone remains in the alcohol-ether solution while the insoluble fraction is inert. After filtration and evaporation of the solvents, this process is repeated. The final resin is extracted with 1,4 dioxane which dissolves the hormone leaving an inert residue. The dioxane solution is displaced with physiological solution and filtered after thorough cooling in the ice chest over night. The product thus obtained after sterilization by filtration has been found suitable for parenteral administration.

The final aqueous solution obtained, as described above, contains relatively little solid matter aside from the added salt. Thus 10 kilograms of fresh beef adrenals gave 20 cubic centimeters of a final extract which when assayed was found to contain 500 rat units (as defined in the next chapter), and

had a solid content of 2.5 milligrams per cubic centimeter, exclusive of the added salt.

EFFICACY OF THE ADRENAL CORTICAL HORMONE

The efficacy of the preparations of the adrenal cortical hormone described in the preceding sections has been demonstrated by the fact that they offer a complete replacement therapy in adrenalectomized mice, rats, cats, and dogs. When these animals are maintained on adequate doses of the hormone they survive the effects of a complete adrenalectomy and manifest no signs of any deficiency. Since the purified extracts maintain all the normal physiological functions of such animals, it is unnecessary to assume, as some have done, that the adrenal elaborates several hormones, the combination of which must be administered to maintain normal behavior in the absence of the adrenals. Nor is it necessary to administer with the extracts an excess of salt over that normally present in the animal's food. Until definite evidence to the contrary is brought forward, there is no reason, therefore, to hypothesize that the adrenal cortex produces more than one hormone. The published evidence which purports to demonstrate a multiplicity of hormones is not convincing and is probably due to poor experimental data and the use of impure and impotent extracts.

In Figure 13 are reproduced typical growth curves of rats treated for 60 days following complete adrenalectomy with the charcoal-hormone combination. The animals grew normally to their adult size and manifested all the signs of perfect health and normal physiological function. The charcoal-hormone preparation is thus capable of supplying a complete replacement therapy for the adrenal cortex. After cessation of the treatment the animals of Figure 13 died within one to two weeks and careful examination of microscopic sections of the adrenal sites revealed no remnant of active or exhausted cortical tissues.

Results similar to those of Figure 13 are obtained when the eluted hormone or the extract prepared for parenteral administration are utilized as is shown in Figure 16.

DOSAGE

There has been no investigation of the dose of the adrenal cortical hormone required by man. This will obviously be a variable dependent on the severity of the adrenal insufficiency

TABLE 2

The adrenal cortical hormone requirement of various animal species
(Grollman and Firor²⁴⁹)

ANIMAL SPECIES	NUMBER OF ANIMALS	WEIGHT	SURFACE AREA	RAT UNITS OF HORMONE NECESSARY PER DAY FOLLOWING ADRENAL-ECTOMY	RAT UNITS PER SQUARE METER OF BODY SURFACE PER DAY
		<i>kgm.</i>	<i>square meters</i>		
Mouse.....	6	0.02	0.0084	1.0	120
Rat.....	10	0.25	0.036	3.5	97
Cat.....	3	2.0	0.16	20	125
Dog.....	6	8.4	0.46	55	120
Man*.....	—	60.0	1.89	220*	—

* The value for man is estimated from the data obtained on the experimental animals, assuming the human requirement to be proportional to the surface area.

in a given patient. For practical purposes, however, it is essential to know approximately the amount of hormone necessary to supply the needs of an individual. This value may be derived indirectly from the data of Table 2, and is based on the hormone requirement of adrenalectomized mice, rats, cats, and dogs. The fourth column of the table records the average surface area of the animals studied as calculated from their body weight, using Rubner's data for the value of the constant in Meeh's formula. The fifth column records the average dose of the hormone necessary to maintain life in the adrenalecto-

mized animals, expressed in terms of rat units. The rat unit as defined in the next chapter is the amount of hormone necessary to maintain normal growth in a 30 day-old adrenalectomized rat. The requirement for maintaining growth in a young animal as compared with the requirement for maintaining life in an adult of the same species is out of all proportion to their relative weights. Thus, whereas one rat unit is necessary for maintaining growth in an immature rat weighing 30 grams, it requires only $3\frac{1}{2}$ rat units to maintain life in an adult rat weighing 250 grams. Likewise, the requirement for maintaining growth in a one-kilogram puppy was found to exceed the average dose necessary for maintaining life in adult dogs weighing 5 to 15 kilograms.

The last column of Table 2 shows that the adrenal cortical requirement of mice, rats, cats, and dogs is roughly proportional to their surface areas. This proportionality is remarkable when we consider the wide individual variations which one encounters in different animals of the same species. Since the surface area is roughly proportional to the active mass of an individual and to his metabolism, it is not inconsistent that the adrenal cortical hormone requirement should also be proportional to this body function. If we assume the validity of this proportionality, the data on mice, rats, cats, and dogs permit us to estimate the human requirement as being about 220 rat units per day. This amount will probably seldom be necessary clinically since the diseased individual, unlike the experimental animal, still possesses some residual adrenal function.

One rat unit as defined in the following chapter corresponds to the hormone content of 10 to 30 grams of whole beef adrenal glands, depending upon the freshness of the glandular material. We thus see that a relatively large amount of glandular material is necessary for obtaining a sufficient amount of the hormone to be effective therapeutically.

No valid evidence has been adduced to show that any effects

follow the administration of excessive quantities of the cortical hormone to normal animals. The claims of Britton and his collaborators that the hormone produces changes in the blood sugar⁸⁹ or the functional activity of the reproductive system¹²⁷ have been shown to be erroneous.^{267, 321} Other effects which have been described are also probably to be attributed to the presence of impurities present in the extracts. The adrenal cortical hormone, as judged from preliminary studies of its chemical nature, is of relatively small molecular weight, and any excess in the body is probably rapidly destroyed or excreted in the urine. As long as the hormone is administered orally, the danger of toxic effects from slight amounts of epinephrine or other impurities is also minimized. For parenteral administration, only highly purified extracts free from phenolic derivatives, epinephrine, histamine, choline, and extraneous lipids should be employed. Except in severe crises or where oral therapy is impossible, frequent, divided doses given orally should furnish all the requirements of the organism for the adrenal cortical hormone.

CHEMISTRY OF THE CORTICAL HORMONE

Several preliminary reports have been made of the isolation of the adrenal cortical hormone in crystalline form. However, it is unwise to accept any product as the hormone until detailed evidence is presented to show that the compound is a chemical entity and not a mixture of substances and that, when administered to a completely adrenalectomized animal, the crystals supply a complete replacement therapy. No complete report of such evidence has as yet been published. Kendall³⁴⁹ in a preliminary communication states that the hormone has the empirical formula $C_{21}H_{34}O_5$ but Wintersteiner and Pfiffner⁶⁸² isolated what is apparently the same compound and found it to be inactive. More detailed and convincing evidence must be presented before we can accept the cortical hormone as having been obtained in crystalline form. The

chief difficulty of obtaining such evidence, in the author's experience, lies in the extremely small yield of the crystalline products which are obtained from the glands, as is also the case in the isolation of other hormones. It requires the extraction of about 200 kilograms of fresh glands to yield approximately one gram of the crude hormone. To one having the facilities for obtaining this material the eventual isolation of the hormone should not be difficult. The non-protein nature of the hormone should make it amenable to crystallization and its reactivity should permit the formation of easily crystallizable derivatives.

The results of chemical investigations based on the use of extracts are always uncertain. Only results obtained on pure crystalline products are acceptable as truly characterizing the hormone. The presence of any impurities will always vitiate the validity of results obtained on heterogeneous extracts. We shall not therefore enter into great detail in an enumeration of such chemical properties of the cortical hormone as have hitherto been described. Several properties of the hormone which are of importance should, however, be mentioned here. The hormone is not a protein. This is demonstrated by its solubility relations and its effectiveness when administered orally. The purest extracts obtained by the author have always been readily soluble in polar liquids (ethyl or methyl alcohol), less so in non-polar solvents (ether or benzene). The presence of extraneous lipids alters these solubility relationships so that we find impure extracts to be readily soluble in ether and insoluble in water, whereas the reverse is true of the purer preparations. This apparent paradox is frequently observed in dealing with biological mixtures. The lipids of the adrenal can tenaciously hold the hormone and determine its apparent solubility. Thus the hormone may be extracted from its crude aqueous solution by benzene. On the other hand, benzene or ether will not readily extract the purified hormone from aqueous solution, for when purified it is no longer being held by extraneous lipids.

Several workers have reported physiological experiments, the results of which they attributed to the cortical hormone because the effects were not elicited after heating the extract to boiling. As a matter of fact, the hormone is not readily destroyed by heat. At a pH of 6.7 one can maintain the extract at its boiling point for ten minutes without appreciable destruction of the hormone. Heating strongly acid or alkaline solutions of the hormone, on the other hand, leads to its rapid destruction. In fact, if kept in an alkaline aqueous solution in the refrigerator, the hormone will be found to become inactivated. Apparently it undergoes a molecular rearrangement or other spontaneous chemical change in the presence of alkali. This destruction must be guarded against by keeping all aqueous solutions of the hormone at a pH of about 6.8 to 7.0.

One of the most striking chemical properties of the purified extract of the hormone is the ease with which it reduces a number of the common reagents,—permanganate, silver nitrate, phosphomolybdic acid, *etc.* If this property proves to be inherent in the hormone, and not a result of contaminating agents, it will offer a chemical method for estimating the hormone. The fact that extracts free of ascorbic acid, uric acid, and epinephrine still reduce the Folin-Denis reagent makes it impossible to use this colorimetric method for the determination of the epinephrine content of cortical extracts. Extracts in which epinephrine may be shown to be absent by the usual biological tests, still give the blue coloration when tested by this method.

Wintersteiner and his collaborators⁵¹² have recently described a procedure for concentrating the cortical hormone. Their final product is a yellow syrup containing the hormone, which they believe to be an α - β -unsaturated ketone. The evidence adduced to substantiate this view is not, however, conclusive.

CHAPTER XVI

THE ASSAY OF THE ADRENAL CORTICAL HORMONE

No chemical method being as yet available for detecting the cortical hormone, it is necessary to depend upon some biological method for the assay of adrenal cortical extracts. The accurate determination of such assays is extremely important. Chemical studies on the hormone can only be made if one possesses a relatively simple method of assay with which to follow the effects of various manipulations. In therapeutic applications of the hormone it is necessary to know the potency of the extracts which are administered. Much of the confusion which exists at present is a result of erroneous ideas as to the hormonal content of a given extract. Thus, the observed effects of a given extract may be attributed to its assumed high potency, when, in reality, careful assay may demonstrate almost the complete absence of the hormone in the extract. The futile use of the same extract clinically may lead to the erroneous conclusion that the cortical hormone is useless in disease.

The various methods suggested for assaying the adrenal cortical hormone may be divided into three groups: 1) methods based on the increased susceptibility of an adrenalectomized animal (usually the rat) to a drug or abnormal physical condition; 2) methods based on some change in the chemical composition of the blood of adrenalectomized animals; 3) methods based on the determination of the amounts of hormone necessary for maintaining normal physiological activities in adrenalectomized animals.

METHODS BASED ON THE DIMINISHED RESISTANCE OF ADRENALECTOMIZED ANIMALS

Perla and Gottesman⁴⁹⁷ have suggested the increased susceptibility of adrenalectomized rats to histamine as the basis

for the assay of cortical extracts. De Meio and Lewis recommended the use of morphine as the toxic agent. As shown by Leloir and Novelli,³⁸⁷ the use of morphine does not lend itself for an exact assay. This is also true of the use of histamine, diphtheria toxin, and other drugs or physical agents. The weakness of these methods lies in the fact that the sensitivity of different animals (normal or adrenalectomized) varies greatly towards a given drug. An excessively great number of animals must consequently be used in order to obtain approximately representative results. The reaction of the adrenalectomized animal will be even more variable because it will depend upon the degree of insufficiency existing at the time of injection of the drug. This is an extremely variable quantity. Even if one were to fix empirically the conditions of the experiment with a high degree of constancy, the method would still remain inexact and require the operation and sacrifice of scores of animals in order to attain even approximately accurate results. A fundamental defect of all methods based on the increased susceptibility to toxic agencies after adrenalectomy is the fact that such increased sensitivity is in part due to the secondary effects of adrenal insufficiency in lowering the general resistance of the body. The methods, therefore, only indirectly measure the degree of adrenal insufficiency.

The working capacity of adrenalectomized rats is decreased in adrenal insufficiency. It has been suggested that this property be used to standardize cortical extracts.¹⁹¹ The objections raised in the preceding paragraph are also applicable to this method of assay.

METHODS BASED ON THE NON-PROTEIN NITROGEN LEVEL OF THE BLOOD

The second method which has been used in assaying adrenal cortical extracts is based on determining the non-protein nitrogen of the blood of the adrenalectomized dog, as suggested

by Harrop, Swingle, and their collaborators.²⁶⁴ Adult dogs adrenalectomized in two stages are given a dose of hormone which maintains the non-protein nitrogen at its normal level. When constant conditions are attained, the dosage is progressively reduced at 7 to 10 day intervals until the blood non-protein nitrogen rises to a level above 50 mgms. per 100 cc. The potency of the extract is expressed in terms of the dog unit which is defined as the minimum daily kilogram dose necessary for maintaining a dog for a period of 7 to 10 days without loss of body weight and elevation of the non-protein nitrogen level of the blood.

Although superficial considerations would indicate that this method should prove satisfactory, it suffers in practice from several vital defects. The period of 7 to 10 days, recommended for the maintenance of the animal on a given dose of extract, is entirely too short. Adrenalectomized dogs, maintained on an adequate dose of hormone for some time will often not manifest any signs of insufficiency for 15 to 25 days. This is not unexpected when one considers the survival period after adrenalectomy. Cessation of therapy does not involve any operative trauma or the use of anesthetics and hence the survival period in good health is much longer than it is following adrenalectomy. Consequently, to apply correctly the method of Harrop and his colleagues would involve periods of observation of about a month and necessitate the use of at least several animals to avoid individual variations. For a dependable assay it would also be necessary to sacrifice the animals at the end of the experiment and prove the absence of accessory tissues or remnants of the adrenals left at operation. To boast that one has used the same animal over a period of years (as has been done) is to display a naive confidence in the operator's infallability, which is undeserved.

The assay of cortical extracts by the dog method, if accurately performed, would involve so much labor, require such an immense amount of extract, and be so long and tedious as

to be entirely impracticable. The same objections as have been outlined above are applicable to assay methods based on the changes occurring in other blood constituents (*e.g.*, the sodium chloride) of adrenalectomized animals. In general, methods involving the use of adult adrenalectomized animals are much less satisfactory than those based on the use of young immature animals. Extraneous factors, such as the administration of excessive amounts of salt in the diet, will give deceptive results in adult animals. In young animals, on the other hand, such factors are less apt to vitiate the results, as shall be demonstrated in the next section. Many erroneous conclusions found in the recent literature are to be attributed to a failure to appreciate the ease with which an assay method may be vitiated by the factors outlined above.

THE RAT METHOD OF ASSAY

The crucial and direct test of the potency of an adrenal extract is its ability to maintain an adrenalectomized animal in perfect health. To apply such a test to an adult cat or dog would require, as we have seen, a period of observation of at least a month on several animals and involve the use of large quantities of extract. The use of the month-old-rat, according to the technique described in the present section, affords a simple and accurate method of assay which involves the use of minimal amounts of extract, can be performed with a modicum of effort, and gives results which are reproducible with a variation of less than 15 per cent. The method depends upon determining the growth curve of month-old adrenalectomized rats for a period of one week during which they are maintained on the cortical hormone. Month-old rats are used because complete adrenalectomy is relatively easily performed on these animals, their normal survival period is short (less than a week), and their normal rapid growth ceases following adrenalectomy. Moreover, the results obtained on immature animals are less apt to be vitiated by extraneous factors, such

as the presence of excess salt in the diet. It is only necessary to determine the amount of a given extract necessary for maintaining normal growth during the week following adrenalectomy, to obtain an accurate estimate of the potency of the extract.

The operative technique. Complete adrenalectomy is much more easily performed in the month-old rat than it is in adult animals. The muscles and connective tissue are so delicate and thin that they are practically transparent. The blood vessels are so small that ligatures or other forms of hemostasis are unnecessary. The rat is not prone to pyogenic infections and hence an aseptic technique is also unnecessary.

The animal is anesthetized by an intraperitoneal injection of a solution of one of the barbiturates. Nembutal has been found satisfactory for this purpose. About 0.8 mgm. of this drug is required for a month old rat. There is a considerable individual variation in the amount of anesthetic necessary and hence it is desirable to inject first the minimal dose producing anesthesia and then repeat with a second small dose when necessary.

When the animal is deeply anesthetized it is placed on a small block and the hair over the lumbar region is moistened with water. Shaving the operative site is not necessary. The skin over the spine is now lifted with a forceps and a single transverse incision is made through the skin, at the level of the costal margin and extending for about 2 centimeters across the back (Figure 14).

To facilitate the removal of the adrenals, it is desirable that the incision through the lumbar muscles be made as nearly as possible over the site of these glands. On the left side, the spleen is used as a convenient landmark. This organ appears as a black shadow through the thin lumbar muscles. A tiny incision⁶ is made into these muscles at the posterior border and about 5 mms. below the upper margin of the splenic shadow. The incision is widened by inserting and spreading the scissors.

FIG. 14. ADRENALECTOMY IN THE RAT

Showing the size and position of the lumbar skin incision in a month-old rat

The left adrenal in the rat lies superficially, just anterior to the kidney. It comes readily into view when the incision in the lumbar muscles is held open by spreading the forceps.

In order to perform a complete extirpation of the glands in the rat, it is essential to avoid grasping the gland or its capsule. The gland is very friable and it is an easy matter to leave a bit of the capsule attached to which is a thin layer of cortical cells. These if left in the body multiply and may maintain the animal alive indefinitely, particularly after a short period of therapy with salt or the cortical hormone. The microscopic nests of accessory bodies are present in the veil of loose connective tissue which surrounds the adrenals. This veil of mesentery must therefore be removed together with the main gland by making a wide excision of the connective tissue surrounding the glands. This is easily done by using a curved forceps with which one grasps the pedicle of the gland and thereby includes the above mentioned mesentery which contains any accessory cortical tissue. Should it be necessary to grasp the gland more securely, this is done by applying a second forceps proximal to the first. The adrenal is now gently brought forward and the pedicle is cut with a scissors leaving the gland and its attached connective tissue in the grasp of the forceps.

The right adrenal is situated somewhat more anteriorly than the left and it is covered by the liver. Its removal is therefore somewhat more difficult to effect. The incision through the muscles should be made along the costal margin by the method described for the left gland. This incision is held open with a straight forceps while the kidney is gently depressed with the curved forceps. The adrenal is then seen lying anteriorly to the kidney and covered by a lobe of the liver. While the gland is kept in view by depressing the kidney, the forceps is projected and is made to embrace the gland and its surrounding connective tissue as in extirpating the left gland. By gentle traction the gland is raised, and the pedicle is cut.

The incisions in the lumbar muscles, which should be about

5 mms. long, are closed by a single suture with silk thread. The skin incision is then closed by several interrupted sutures.

With experience, the above operation can be performed with ease in less than 5 minutes. If one is careful not to use an overdose of the anesthetic and avoids tearing any of the large blood vessels or injuring the kidney or liver, the mortality from the operation is practically nil. The animals recover and within several hours appear perfectly normal. In animals dying within 48 hours following the operation, the cause of death is to be attributed to gross mishandling with consequent traumatic shock.

By skillful technique it is possible to perform a complete adrenalectomy and removal of all the accessory cortical tissue in over 95 per cent of all animals. Occasionally, a slip of the forceps, or failure to include the necessary amount of the connective tissue results in leaving an accessory body. Such unsuccessful operations are detected by the prolonged survival of the animal after cessation of treatment and do not result in self-deception as regards the potency of a tested preparation.

Food and care of the animals. In order to avoid any variations in the food intake due to changes in the diet, the animals should, when weaned at the age of 20 or 21 days, be placed on the same diet as they receive during the assay period. Any of the well-known diets which furnish all the essentials for normal growth is satisfactory. The diet should be in powder form to permit easy mixture of the hormone preparation with the food. The following modification of the Steenbock diet is suitable for the purpose:

	<i>Grams</i>
Finely ground yellow corn.....	2280
Powdered whole milk powder.....	1000
Linseed oil meal.....	480
Cascin.....	150
Alfalfa meal.....	60
Sodium chloride.....	15
Calcium carbonate.....	15

Since the survival of animals is determined to some extent by the diet used, this should be standardized and remain unvaried throughout the period of assay. Rats, even in adrenal insufficiency, may be tempted by choice morsels which will give the false impression of growth. Since only a short time is necessary for the completion of the assay, the addition of lettuce to the above described diet twice weekly, as ordinarily done in protracted experiments, is unnecessary.

In assaying a given preparation, a measured amount is injected or preferably mixed with the animal's food. The author has found that the use of six rats for each test precludes the vitiating factor due to individual variations, if one has available a stock of animals of uniform quality. When the hormone is orally administered one obviously must make allowance for the daily food requirement of the animals. On the day following operation, each of the animals consumes about 5 grams of the mixture described above. During the course of a week of treatment their food intake gradually increases to about 10 grams per rat per day. With practice one easily estimates the quantity of food necessary for a day and this amount is mixed with a given dose of the hormone daily. The rats are weighed daily at the same hour. It may be objected that with oral therapy as just described each rat does not necessarily ingest an equal share of the hormone nor is the daily allotment always exactly equal to the daily food requirement. As a matter of fact, these objections are not of great practical significance. Any slight over- or under-estimate of the daily food requirement is compensated for by the next day's feeding. Individual variations in the food intake of the different animals can be avoided by maintaining each rat in a separate cage.

In Figure 15 are reproduced typical curves showing the average gain or loss in weight of groups of six rats maintained under various conditions. The lower curve demonstrates the

FIG. 15. TYPICAL GROWTH CURVES OBTAINED IN THE ASSAY OF THE ADRENAL CORTICAL HORMONE

The growth curves of groups of six rats maintained on a constant diet. With the exception of the animals of the "Control Operation" curve, all the rats were adrenalectomized at one sitting at the point indicated as usual on the abscissa. The animals of the "Control Operation" were subjected to a dummy operation in which the usual incisions were made without interfering with the adrenals. Except for the group of "Adrenalectomized Controls" which received no treatment, each animal received daily for seven days an amount of the *charcoal-hormone* combination derived from 3, 5, 8, 10, 13, 15, 20 and 30 grams, respectively, of whole beef glands as indicated on the respective curves. *x* indicates the death of an experimental animal.

slight loss of weight suffered by rats receiving no therapy. The other curves show the gain in weight of rats receiving various doses of the same *charcoal-hormone* preparation for seven days. As the amount of hormone administered is increased, the growth curve approximates more and more that of a group of animals subjected to a "control" operation in which the skin and muscle incisions were made without interfering with the adrenals.

As seen in Figure 15, it is easier to determine the amount of hormone necessary for allowing a daily increment of about 1.5 grams in weight than to determine the amount necessary for permitting the same rate of growth as is observed in normal animals. Hence, rather than determine the latter quantity, which may be defined as a rat unit, it is easier to determine the minimum quantity of hormone which allows a gain in weight of 1.5 grams per rat per day. This quantity, as may be seen from Figure 15, is about one-half of a rat unit.

In assaying a given preparation, the number of groups of animals to be used will obviously depend upon the accuracy desired. For an accurate assay of a large batch of available hormone, several groups of animals are maintained on various amounts of hormone as exemplified in Figure 15. Where one is only interested in obtaining an approximately quantitative result, a single group of animals suffices and the dose may be altered from day to day until one approximates the dosage which results in a daily gain in weight of about 1.5 grams per rat.

After the expiration of the period of treatment (7 to 10 days) the animals are maintained on the same diet without addition of the hormone and their weight curves followed, as shown in Figure 15. The cessation of growth after stopping treatment and the subsequent death of the animals in the course of five to ten days is an indication of the completeness of the extirpation. If the adrenalectomy is incomplete the animals continue to grow and live for long periods. One thus has a check upon the completeness of the original operation and

avoids deceiving oneself by results based on an incomplete operation.

THE SUPERIORITY OF ORAL OVER PARENTERAL ADMINISTRATION OF THE CORTICAL HORMONE

The parenteral administration of any therapeutic agent suffers from the disadvantage that it necessitates the preparation of a highly purified product. The presence of toxic impurities may easily mask the beneficial effects of any hormone present in a given extract. Thus, in an experiment in which an incompletely purified extract was administered intraperitoneally to adrenalectomized rats, doses of one cubic centimeter per rat per day failed to provide complete replacement therapy. The same extract administered orally by mixing with the animals' food served as a complete replacement therapy in doses as small as 0.05 cubic centimeters. The noxious impurities present in this extract were without deleterious effects when administered orally.

Another important advantage favoring oral therapy in administering the adrenal cortical hormone is the fact that this hormone is much more effective when administered in multiple doses than when an equal amount is administered in a single dose. This advantage of divided doses at frequent intervals is attained practically only with an oral method of therapy.

The early work on the relative efficacy of oral as compared to parenteral administration of the cortical hormone indicated that it required about five times as great a dose for oral use as for intraperitoneal injection.²⁴⁶ This apparent superiority of the parenteral mode of administration was based on a comparison of the effects of a single dose administered by stomach tube with the effects of a single injection. However, if one compares the effects of a single parenteral injection with the results which follow the administration of the hormone in the animals' food, one finds that the oral method of administration yields better therapeutic results than the parenteral administration of the

FIG. 16. A COMPARISON OF THE ORAL AND INTRAPERITONEAL METHODS OF ADMINISTERING THE ADRENAL CORTICAL HORMONE AND THE EFFECTS OF SALT THERAPY

The growth curves of groups of six "month-old" rats adrenalectomized at one sitting on the day indicated as 0 on the abscissa. The animals in the upper curve received daily for seven days a total of 1 cc. of an extract of the cortical hormone which was incorporated with the animal's food. The animals in the second curve were injected with an equal amount of the same extract intraperitoneally once daily. Comparison of the two upper curves illustrates the more favorable growth response which follows oral as compared to parenteral therapy. The lowest curve shows the failure of growth in the untreated control animals. The adjacent curve shows the favorable effects of therapy with a mixture of sodium chloride and bicarbonate. *x* indicates the death of an experimental animal.

same dose of the hormone. In Figure 16 are reproduced the average growth curves of groups of six adrenalectomized rats. One group of six rats was given an intraperitoneal injection of a purified extract. The food of a second group of six animals was mixed with an amount of the extract exactly equal to that injected into the first group. As is evident from Figure 16 the oral form of therapy gave a markedly superior therapeutic effect as compared to the injection of an equal amount of the same extract.

ACCURACY OF THE RAT ASSAY METHOD

One can assay preparations of the adrenal cortex by the rat method with a degree of accuracy which is unusually good for a biological method. Obviously, for the best results, certain rigid conditions must be maintained. The animals used should be uniform in quality and in good health. They should be maintained under constant conditions at a moderate and uniform room temperature (25°C.).

One would hardly expect different workers under varying laboratory conditions and using diverse operative techniques, to obtain perfectly identical results. To render the results of different workers comparable it would be necessary to have some standard as a unit of comparison. Unfortunately, such a standard is not available at present. However, the white rat is relatively uniform in its reactions and by using a standardized technique, comparable results should be obtainable by different workers. In the author's experience, the rat method of assay as outlined above is reproducible to within $\pm$ ten per cent.

As shown in Figure 16, the administration of an excess of sodium chloride and bicarbonate in the diet does not permit normal growth in young adrenalectomized rats. The same is true of the administration of Ringer's solution in place of drinking water, or the injection of large amounts of physiological saline or Ringer's solutions. Only when the operation

has been incomplete and residual fragments of the adrenals or accessory glands have been left does the animal grow normally. In the case of adult animals the ability of adjuvant therapy with salt to maintain animals in relatively good condition for extended periods may lead to fictitious results in evaluating the effects of therapy with adrenal extracts. Young animals which grow normally under such adjuvant therapy may be shown to possess some functional interrenal tissue.

CHAPTER XVII

THE RELATION OF THE ADRENALS TO TOXINS, INFECTION, AND IMMUNITY

Adrenalectomized animals are exceedingly sensitive to toxins.^{52, 138, 155, 394, 479, 629} They are prone to infections and rapidly succumb to disease processes which have only trivial effects in normal animals. This same loss of tolerance to toxins and infections is manifested in Addison's disease, in which minor ailments may precipitate the patient into a crisis. Because of these oft-observed hypersusceptibilities of the organism in adrenal insufficiency it has been thought that the adrenals are in some way responsible for the production of those immunological bodies which protect the normal individual.⁶¹

Further evidence implying a possible relation of adrenal function to infectious processes is found in the changes which the adrenals undergo as a result of various types of toxic agents and infections. Many of the anatomical changes described in the literature as associated with infectious processes are undoubtedly merely artifacts due to an imperfect technique or to *post-mortem* changes which are particularly marked after death from certain diseases. Nevertheless, there exists sufficient valid evidence to substantiate the idea that toxins and many infectious processes induce definitely recognizable changes in the histological appearance of the adrenal glands. It does not necessarily follow, however, that these histological changes indicate an intimate relation between the function of the adrenals and infectious or toxic processes.

HISTOLOGICAL EVIDENCE OF ADRENAL INJURIES

The adrenal after various forms of injury undergoes changes which differ according to the agent used in producing the in-

jury and the dosage employed. With chloroform narcosis,²³⁷ an immediate damage is produced which is rapidly repaired. The earliest changes observed are congestion of the medulla and inner cortical zones followed by degeneration of the cells of the *zona reticularis*. A large proportion of the cortical cells may be ultimately destroyed. Recovery sets in within 48 hours during which the undamaged cells in the more peripheral layers of the cortex show active mitosis. This regenerative process is similar to that observed following necroses due to infectious processes. The regenerative capacity of the adrenal is entirely comparable in its extent with that of other organs as, for example, the liver. The *zona glomerulosa* and the outer portion of the *fasciculata* constitute the growth center where regeneration occurs, the newly formed cells being displaced inwardly to supply the loss at the inner layers.

In contrast to the effects of chloroform inhalations, the injection of diphtheria toxin produces a progressive injury of the adrenals the repair of which is slow.³⁰¹ Large doses of the toxin produce hemorrhages and focal necroses (chiefly in the *reticularis*) which during repair are replaced in part by connective tissue. Small doses decrease the lipid content (as observed microscopically) of the cortex and cause widening of the reticular zone at the expense of the *fasciculata*. Diphtheria toxin also causes a marked reduction in the epinephrine content of the adrenals but this decrease, as Edmunds and Johnson¹⁷² showed, is not the cause of the circulatory collapse observed in the terminal stages of animals poisoned by this toxin.

In scurvy and other diseases of inanition there is a marked increase in the lipid content of the cortical cells with granulation of the mitochondria. Administration of vitamin C to scorbutic animals causes active regeneration of the injured tissue.³⁰¹

From observations in a number of diverse diseases, Elliott¹⁸¹ showed that the storage and disappearance of the cortical lipid occurred under conditions entirely different from those control-

ing the body fat in general. The cachexia of cancer or diabetes, though depleting the store of fat from the body, leave unchanged the lipid of the adrenal cortex. Pathological intoxications, such as diphtheria, increase the apparent fat in the heart and kidney while they exhaust the cortical lipids. The cortical lipid may be unchanged in conditions in which the epinephrine store of the medulla is depleted. The greatest loss of epinephrine from the adrenals was observed by Elliott in cases of afebrile acute cardiac failure.

The world-war afforded an opportunity of studying the histological appearance of the adrenals of individuals suddenly killed in the prime of health and comparing the results with those observed in their comrades dying of various toxic or infectious processes. In this way Dietrich¹⁵⁶ obtained consistent data on the changes in the adrenals as a result of wound infections. In no case of death following these infections were changes in the adrenal cortex not present. These changes varied in intensity from a simple disappearance of the lipid to an actual disintegration of the normal cell structure, or consisted simply of circulatory changes such as hyperaemia, hemorrhages, infiltration of leucocytes, or thrombosis. These changes in the adrenals were found in all cases of wound infections whereas there was only an occasional appearance of similar inflammatory changes in other organs. The morbid changes of the adrenal constituted the first and most pronounced findings observed at autopsy. The adrenals were most markedly affected in infections marked by a particularly toxic nature such as in the edema of gas poisoning or in peritonitis. Although the observed changes were not specific for any particular disease, they were related to the intensity and nature of the infectious process.³⁷⁶ Injury of the adrenals following the toxic and infectious agencies just described is limited chiefly to the cortical cells. The cells of the medulla undergo relatively slight changes as compared to the cortex. Prolonged injury results in the production of "colloid-droplets"

but mitoses are rarely observed in the medullary cells following such injuries. However, Graham²³⁷ has reported active regeneration of the medullary cells in a case of fatal "shock."

EFFECT OF TOXIC AGENTS AFTER ADRENALECTOMY

Adrenalectomized animals are not only hypersensitive to such extraneous influences as excessive heat or cold, muscular exhaustion, excitement, *etc.*, as pointed out in Chapter VIII, but they also show an abnormal sensitivity to various drugs and toxic agents. This hypersensitivity has been demonstrated for histamine, curare, strychnine, morphine, nicotine, acetonitrile, adrenaline, diphtheria or tetanus toxins, cobra venom, typhoid or staphylococcus vaccines, foreign proteins, and other toxic agencies. In many cases this hypersensitivity is very marked.^{116, 147, 155, 233, 394, 479, 629}

The increased sensitivity of adrenalectomized animals to toxins will obviously depend upon the degree of adrenal insufficiency from which the animals are suffering at the time of the injection of the toxin. Animals manifesting a marked degree of insufficiency will succumb to doses which are only a fraction of those tolerated by normal animals, while animals manifesting only mild or no symptoms of insufficiency after an incomplete adrenalectomy will obviously not manifest an appreciable hypersensitivity. This variability in the response of adrenalectomized animals has led to the conflicting findings of different investigators. Thus the reported normal resistance of adrenalectomized rats to morphine or tetanus toxin is explained by the fact that the animals were incompletely adrenalectomized (as evidenced by their prolonged survival) and hence despite the absence of macroscopically visible accessory tissue, were not suffering from severe adrenal insufficiency.

As first demonstrated by Dale and Laidlow,¹⁴⁸ adrenalectomy greatly enhances the sensitivity of animals to histamine. It is possible, however, that this hypersensitivity is in part (but not completely as claimed by Wyman⁶⁹¹) attributable to the

extirpation of the medulla. Epinephrine and histamine are marked antagonists in their effects on the circulation. One would anticipate, therefore that the secretion of epinephrine called forth by an injection of histamine into a normal animal would raise its tolerance to this drug compared, for example, to a partially adrenalectomized animal in which no cortical insufficiency was manifest. In the case of most drugs hitherto investigated, however, the absence of medullary secretion is not responsible for an appreciable part of the hypersensitivity to toxic agents for such hypersensitivity can be counteracted by the maintenance of the animal on an adequate dose of the cortical hormone.⁶⁸⁶

Adrenalectomized animals are also abnormally susceptible to anaphylactic shock as produced, for example, by the injection of horse serum in rats or guinea pigs.^{200, 350} This effect is obtained if the animals are sensitized either before or after the removal of the adrenals, and is related chiefly to the degree of adrenal insufficiency.

Rabbits with a high grade but sublethal adrenal insufficiency, after immunization with sheep blood-corpuscles, show hemolysin titers more than twice as high as in control animals. This increased antibody formation was attributed by Také and Marine⁶²⁰ to a loss of some regulatory and inhibitory influence which the cortex of the adrenal normally exerts on the irritability and susceptibility of the body cells. If the amount of antibody produced by an animal depends on the concentration of the dosage of antigen, increased formation of hemolysin in adrenalectomized animals would be explained by the failure of the body to detoxify the antigen in adrenal insufficiency thus maintaining it in a high concentration. There is also hypertrophy of the lymphoid tissues in adrenal insufficiency and these may be concerned in antibody formation.⁶²⁰

Rats immunized with typhoid vaccine within three weeks following adrenalectomy were found by Jaffe and Marine³³⁶ to have agglutinin titers averaging two to three times that of

their controls. Although the resistance of rats to large doses of typhoid vaccine was still somewhat below that of normals six weeks after adrenalectomy, no difference in their agglutinin response could be determined. The increased antibody formation in recently adrenalectomized rats is a reflection of their decreased resistance to the antigen. The increased antigenic effect gradually disappears with resumption of normal cortical activity brought about by a regeneration of bits of tissue and accessory bodies left at operation.

The above described effects are entirely the result of the removal of the cortex for epinephrine has no effect upon the opsonic properties of the blood or upon sheep-cell hemolysin or typhoid agglutinin production in rabbits. On the other hand, traumatization of the periadrenal tissues, as Perla and Gottesman⁴⁹⁶ have shown, produces the same effect as adrenalectomy on the formation of antibodies in rats.

THE EFFECT OF THE ADRENAL CORTICAL HORMONE IN INFECTIONS

One can attribute the marked sensitivity of the adrenals to toxins and infections to the unique chemical composition of these glands. Their high lipid content would obviously make them susceptible to attack by lipid solvents such as chloroform, ether, phenol, and the like. It is unnecessary to infer that the adrenals suffer because of their assumed anti-toxic function. On the other hand, one might be justified in assuming that the observed anatomical changes disturb the normal functional capacity of the glands and thereby play a part in producing some of the clinical manifestations of the toxic or infectious process. This has, however, not been proven and awaits further investigation particularly the study of the therapeutic effects of administering the cortical hormone in these conditions.⁷⁸ Scott and his collaborators⁵⁶³ found their preparation of the cortical hormone to have no influence on the action of diphtheria toxin in guinea pigs, trypanosome infection in rats, and pneumonia in mice. Firor and the author in a few prelimi-

nary experiments also found the injection of small doses of the cortical hormone to have no demonstrable influence on the mortality of mice infected with mouse typhoid. Nor did the infusion of 100 cc. of the cortical extract exert any demonstrable influence on a patient dying of peritonitis (probably pneumonic in origin).

The above described experiments were too few in number and involved the use of insufficient doses of the hormone to make the results decisive. The reports of authors describing therapeutic effects in various infections of man are based on the use of extracts whose potency we know to be insignificant and hence must be viewed with scepticism. Further experiments using large doses of hormone are desirable to determine if exhaustion of the adrenals in certain infections is in part responsible for the manifestations of these diseases. The evidence available at present does not suggest that this occurs except perhaps in rare cases.

The fact that adrenalectomized animals succumb more readily than normal animals to the effects of toxic agents is no evidence, however, that the adrenal glands themselves play any direct rôle in conferring natural immunity on the normal animal. The facts only prove that in adrenal insufficiency the organism is incapable of its normal response to such toxic influences due possibly to the incapacitation of the organs (*e.g.*, the liver or kidney) which normally excrete, destroy, or detoxify injected toxins or to a lowered resistance of the tissues generally induced by the adrenal insufficiency. It would not follow, therefore, that the adrenal cortical hormone will manifest antidotal actions against toxic agents or, as has already been indicated, that the hormone should manifest a therapeutically beneficent effect when administered in infectious diseases. The relation of the adrenals to the reaction of the organism to toxins and infections offers an interesting field for further study of the normal mechanism of these reactions.

CHAPTER XVIII

THE ADRENALS AND SURGICAL SHOCK

Our ignorance of the function of the adrenals has made these glands the natural butt of theories purporting to explain the condition of shock. At first, dysfunction of the medulla was held responsible for the development of shock. With the shift of interest in the adrenals from the medulla to the cortex, these glands were again linked with shock, the absence of the cortical hormone being now held responsible for its development instead of epinephrine as in the older theories.

Shock is a rather loosely defined term applied to a condition marked by a failure of the circulation manifested particularly by a fall in blood pressure. Shock occurs under a variety of conditions—after surgical operations, traumatic injuries, in toxic conditions, after excessive hemorrhage, in psychic disturbances, and the like. Many theories have been advanced to explain the underlying pathology which brings about a state of shock but none of these theories has proven satisfactory. Shock, like prostration, asthenia, or the like, is a term describing a condition rather than a disease entity and results undoubtedly from a variety of causes quite unrelated to one another but having in common only the fact that they all cause a serious collapse of the mechanism which normally maintains the circulation. One can explain the occurrence of shock under most conditions by a consideration of the underlying pathology accompanying the condition. Thus in a sudden widespread dilatation of the capillaries, the great increase in the capacity of the circulatory bed must necessarily result in a dangerous drop in blood pressure and a condition of shock. Similarly a sudden failure of the heart or the loss of a large volume of the circulating fluid (as in hemorrhage) must result in shock.

A large group of cases of shock occur under conditions in which simple hemodynamics does not explain the condition as easily as in the above described cases. Thus in the so-called cases of surgical shock, or in the shock following excessive traumatic injuries, the local changes are not sufficient to account for the observed collapse of the circulation. In cases of this sort in which emboli (fat or air) have been released into the circulation it is obviously to embolism that we must attribute the observed shock. In certain cases, however, neither emboli nor the release into the circulation of toxic products can account for the development of shock. Thus crushing of the testicles has been utilized experimentally to reproduce the condition in animals. In this form of shock, which also includes many cases of surgical and traumatic shock, we must assume that the mechanical injury has released a stream of impulses which have resulted in the collapse of the circulation. The existence of such reflex impulses in response to trauma is easily conceivable when we consider the normal responses of the circulation to minor stimuli.²⁴³

The present discussion of the supposed rôle of the adrenals in the causation of shock has been prefaced by the above considerations in order to indicate that shock is a symptom complex due to a variety of possible causes and is not to be considered as a disease entity whose causation must be sought in some particular gland or condition. A failure to appreciate this has undoubtedly led to the many theories advanced for the condition, none of which has withstood the test of time. It would be wise perhaps if we were to cease using the term shock with the connotation of a disease entity of mysterious etiology and always modify the term by some expression indicative of its origin.

The fact that epinephrine raises the blood pressure so spectacularly when injected and the fact that hypotension is one of the chief symptoms of shock led to the earlier theories relating

the adrenals and shock. It was assumed that the abnormal stimulation which leads to shock, depletes the adrenal stores of epinephrine and thus leads to the observed drop in blood pressure. We have seen, however, that normally epinephrine plays no part in maintaining the vascular tone and that animals deprived of their entire medullary substance maintain a normal blood pressure. We can thus dismiss the adrenal medulla and epinephrine as involved in the causation of shock. If shock really resulted from a depletion of the epinephrine of the adrenals, one would expect a marked rise in blood pressure to precede the fall. Such a rise is, however, not observed. Stimulation of the splanchnic nerves,¹⁷⁸ exposure of the viscera,³⁰⁷ and sensory stimulation^{105,546} may cause an increase in output of epinephrine but it is doubtful if this secretion has anything to do with the ultimate development of shock in these conditions. The depletion of epinephrine from the adrenals in fatal cases of operative shock has been reported by Bainbridge and Parkinson²⁷ and Elliott,¹⁸¹ but other workers have not confirmed these results.⁶³⁹ Stewart and Rogoff⁵⁸⁸ found that the output of epinephrine from the adrenals in their animals was the same during shock as under normal conditions but, as we have seen (Chapter VI), their values for the normal are probably too high. According to other investigators epinephrine secretion is increased during shock.⁵⁴⁷ In any case, these results would not prove the existence of any relation between the adrenal medulla and the development of shock.

Rich⁵¹⁹ subjected normal and adrenalectomized animals to uniform intestinal manipulation and showed that the time required for the production of shock and the character of the blood pressure curves were the same in both cases. These experiments indicate that excessive epinephrine secretion is not responsible for the development of shock, but they do not exclude the possibility that the cortical hormone is involved.

One can assume that in Rich's experiments sufficient amounts of the hormone remained in the organism after adrenalectomy to prevent the onset of shock for some time.

Austmann, Halliday, and Vincent²⁵ kept dogs under ether for as long as 40 hours after removing both adrenals without any marked fall in blood pressure. Bazett,⁴⁷ on the other hand, found that although excision of the adrenal glands in cats causes no immediate fall in blood pressure, the extirpation hastens the onset of the fall which ultimately occurs in animals maintained for long periods under anesthesia (urethane) or after decerebration. This was particularly striking in the decerebrate preparations which normally maintain their blood pressure for several days if the body temperature be kept normal. After excision of the adrenals, however, the blood pressure falls after about an hour and death occurs within ten hours after the operation.

Injection of histamine causes a drop in blood pressure similar to that observed in shock. The adrenalectomized cat, as Dale¹⁴⁷ showed, is extremely sensitive to histamine. Although a normal, unanesthetized cat can tolerate an infusion of histamine in doses up to at least 10 mgms. per kilo., without severe effects, the same animal on the day following bilateral adrenalectomy is killed by doses as small as 0.16 mgms. per kilo. Anesthesia, likewise reduces the resistance of the cat to histamine. After an hour's administration of ether, 1 or 2 mgms. of histamine per kilo of body weight will induce the fatal shock characteristic of histamine poisoning.

Dale pointed out that the "circulatory condition developing in the 24 hours following removal of the adrenals has features in common with that produced by histamine, including a pronounced increase in the proportion of corpuscles in the blood." As Dale recognized, it was impossible by his experiments to determine whether the absence of the medulla or the cortex was responsible for the decreased resistance towards histamine of the adrenalectomized animal. Histamine stimulates epineph-

rine secretion (*cf.* Chapter VII) and this liberated epinephrine may play a part in protecting the normal animal against histamine poisoning.

Swingle and Parkins⁶¹¹ have also studied the effect of various types of trauma on the arterial blood pressure of dogs with and without adrenal glands. Unlike the experiments of previous authors these were performed on animals adrenalectomized some days previously and kept alive by injections of cortical extracts. Swingle and Parkins found their adrenalectomized animals, supposedly maintained in good health on an adequate supply of the cortical hormone, to be extremely susceptible to traumatic shock. Administration of cortical hormone restored the adrenalectomized animals from a state of shock. The authors concluded from their experiments that depletion of the adrenal cortical hormone is the causative factor in surgical shock.

Swingle and Parkins' conclusion is not proven, however, by their experiments. It is well known that animals after adrenalectomy go into profound shock under the effects of minor stimuli, an observation which has been noted by numerous workers. It is obviously necessary to conclude therefore that adrenalectomy (or more specifically the loss of adrenal cortical function) leads eventually to a state of shock which resembles in many ways that observed from a variety of other causes. To conclude, however, that shock in general is due to inadequacy of the adrenal cortex, as Swingle and his collaborators claim, is not valid. The hypersusceptibility of their adrenalectomized animals to trauma is evidence only of inadequate therapy with the cortical hormone as the results of their own experiments, in which injection of hormone prevented shock, indicates. Restoration of adrenalectomized animals from shock by treatment with the hormone also proves only the long known fact that adrenalectomy reduces the tolerance of animals to influences which precipitate an animal into a state of shock. As has already been stated in Chapter IX, there is

no evidence of any abnormal permeability of the capillaries in adrenal insufficiency, as claimed by Swingle and his collaborators, to account for the loss of blood volume which they assume is the cause of the development of shock in adrenal insufficiency.

Heuer and Andrus²⁹⁵ have recently reported that the administration of adrenal cortical extract controlled the shock which follows the injection of aqueous extracts of closed intestinal loops. Their results, however, do not appear convincing and the commercial extract which they utilized is of doubtful potency as regards its hormone content. Moreover, the fact that they were unable to obtain their results after heating the extract to its boiling point indicates that they were deceived in attributing their observed results to the hormone, for as we have seen (Chapter XVI), the hormone is not so readily destroyed by heat.

The cause of the state of shock which ultimately develops in adrenal insufficiency is still problematical. According to many authors the shock is due to the loss of fluid from the circulation, as described in Chapter XI. In some cases this may be true, for, occasionally, an intravenous injection of saline will induce a remarkable improvement in animals in severe adrenal insufficiency. In most cases, however, such injections are of no avail which is inconceivable if one accepts the view that adrenal cortical insufficiency expresses itself solely in the development of a state of circulatory shock. Other factors undoubtedly play a part and, except in those cases in which loss of fluid from the circulation results in a state of shock, we must look upon the development of this condition in adrenal insufficiency as an agonal manifestation comparable to that seen in many other fatal diseases.

The question finally arises as to the implication of the adrenals in the development of shock by influences other than adrenalectomy. It is quite conceivable that many conditions may deplete the supply of the cortical hormone from the body. In such cases the ultimate development of a state of shock may

be comparable to that which follows adrenalectomy. A long period of etherization, protracted fevers, burns, *etc.* may possibly lead to such a state. However, in most cases of shock it is difficult to see how the adrenals are implicated. Thus in such injuries as crushing of the testicles or manipulation of the intestines, it is unlikely that the adrenals should suddenly cease secreting the vital cortical hormone, or, if they do, that the supply of the hormone should be depleted so rapidly from the body. It is more logical and in accord with the known facts to attribute the shock which develops in such conditions to nervous impulses from the site of injury which reflexly cause a paralysis of the vasomotor system.

CHAPTER XIX

SURGERY OF THE ADRENAL

Besides the complete excision of the adrenal glands, described in Chapter VIII, a number of other surgical procedures have been utilized for the experimental study of these glands. In order to simulate the condition obtaining in Addison's disease, efforts have been made to reduce the cortical tissue sufficiently to induce a chronic deficiency of the vital hormone without causing death from acute insufficiency. Partial excision of the glands has been performed with this purpose in view. The results, however, have been unsatisfactory for the regenerative capacity of the cortical tissue soon leads to hypertrophy of the remaining fragments and the resumption of normal adrenal function.^{413, 571, 582, 594}

Destructive lesions have also been produced experimentally by the injection of bacteria, bacterial toxins (diphtheria, tetanus, pneumococcus, streptococcus, *etc.*),⁴⁸² or chemical substances (arsenic, mercury, chloroform, phenol, *etc.*).⁵² The lesions of the cortex produced by these substances have already been discussed in Chapters III and XVII.

Martinotti⁴⁴¹ first observed the marked histological changes occurring in the adrenal after ligation of the lumbo-adrenal vein, and subsequent workers have utilized this procedure in an attempt to produce a chronic functional insufficiency.^{184, 248, 273} Ligation of all the vessels to the adrenal will usually result in a rapid onset of an acute adrenal insufficiency. In cats, however, there exists a direct vascular connection between the adrenals and the kidneys and if this channel for venous drainage be left untied, one can produce a degree of chronic insufficiency. Koudintzeff²⁶⁹ ligated the efferent vessels of dogs. His animals survived 16 to 24 days, had an elevated temperature, lost weight, and died in convulsions.

Nothnagel⁴⁷⁴ attempted to produce Addison's disease in animals by crushing the adrenals. He described the development of pigmented spots on the mucous membranes of rabbits in which this operation was performed. Abelous and Langlois⁵ ligatured the adrenal pedicle and crushed or cauterized the glands in an attempt to induce a chronic insufficiency. Marine⁴³⁴ and his coworkers froze the surface of the glands by a spray of ethyl chloride in order to induce partial cortical insufficiency. These methods have been only partially successful. It is difficult to adjust the degree of injury so that the animal will not die of an acute insufficiency or else survive without any demonstrable deficiency.⁵⁶¹

With a preparation of the cortical hormone available, the most satisfactory method of producing a partial insufficiency would seem to be the following: After complete adrenalectomy the animal is maintained on an adequate dose of the hormone until the operative wounds have thoroughly healed. The dose of hormone is now gradually reduced until a definite degree of insufficiency is clinically evidenced by a loss or failure to gain weight, a reduced body temperature, and general inactivity. By this method the author has succeeded in keeping animals (rats, cats, and dogs) in a state of chronic insufficiency for several months, during which time they developed the changes described in Chapter XIV.

Many methods have been devised for destroying the medullary tissue without fatal injury to the cortex in order to study the effects of exclusion of epinephrine from the circulation. The adrenal may be scooped out, as in the experiments of Houssay and Lewis,³¹¹ or destroyed by inserting glass capillaries containing radium or its emanations.^{41, 373} The possibility of destroying the medullary cells by a specific cytotoxic serum, as is reputed to have been done,³⁸⁹ is very doubtful. Extirpation of one adrenal and denervation of the other has been the method most commonly used for excluding the medullary function, but it has not been proven and it is

improbable that this procedure entirely prevents the secretion of any epinephrine from the denervated gland.

SURGERY OF THE HUMAN ADRENAL

Surgical operations on the human adrenal have involved chiefly the removal of the glands for tumors. Denervation of the glands or their resection for hypertension or hyperthyroidism are based on such ill-defined grounds that they need not be considered here.^{136*}

Surgery of the adrenal has in the past been attended with an exceedingly high mortality. This is to be attributed probably to the acute cortical insufficiency which the patients suffer as a result of the combined effects of the trauma, anesthetic, reduction in the amount of active cortical tissue, and injury of the remaining gland by manipulation. The administration of large doses of the cortical hormone following the operation should offer a means of maintaining the patient in good condition until the residual cortical tissue has hypertrophied.

In the surgical approach to the adrenals in man either a lateral, posterior lumbar incision, or a transverse, abdominal incision half-way between the ensiform cartilage and umbilicus are most commonly employed. The former approach is similar to that used in operations on the kidney and avoids opening the peritoneal cavity. The second approach has the important advantage that it permits examination of the two glands. This is often of vital importance. An enlarged gland, assumed to be a tumor, has been removed with fatal outcome. *Post-mortem* examination proved the extirpated gland to be an hypertrophied gland while the other gland was atrophied. An abdominal approach also permits examination of the ovary for suspected tumors.^{95, 655}

Crile⁶⁵⁵ has emphasized the danger of handling the glands, the delicacy of which we have already discussed in Chapter III. Handling may cause a fatal apoplexy of the adrenals and Crile therefore recommends that the examination of each

* Cf. Jour. Am. Med. Assoc. vol. 106, p. 279.

gland be made at a separate operation. Judging from the results of adrenalectomy in animals this would appear to be a wise procedure.

The discovery of a technique by which to differentiate the androgenic tissue which gives rise to the tumors described in Chapter XXIII from normal cortical tissue is very desirable. Broster and Vines⁹⁵ have described such a technique but it has not as yet found general acceptance. When such differentiation shall be possible, the removal of a slice of tissue for biopsy will permit an accurate diagnosis and determine the nature of the operation to be performed. At present the surgery of the adrenal is handicapped by the inability of the operator to judge the nature of the pathological process involved, unless there be an obvious fungating tumor. An enlarged gland which appears to be a tumor may in reality prove to be a normal hypertrophied organ. The surgeon may remove the greater part of a gland and yet leave a large amount of the offending tissue. If the adreno-genital syndrome be due to hypertrophy of the juxta-medullary androgenic zone, as indicated in Chapters IV and XXIII, it would be futile to remove one adrenal and part of the other to cure the condition, for the remaining androgenic tissue will continue to grow. Where only one gland is involved, unilateral extirpation should prove adequate. When the affection is bilateral, as it apparently frequently is, it will be necessary to devise a technique whereby the internal layers of the cortex (consisting of the androgenic tissue together possibly with the medulla) are scooped out, leaving the normal and vital external layers of the interrenal tissue.

ADRENAL GRAFTS

Transplantation of adrenal tissue has been performed by a number of investigators in both man and the lower animals. Most of the attempts in the human subject were performed many years ago when grafting of tissues was undertaken with

an optimism that we now would deride. Thus Bra¹¹⁹ transplanted the adrenals of a dog into the cellular tissue of the abdomen in a child with Addison's disease. Death followed in three days. Jaboulay's two cases died within 24 hours following a similar transplantation as did also the patient of Courmont.¹¹⁹ In view of the fact that the transplantation of human tissue is fraught with the greatest of difficulties, one is forced to disregard the claims of all early investigators who optimistically report favorable results from transplants of pig, sheep, and dog adrenals into patients suffering from Addison's disease. The introduction of foreign protein and the necessary operative procedures could only have aggravated the adrenal insufficiency of these patients. The small amounts of hormone present in the transplanted tissues were too minute to exert even any temporary amelioration which, when it occurred, must have been due to the natural remissions so common in Addison's disease.

Attempts have also been made to transplant the adrenals of premature fetuses into patients suffering from Addison's disease. In most cases the patients were in the late stages of the disease and the shock of the operative procedure hastened the onset of death. In only one case was success reported with recovery of the patient but when the latter, some years later, came to autopsy it was found that his adrenals were normal, that he had never had Addison's disease, and that the assumedly successful grafts into the testicle had undergone atrophy.¹²² Other cures have been reported but must be viewed with scepticism.¹⁴⁵

In animal experiments, on the other hand, successful grafts and transplants of the adrenals have been made by several investigators, despite the failures of many of the earlier workers who failed to appreciate the difficulties attendant upon transplanting any tissue to a new host. Boinet's intraperitoneal grafts in rats were unsuccessful as were also those of Strehl and Weiss,⁶⁰⁰ and Hultgren and Anderson's³²³ intramuscular

grafts in cats and rabbits. In Poll's subcutaneous grafts some regeneration of the cortex occurred while the medullary tissue disappeared. Similar results were obtained by grafts in the omentum of the rat by H. and A. Cristiani.¹³⁷

Stilling⁵⁹⁷ found cortical tissue grafted into the testicle of a rabbit to be active after three years. Busch, Leonard, and Wright¹⁴⁵ made successful grafts into the kidneys of rabbits which maintained life after bilateral adrenalectomy. Death followed the removal of the grafts. In none of the above cases was medullary activity demonstrable, the medulla always disappearing in the transplanted tissue.

Abelous and Langlois⁴ successfully transplanted adrenal tissue into the lymph sacs of frogs. Abelous was also successful in producing homotransplants into the ileo-coccygeal muscles, the subsequent removal of which in previously adrenalectomized animals caused the frogs to manifest the typical symptoms of adrenal insufficiency.

In recent years, grafting of adrenal tissue has been performed successfully in guinea pigs and rats. Elliott and Tuckett¹⁸³ found the subcutaneous tissues of the guinea pig to be peculiarly sensitive to adrenal grafts. The irritant substance inducing this tissue reaction to grafts of guinea pig adrenals, occurred chiefly in the medulla but was almost absent from the adrenals of carnivores and was not, therefore, epinephrine. Jaffe,³³⁴ however, found that autoplasmic transplants into the abdominal wall of guinea pigs remained for months, growing to fairly large size. Homoplasmic transplants, on the other hand, usually degenerated after a few months. Small autoplasmic transplants maintained the life of completely adrenalectomized animals for weeks while large transplants maintained them indefinitely in good condition. In these experiments Jaffe avoided the transplantation of medullary tissue, transferring small bits of the cortical tissue, after washing in saline, into pockets in the muscle of the abdominal wall.

In the rat, Jaffe³³³ and Wyman and Suden⁶⁹² have obtained

successful autoplasmic transplants in a large fraction of their animals. Homoplastic transplants are also successful in the rat but heteroplastic transplants have not been achieved. The growth of transplants is contingent upon a physiological need for their secretion and hence such transplants can not be used to induce hyperfunction of the cortical tissue. The total amount of tissue regenerating is fairly constant and limited by the needs of the organism and the amount of functional tissue already present in the animal.⁶⁹²

The oft-quoted successful transplants of Haberer²⁶¹ are not really grafts but consist merely in transferring the whole adrenal after dislocating it from its peritoneal attachments to an incision into the kidney. The blood vessels to the gland are left intact. Such dislocated glands continue to grow in the kidney without suffering degeneration even of the medullary tissue.

The medullary tissue shares the property of nervous tissue generally, to which it is embryologically related, in showing little or no regenerative capacity. Hence in transplants of whole glands the medulla soon degenerates.¹⁴¹ Undoubtedly the liberation of epinephrine from the degenerating medulla will inhibit growth of the cortical cells and hence one should only use the outer zone of the cortex (which constitutes the active regenerative tissue) in transplants. The abdominal muscles, ovaries, testicles, kidneys, and subcutaneous tissue of the axilla have been favored as the most practical sites for successful transplants.

In recent years, the effort to transplant cortical tissue into patients with Addison's disease has been revived, but thus far no convincing report of any success has been published. One of Beer and Oppenheim's⁴⁹ two patients showed microscopic areas of functional tissue at autopsy two weeks after the transplant. The other patient improved after the transplant, but such improvement must be attributed to a natural remission for it is inconceivable that the minute amount of

hormone present in the transplanted fragments could have had any immediate remedial effects.

A method for the successful transplantation of cortical tissue in clinical cases is highly desirable. Unfortunately the transplantation of all glands is still in the experimental stage. The difficulty of obtaining healthy material for the transplant and the desensitization of the recipient to the foreign tissue are the two outstanding problems which must be solved before one can hope for a successful general application of tissue grafting. The great need and value of a method for transplanting cortical tissue in man make it desirable that further studies in this field be inaugurated.

CHAPTER XX

OTHER CHEMICAL CONSTITUENTS OF THE ADRENALS

Besides epinephrine and the cortical hormone, which we have considered in previous chapters, the adrenals contain a number of other compounds in relatively high concentrations. The presence of these constituents of the adrenals has excited the curiosity of many observers who have attributed to them an importance which the factual evidence at hand does not justify. In every tissue are found numerous compounds the significance of which to the animal economy is unknown. Many of these compounds (*e.g.*, the lipids) are an essential part of the structure of the cell. The presence of others is perhaps incidental and dependent upon solubility relationships. Substances will distribute themselves throughout the organism in accordance with the physico-chemical laws of solution. Hence a lipid-soluble vitamin will be concentrated in glands having a high lipid content rather than in muscular or connective tissues. The mere presence of a compound in the adrenals does not therefore justify the assumption that it is necessary for the proper function of these glands or that it is produced by these glands. In the present chapter we shall consider a group of substances found in the adrenals and evaluate the available evidence as to the significance of their presence in the glands.

The isolation of a chemical compound from the adrenals does not prove that it was present in the normal gland for its presence may be the result of *post-mortem* changes or of the chemical manipulations utilized in its isolation. The adrenal is noteworthy for the rapidity with which it undergoes autolytic changes. If one extracts a fresh gland with a solvent such as alcohol or acetone the solution thus obtained is light yellow

and free of many constituents present in the dark brown solution obtained when glands are extracted which have stood for sometime at room temperature after the death of the animal. Obviously autolysis results in the production of a number of substances not present as such in the normal living organ.

GENERAL COMPOSITION

Even superficial examination of an adrenal gland will show its exceedingly high lipid content. In an analysis by Biedl,⁵⁶ the adrenals of the pig showed 74.61 per cent water and 25.39 per cent dry residue. Of the latter, 61.12 per cent was protein-like in nature while 38.88 per cent was of a lipoidal or fatty nature.

The water content of the adrenal glands undergoes wide variations which are dependent on the lipid content of the gland. The greater the lipid content, the less is the water content of a given gland. Materna and Januschke⁴⁴⁵ found the water content of the human adrenal to vary from 58 per cent to 85 per cent of the gross weight of the gland. This represents an unusual variation not frequently encountered in other organs.

The adrenals are also characterized by an unusually high sulfur content, 3.77 per cent of their dry residue being composed of this element. Only the epidermis and its horny derivatives (hair and nails) have such high sulfur contents. This high sulfur content led Loeper and his coworkers and Aufrecht and Dresing¹³⁵ to assume that the adrenals are involved in the sulfur metabolism of the body, but other evidence for this view is lacking.

The nature of the sulfur compounds present in the adrenals is not entirely clear. A great part of the sulfur is present as glutathione which is found in higher concentration in the adrenal glands than in other organs. Thus in the dog Blanchetière *et alii*⁶² found the adrenals to contain 4.8 mgms. of glutathione per gram of adrenal tissue, while the liver and

striated muscles contained only 3.1 and 0.7 mgms. per gram, respectively. In the rat, Houssay and Mazzoco³¹⁴ found the adrenals to contain 12 mgms. of glutathione per gram of gland. The venous blood draining the adrenals is particularly rich in glutathione. Perfusion of an adrenal with solutions containing glutamic acid and cystine gives rise to the synthesis of glutathione, according to Blanchetière and his coworkers.⁶² On the other hand, Houssay and Mazzoco³¹⁴ found the reducing action of muscle and liver to be increased after adrenalectomy which they attribute to an increased glutathione content of these tissues. Obviously one would not expect such a result if the adrenals were the site of synthesis of glutathione. Further work is necessary to elucidate the exact relation between the adrenals and glutathione metabolism.⁴⁴⁹

A crystalline compound, having the empirical formula— $C_{35}H_{74}O_8N_2S$, has been obtained from the adrenals but the evidence adduced is so meager as to lead one to doubt that the compound is in reality a pure substance. Elementary sulfur is easily eliminated from the compounds in which it occurs in the adrenals. Deposits from crude adrenal extracts have often been found by the author to contain beautifully formed diamond-shaped crystals which Hendricks and Milner demonstrated to be elementary sulfur.

LIPIDS

As we have already noted, the adrenals are characterized by an unusually high lipid content and investigators since Virchow⁶⁵⁰ have devoted much attention to the study of these lipids both from an anatomical as well as from a chemical viewpoint. These two methods of study often give results which superficially seem to be irreconcilable. For example microscopic observations will often reveal an apparent disappearance of the lipids from the adrenals in certain pathological conditions. Chemical analysis may show, however, only insignificant changes in the lipid content of the glands. The

lipids are thus able to assume forms which differ as regards their staining and morphological appearance and it is necessary to bear this fact in mind in interpreting anatomical observations. There is a considerable species difference regarding the distribution as well as the composition of the lipids. In some animals (including man) the lipid shows double refraction but this optical evidence of anisotropy is absent in the adrenals of some of the lower animals.

In man the lipids, as observed histologically, seem to be present chiefly in the fasciculata. The reticularis contains smaller droplets while the glomerulosa appears to be free of visible lipids. The fat droplets have a peculiar glistening appearance and show the double refraction referred to above, when observed under a polarizing microscope. After fixation in formalin or bichromate, the lipids are solidified but melt again when heated to 56°C.¹⁵⁷

In the dog, the glomerulosa is the chief depository of lipids while in the Cheiroptera (bats) the lipids are present chiefly in the reticularis.³⁶²

Various stains are utilized to demonstrate the presence of the adrenal lipids histologically. Osmic acid stains the droplets black or greyish brown; sudan and scarlet red, a brilliant orange or red; Nile blue, violet or blue. Like myelin, the lipid is stained blue by Weigert's method.

The adrenal lipids are also characterized by the relative ease with which they are removed from the cells by such solvents as xylol, ether, or chloroform.

The chief constituent of the anisotropic lipid is cholesterol and its esters. Wacker and Hueck⁶⁵³ found each human adrenal to contain 0.4 per cent of free cholesterol and 0.1 to 0.15 per cent of cholesterol esters. However, other substances are also present which contribute to the anisotropic property of the adrenal lipids. Thus Rosenheim and Tebb¹³⁵ have isolated stearic and palmitic acids, sphingomyelin (a phospholipid), and phrenosin (a phosphorous-free galactosid) from

the adrenal cortex. They believe the anisotropic substance in the cortex to be free stearic and other fatty acids, as well as cholesterol esters. The existence of a cannaubic acid ester as claimed by Biedl⁵⁶ is questionable. With the exception of the central nervous system, no organ contains as much lecithin-like substances as the adrenals.¹³⁵

The lipid content of the adrenals undergoes marked changes in response to various physiological and pathological conditions, but the significance of these changes is not entirely clear. During muscular exercise, according to Elliott and Tuckett,¹⁸³ the lipids diminish in amount, reappearing during rest. By feeding with cholesterol, the anisotropic fat content may be markedly increased. The adrenals apparently reflect the lipid content of the blood. Thus small doses of saponin which increase the cholesterol content of the blood also increase the amount of lipids present in the adrenals, while large doses of this poison diminish the cholesterol content of both the blood and the adrenals.

The cortical lipids also undergo changes in various pathological conditions, but the significance of these changes is not clear. There is a disappearance of the lipids in infectious or toxic processes⁷⁸ as well as in anemia and severe hemorrhage. In chronic cardio-vascular-renal disease, in inanition, and cachexic conditions generally, the lipids are often increased.¹⁵⁶

There has been considerable speculation regarding the rôle which the lipids play in the adrenals. We have already considered (Chapter XI) the changes in the cholesterol content of the blood in adrenal insufficiency and their probable relation to adrenal physiology. Lipids are widely distributed throughout the organism and constitute a fundamental part of the architecture of all cells. It is doubtful therefore if the lipids of the adrenal play the specific rôle which earlier writers attributed to them.

The views that the cortex is the seat of manufacture of the lipids of the body, that the adrenal regulates the lipid me-

tabolism, or that the lipids of the adrenal perform an indispensable function in the organism are no longer tenable. The fact that adrenalectomized animals maintained on lipid-free extracts manifest no abnormalities attributable to the absence of the adrenals proves that although the adrenal lipids are probably essential parts of the cells, they are neither the product of these cells nor is the adrenal tissue *per se* necessary for the normal lipid metabolism of the organism.

CHOLINE AND ACETYLCHOLINE

Hunt³²⁴ first demonstrated the presence of choline in extracts of the adrenals and demonstrated that such extracts contained a substance which readily yields choline on chemical manipulation. Since choline is a product of the hydrolysis of the phosphatides, it is found wherever the ubiquitous lecithin occurs and it is often very difficult to decide whether the choline obtained from an organ was originally present as such or was derived from lecithin.

Feldberg and Schild¹⁹³ found acetylcholine to be more abundant in the medulla than in the cortex, the proportion varying from 2.5:1 to 4.5:1. The reverse relation held for choline. These authors (Chapter VI) also demonstrated the secretion of choline from the adrenals during stimulation of the splanchnic nerves.

The presence of choline in the adrenals has led authors to assume that this substance is an important product of the gland and some have even hypothecated that it is the cortical hormone. There is nothing to support these views nor is there, except for the experiments just cited, any evidence to indicate that choline or its esters play any significant rôle in the adrenals. These substances may, however, contaminate crude adrenal extracts. Some of the pharmacodynamic effects of such extracts described in the literature are undoubtedly to be attributed to the presence of choline rather than to the cortical hormone.¹⁶⁸ The biological assay of the epinephrine content

of adrenal extracts may also be vitiated by the presence of choline and its esters since the pressor effect of epinephrine is easily antagonized by small amounts of these substances.

VITAMINS

The adrenals contain appreciable quantities of vitamins A, B, C, G, and probably other vitamins in lesser concentrations. The idea of the existence of an intimate relationship between the adrenal cortex and vitamins A, B, (B₁), C, and G (B₂) has received much attention in recent years. This supposed relationship has been based upon: 1) the high concentrations of the vitamins (particularly A and C) occurring in the adrenal; 2) the changes occurring in the adrenal during avitaminosis (atrophy in avitaminosis A; hypertrophy in avitaminosis B and C); 3) the increase in susceptibility to infectious disease in avitaminosis and in adrenal insufficiency; 4) the similarity between certain of the clinical manifestations of avitaminosis and of adrenal insufficiency; 5) the alleged ameliorating effects of cortical extracts in avitaminosis B and C; 6) the similarity between certain of the chemical properties of the adrenal cortical hormone and vitamin G (B₂).²⁴⁷

Vitamin A. The high concentration of carotene (pro-vitamin A) in the cortex may be attributed to the high lipid content of the gland. Other lipoidal tissues, such as the corpus luteum also contain equally high concentrations of pro-vitamin A, and we may therefore consider the existence of pro-vitamin A in the adrenal to be due to the laws of distribution based on simple solubility relationships. The adrenal atrophy occurring during avitaminosis A may be considered as part of a general reaction to the lack of a vital factor necessary for the well-being of many organs and tissues. There seems no reason, therefore, to assume the existence of an intimate relationship between vitamin A and the adrenal cortex.⁴⁵⁶

Vitamin B (B₁). Pico-Estrada⁵⁰⁶ demonstrated the increased susceptibility of adrenalectomized rats to avitaminosis

B. It is well known, however, that adrenalectomized animals are hypersensitive to any abnormal condition—the administration of drugs, dietary deficiencies, temperature extremes, excitement, *et cetera*. Hence his observations indicate no special relationship between the adrenals and vitamin B.²⁴⁷

Schmitz and Kühnau and Lockwood and Hartman claimed that they could ameliorate the symptoms of vitamin B deficiency by administration of cortical extracts.²⁴⁷ However, since the adrenal cortex is rich in vitamin B content, it is imperative to remove all traces of this vitamin before one can conclude that the effects observed after administering a given extract are due to the cortical hormone rather than to simple contamination by the vitamin. The cortical hormone as prepared by these authors was a relatively crude extract containing a number of constituents. When one considers the difficulty of removing the last traces of vitamin B from relatively pure casein or from refined lactose it is not unexpected that crude cortical extracts should contain this vitamin. That this contamination of the extracts was probably responsible for the observations of Schmitz and Kühnau and Lockwood and Hartman was demonstrated by Grollman and Firor.²⁴⁷ The last named authors demonstrated that the administration of adrenal cortical extracts did not ameliorate the symptoms of avitaminosis B and hence the assumption of an interrelation between the hormone and the vitamin was unfounded. The gross and histological changes occurring in the adrenals of animals suffering from avitaminosis B is no more striking than that which occurs in other endocrine glands and that might be anticipated to result from the inanition and retardation in growth which accompany vitamin B deficiency.

Vitamin C (ascorbic acid). Vitamin C was first isolated in crystalline form from the adrenal glands. While seeking the active principle of the adrenal cortex, Szent-Györgi⁶¹⁵ isolated a crystalline compound having the formula, $C_6H_8O_6$, which subsequent work showed to be identical with vitamin C.

Szent-Györgi named the compound ascorbic acid but the name cevitamic acid has been adopted by the Council of the American Medical Association to avoid any therapeutic implications.

Ascorbic acid is present in both the cortex and the medulla. Reduction of silver nitrate to give a black precipitate has been used to indicate its presence but this test is interfered with by the epinephrine of the medulla.³²⁶ Hence the conclusion of earlier workers that ascorbic acid was present in the cortex only is incorrect. The concentration of ascorbic acid differs in different parts of the gland. Glick and Biskind²²⁵ found 1.2 to 1.3 mgms. of the vitamin per gram of medullary tissue in beef adrenals. In the fascicular layer of the cortex, it was present in a concentration $1\frac{1}{2}$ times as great as in the medulla.

The structure of ascorbic acid has been determined and its synthesis accomplished. Its distribution in the organism and its chemical, physiological, and therapeutic properties have been widely investigated.

The ascorbic acid content of the adrenals of various animals ranges from 0.76 mgms. per gram for the beef to 5.2 mgms. per gram for the rat.⁶⁰⁷ The ascorbic acid content of the liver varies from 0.08 mgms. per gram for the rabbit to 0.46 mgm. for the sheep and the rat. Considering the relative sizes of the adrenal and the liver it is obvious that the latter organ is the chief store for ascorbic acid in the body. Other organs also contain an appreciable amount of ascorbic acid. Thus Svirbely⁶⁰⁷ found the following average values for the ascorbic acid content of the guinea pig, expressed in mgms. per gram of different organs: adrenal, 0.25; liver, 0.02; kidney, 0.02; heart, 0.005; spleen, 0.08; leg muscle, 0.005.

The presence of vitamin C in the adrenals led earlier workers to assume that these glands were intimately associated with the production of scurvy. Experiments were adduced which reputed to show that the administration of the cortical hormone ameliorated the symptoms and protected animals against scurvy. It was demonstrated by Grollman and Firor,²⁴⁷ how-

ever, that ascorbic acid has no value as a replacement therapy in adrenal insufficiency. Nor conversely does the adrenal cortical hormone act in preventing the development or in mitigating the symptoms of scurvy. The results of previous authors who had claimed otherwise were due in part possibly to the contamination of their crude extracts with ascorbic acid, and in part to a misinterpretation of the significance of their observations.³³⁰

Vitamin G (B₂). Purified adrenal cortical extracts have no demonstrable effect on the development of avitaminosis in rats deprived of vitamin G. The failure of young animals maintained on a vitamin-G-free diet to grow can not be attributed to adrenal insufficiency, for administration of the cortical hormone does not stimulate growth in such animals. The presence of this vitamin in the adrenals must be considered in the same light as has been indicated in our discussion of vitamins A, B, and C.²⁴⁷

As already indicated the vitamins are also found in as high a concentration as they occur in the adrenals in other organs. Moreover, adrenalectomized animals maintained on adequate doses of the cortical hormone manifest no symptoms of vitamin deficiencies. We are justified in concluding, therefore, that it is only incidental that these vitamins occur in the adrenals. The susceptibility of the adrenal gland to conditions of avitaminosis is also not unique, for other endocrine organs (thyroid, pituitary, sex glands, *etc.*) are equally affected by dietary deficiencies. The vitamins, apparently, are essential for the well-being of a great number, if not all, of the organs and tissues of the body. The similarity between certain clinical manifestations of dietary deficiencies and of adrenal insufficiency are due to the fact that the cortical hormone is also necessary for the well-being of a number of tissues and organs. Hence, deficiency due to either vitamin or hormone leads to a multiplicity of manifestations (in the gastro-intestinal, nervous, cardiovascular, endocrine and other systems) and to a super-

ficially similar symptomatology. The increased susceptibility to infectious disease in avitaminosis and in adrenal insufficiency is only an indication of a general lowering of body-resistance—a condition occasioned by a number of causes.²⁴⁷

OTHER CONSTITUENTS

Small amounts of neurin are found in the adrenals and it has been suggested⁶⁸ that epinephrine may be derived from this neurin and pyrocatechol. Neurin is closely related to cholin which on decomposition yields hydroxy-ethyl-amine, the side group of the epinephrine molecule.

Histamine is present in extracts of the adrenals but it is probable that it is formed during extraction, from labile mother-substances. It is to histamine, choline, and acetylcholine that we must attribute the fall in blood pressure observed after injecting impure adrenal cortical extracts.

Jecorin, originally thought to be a pure substance, was isolated from the adrenals by Menasse, but subsequent work has shown this substance to be an adsorption compound of kephalin, inorganic salts, sugars, and other organic substances.¹³⁵

Adrenal extracts have been shown to manifest a number of enzymic actions. Thus the power of such extracts to oxidize salicylic aldehyde have been attributed to the presence of an aldehydase.¹³⁵ It has also been shown that the adrenals exert a marked diastatic activity.⁵⁶ The distribution of the lipolytic enzymes in the different zones of the adrenal has recently been determined.²²⁶

The yellow color of the adrenal is due to the presence in the adrenals of carotin, xanthophyll, and lipochromic substances. Infiltration of lipochromes occurs in many conditions associated with anatomical changes in the adrenals. The lipochromes are derived from the ingested food while the melanin pigment observed in the adrenals is endogenous in origin.¹⁹⁶

PART IV. CLINICAL CONSIDERATIONS

The involvement of the adrenals in Addison's disease, in adrenal tumors, and in certain anomalous developments of the reproductive system has been well established. Aside from these three conditions, which will be discussed in the following chapters, the adrenals have been implicated in a number of other clinical conditions on the basis of rather meager or obviously fallacious evidence. It is, nevertheless, very probable that the adrenals are involved in clinical conditions other than those generally recognized at present. Heretofore, pathological evidence and clinical observation have been the sole means whereby one might hope to detect the association of adrenal dysfunction with some clinical condition. Both of these methods are, however, notoriously unsatisfactory. The histological study of the adrenals is fraught (as we have seen in Chapter III) with great difficulty and unless the greatest precautions are taken it is difficult to interpret the significance of any observed abnormalities. Clinical observation is also an unsatisfactory means of gauging adrenal function for the symptoms associated with adrenal insufficiency are shared by a number of diseases in which the adrenals are not involved.

The development of potent extracts of the adrenal cortex has opened a new field of investigation in the clinical study of the adrenals. It is now possible by applying the therapeutic test to determine if insufficiency of the adrenals is responsible for a given clinical condition.

Most of the earlier clinical literature on the adrenals was based on the belief that epinephrine was the sole or at least the important internal secretion of these glands. The literature, even of recent years, contains voluminous reports based on this assumption. There is, however, no valid evidence to indicate that an insufficiency of the adrenal medulla manifests

itself clinically in any way. Despite its wide therapeutic applications, there is no clinical condition which can be proven to be a result of an insufficient secretion of epinephrine from the adrenal medulla.

CHAPTER XXI

ADDISON'S DISEASE

Thomas Addison's⁷ treatise "On the Constitutional and Local Effects of Disease of the Suprarenal Capsules" published in 1855, is one of the classics of medical literature. His publication initiated the modern study of the adrenal glands and remains today, from a clinical standpoint, almost complete in its accurate description of the disease.

Addison's observations were soon confirmed by Wilks,⁶⁸¹ Greenhow,²⁴² Hutchinson, Isaac Taylor, and Trousseau.⁶⁴⁰ The last named author first applied the term "Addison's Disease" in 1856. Addison's original conception of the disease was the one which is generally accepted today: *viz.*, that any lesion of the adrenals sufficient to interfere with their function would give rise to the train of symptoms characteristic of the disease. This view, however, soon became the subject of criticism by Addison's contemporaries who considered the disease as originating from a special lesion of the glands which resulted in secondary effects on the neighboring sympathetic nerves and ganglia. Addison also was led to alter his original views and to regard an involvement of the sympathetic system as a contributing factor in causing the disease. This "nervous" theory of the origin of the disease was in time abandoned when it was found that in many cases of genuine Addison's disease there was no involvement of the sympathetic system.

Wilks,⁶⁸¹ who studied the pathological anatomy of Addison's first patients, reported in 1865 a total of 33 cases which he considered as belonging to the disease entity which Addison had described. He excluded, however, certain of Addison's original cases as not belonging to this category. Wilks first noted the enlarged lymph nodes and lymphoid tissue, and the hypo-

plasia of the heart, which are found in patients dying of Addison's disease. He denied, however, the tuberculous nature of the adrenal involvement, for the variety of pathological forms in which tuberculous lesions manifest themselves was not clearly understood until a later date.

INCIDENCE

Addison's disease is relatively rare. In the United States 300 to 400 persons die annually from this cause, the death rate from Addison's disease being 0.4 per 100,000 population. In the British Isles the death rate is given as 0.6 while in Japan it is said to be only 0.04.²⁶⁰ Only 5 of the 320 persons recorded in 1917 as dying from Addison's disease were negroes which is surprising in view of the high incidence of tuberculosis in this race. It may be that because of the impossibility of detecting the pigmentation, which is so important an aid in diagnosis, most cases of the disease in the dark-skinned races are not recognized.

Addison's disease is one of middle life, the majority of cases occurring between the twentieth and fortieth years of life. Although cases have been reported in infants and in octogenarians, the disease is rare in children under 10 years of age. Except in very rare instances there is no evidence of any hereditary factor involved in the etiology of the disease. About two-thirds of all cases occur in males.⁵⁴⁰

Many predisposing causes have been noted such as worry, emotional shock, alcoholism, heredity, infections—especially malaria, influenza, pneumonia, and tuberculosis,—but it is doubtful if any except the last named play an etiologic rôle. The incidence of Addison's disease was not materially increased following the influenza epidemic of 1918. Hence it is very doubtful if influenza can be considered as a predisposing factor.²⁶⁰ A patient will naturally date the onset of the disease to some illness which may have been incidental or have accentuated a previously latent condition.

CLINICAL COURSE

The onset of the disease and the development of the cardinal symptoms—asthenia, gastro-intestinal disturbances, and pigmentation—are extremely insidious in their development. The patient begins to tire readily on slight exertion and becomes incapable of physical or mental effort. His complexion gradually becomes darker. Gastric symptoms with loss of appetite, nausea, and constipation alternating with diarrhea occur with increasing frequency.

The clinical course of the disease has been aptly described by Addison⁷ as follows:

“The patient, in most of the cases I have seen, has been observed gradually to fall off in general health; he becomes languid and weak, indisposed to either bodily or mental exertion; the appetite is impaired or entirely lost; the whites of the eyes become pearly; the pulse small and feeble, or perhaps somewhat large, but excessively soft and compressible; the body wastes without, however, presenting the dry and shrivelled skin and extreme emaciation usually attendant on protracted malignant disease; slight pain or uneasiness is from time to time referred to the region of the stomach, and there is occasional actual vomiting, which in one instance was both urgent and distressing; and it is by no means uncommon for the patient to manifest indications of disturbed cerebral circulation.” . . . “We discover a most remarkable and, so far as I know, characteristic discoloration taking place in the skin—sufficiently marked, indeed, as generally to have attracted the attention of the patient himself, or of the patient’s friends.”

“The disease develops in the third or fourth decade of life, usually quite insidiously, with adynamia and apathy. To these are added disturbances of the digestive tract (constipation, often alternating with diarrhea), and pig-

menting of the skin and mucous membranes: the patients succumb under a gradually increasing cachexia, not rarely with stormy terminal manifestations; autopsy almost always shows disease of both suprarenals, mostly tuberculous caseation."

Although the typical course of the disease leads to a fatal result in two to four years, chronic types have been reported¹¹⁹ in which patients have lived for ten or twenty years after the onset of the initial symptoms. In the acute forms death may follow in less than a week. Thus in a case due to thrombosis of one adrenal and infarction of the other, death occurred in five days.¹¹⁹ The average duration of the disease is about two years and varies according to the underlying pathological process as discussed later.

SYMPTOMATOLOGY

Asthenia, pigmentation, loss of weight, digestive disturbances (anorexia, nausea, vomiting, constipation, diarrhea), and abdominal or lumbar pain constitute the chief symptoms of complaint of patients suffering from Addison's disease.

The symptoms of the disease will vary depending upon the stage of its development. In an acute crisis the gastro-intestinal disturbances—nausea, vomiting, diarrhea, abdominal pains—may be the outstanding symptoms and lead to an erroneous diagnosis of an acute "surgical" abdomen. Hiccup may precede the onset of a crisis.

In the chronic stages of the disease, asthenia, weakness, pigmentation, hypotension, and decreased resistance to disease are the predominant symptoms.

Asthenia. Asthenia (physical and psychic) is the most characteristic and constant symptom. There is extreme lassitude and constant fatigue, which steadily progresses in its severity except during periods of remission. The patient ultimately is unable to rise from bed or even to perform the slightest movements.⁵⁴⁰

Pigmentation. A peculiar pigmentation of the skin is one of the most striking findings in Addison's disease. Addison⁷ described the pigmentation as a "dingy or smoky appearance or various tints or shades of deep amber or chestnut brown." With progress of the disease, the skin becomes light brown, bronze, dark brown, and ultimately the dark hue of a negro. Although pigmentation has been considered by some authors as an indispensable symptom in diagnosis, Lewin³⁹² found pigmentation in only 72 per cent of his cases. Lewin's estimate is probably too low, but there are a number of recorded cases^{119, 540} in which the diagnosis has been confirmed by autopsy and which during life were typical examples of Addison's disease except for the absence of pigmentation. In acute cases, particularly, pigmentation may be absent. In many cases the pigmentation may be the first symptom to attract the attention of the patient's friends.

The pigmentation is most marked in regions normally pigmented or exposed to light or pressure. It appears first on the face, neck, backs of the hands, and particularly on the knuckles. The skin of the scalp and areas covered by hair usually escape the pigmentation as do also the nail beds, palms, and soles.¹¹⁹

Pigmentation of the mucous membranes of the mouth, conjunctiva, and vagina, and of the serous membranes also commonly occurs. In the mouth this pigmentation usually is found as small spots and streaks of a bluish black color, on the lips, on the border of the tongue, the buccal mucosa, and gums.⁵⁴⁰

Cases of remission in the pigmentation and even its disappearance have been recorded. However, an apparent reduction of the pigmentation is produced by hydration of the skin. This may account for the claimed diminution in pigment observed after various forms of treatment.

Biopsy of the skin in Addison's disease shows the deposition of an abnormal amount of melanin in an epidermis and cutis

of an otherwise normal skin. The pigment is intracellular and forms a cap above the nucleus of the basal cells.⁴⁵⁸

Pigmentation is not confined to Addison's disease but may occur in abdominal growths, pregnancy, uterine disease, hemochromatosis, vagabonds, chronic arsenic poisoning, pernicious anemia, ochronosis, von Recklinghausen's disease, *et cetera* as well as in apparently normal individuals.

The usual explanation of the pigmentation is that it results from disease of the medulla rather than of the cortex. The diseased medulla being unable to retain its normal store of epinephrine is supposed to release this substance into the circulation which deposits it (or possibly its oxidation product or a precursor) in the epidermal layers where it is retained as melanin. This hypothesis would account for the cases of Addison's disease which are unaccompanied by pigmentation by assuming little involvement of the medulla. It would account also for the clinical fact that the more chronic form of the disease is more apt to be accompanied by deeper pigmentation than the acute, rapidly fatal form. In the former, it is the medulla which is most extensively involved, while in the latter, the cortex is chiefly affected.²⁶⁰

There have been several reports of pigmentation in lower animals following adrenalectomy (Nothnagel,⁴⁷⁴ Tizzoni,⁶²⁸ and Marino-Zucco⁴³⁷). Boinet went so far as to see the pigmentation not only in the skin of rats but as hematoidin granules in the blood. The careful observations of recent years on thousands of rats, cats, and dogs have not confirmed the appearance of any pigmentation. This is not unexpected if one assumes that disease of the medulla is responsible for the appearance of pigmentation in man, since in animal experiments not only the cortex but the medulla as well is removed. Only in animals in which the medulla is injured would one expect the pigmentation to appear on shaved portions of the skin subjected to the sunlight. Indeed Kellaway and Cowell³⁴⁸ observed pigmentation under these conditions in two animals in which the

medulla was partially destroyed by the insertion of needles containing radium emanations. In rats in which the medulla and most of the cortex was extirpated, the author failed to observe pigmentation of the shaved skin after exposure for several months to the sunlight. These results as well as the absence of pigmentation in adrenalectomized animals maintained for long periods on cortical hormone would indicate that it is not the presence of a precursor of epinephrine which is responsible for the pigmentation. It is possible of course that in the diseased medulla epinephrine itself or its precursor is converted to a product which is deposited in the skin.

It has recently been suggested that the absence of ascorbic acid in the diseased adrenal is responsible for the pigmentation and a case has been reported in which injection of this vitamin caused a disappearance of the pigmentation. This seems very improbable, however, if one considers the wide distribution of ascorbic acid in the body (*cf.* Chapter XX).

From a chemical consideration (*cf.* Chapter V) and from the arguments cited above, one would incline to the view that disease of the medulla is responsible for the pigmentation. However, in cases of adrenal atrophy in which only the cortex is destroyed while the medulla remains apparently healthy, one still finds marked pigmentation.⁶⁶⁸ Unless one assumes that the diseased cortex acts upon a precursor of epinephrine or interferes with the normal function of the medulla, it is difficult to accept the view that in these cases the medulla is responsible for the pigmentation.

Geenhaw²⁴¹ reported two cases and Bristow¹¹⁹ one case of complete destruction of the glands by carcinoma in none of which was there any pigmentation. The only one of 28 cases described by Barker³⁹ in which pigmentation was absent was one in which some functional medullary tissue was demonstrable.

From a clinical standpoint it is thus difficult to decide as to the anatomical basis for the appearance of the pigmenta-

tion. It has been suggested that the discoloration is due to sulfur derivatives normally removed from the circulation by the adrenals, or to some deficiency of the skin which causes the deposition of melanin, or to a protective mechanism on the part of the skin. The above cited clinical facts do not support these assumptions. Further experimental work is necessary before we can arrive at a satisfactory conclusion as to the mechanism of the pigmentation. The difficulty (if not the impossibility) of producing pigmentation in experimental animals has rendered a solution of this problem difficult. It appears most likely that pigmentation results from a change in the human skin, due to the deficiency of the cortical hormone, and is merely an exaggeration of the normal pigmentary processes. It may not be caused by deposition of epinephrine, its related compounds, or other normal or abnormal products of the adrenal glands (*cf.* Chapter V).

Loss of weight. Loss of weight which is so important a symptom of adrenal insufficiency in experimental animals is also a valuable index in gauging the progress of Addison's disease. The body weight is markedly reduced during exacerbations of the disease and is increased during remissions. Despite the loss of weight, the skin maintains its elasticity and hence does not give the flabby appearance characteristic of other wasting diseases.⁵⁴⁰

Digestive disturbances. In an analysis of 160 cases of Addison's disease, Marañón, Sala, and Arguelles⁴²⁹ found the following digestive disturbances:

	<i>Cases</i>
Intense hunger	3
Inappetence (anorexia)	142
Dyspepsia	61
Nausea	39
Gastric ulcer	3
Diarrhea	45
Constipation	35
Pseudo-peritonitis	11
Hiccough	43
No digestive symptoms	19

Intense hunger which is an infrequent complaint was only observed during the beginning of the illness. Anorexia is the most common digestive complaint. The mere sight of food may arouse nausea. Vomiting is a troublesome symptom particularly in the late stages of the disease.

The severe diarrhea as seen in experimental adrenal insufficiency also occurs in man and may simulate, in its appearance, *cholera nostras*. The pseudo-peritonitis referred to above, is marked by spasm of the abdominal muscles, by a small pulse, and in general may give the appearance of an acute affection of the abdomen.

An aversion to fatty foods has been noted by Rogoff⁵²² as an important early symptom.

One of the common findings in experimental adrenal insufficiency, as we have seen (Chapter X), is inflammation and ulceration of the gastro-intestinal tract and digestive disturbances identical to those observed in Addison's disease. The disturbances observed in man may possibly be due to an inflammation of the gastro-intestinal tract similar to that observed in experimental adrenal insufficiency. In the late stages of the disease there may be a diminution or even absence of the gastric hydrochloric acid and of the ferments.¹¹⁹

Nervous manifestations. Certain nervous and mental manifestations often occur in Addison's disease. The patient may be apathetic, listless, depressed, or irritable; mental activity is often impaired; neuralgic pains occur in the lumbar region, in the epigastrium, and in the extremities; headaches, tinnitus vertigo, rheumatoid pains, and insomnia are sometimes present.

Pain in the back may be an early symptom and was present in every case in Snell and Rowntree's series. Pain on pressure in the costolumbar angle has been considered a noteworthy symptom.⁵²²

In the late stages of the disease, nervous symptoms may become prominent. Delirium, acute mental confusion, convulsions, numbness, and coma are common as terminal symptoms.

The agonal symptoms may be accompanied by clonic convulsions, delirium, and stereotyped movements similar to those observed in insulin poisoning. These symptoms, which are also not infrequently observed in experimental animals, are sometimes probably due to hypoglycemia.^{17, 473}

CIRCULATION

Addison⁷ noted the small, weak, easily compressible pulse of his patients. Wilks⁶⁸¹ observed the extreme hypoplasia of the heart with its tortuous coronary arteries. The heart sounds described by Addison as discernible over the apex are probably attributable to the anemia of his patients. The hypoplasia of the heart and large vessels has been frequently noted, the transverse diameter of the heart being only 8 cms. in a case described by Zondek.⁵⁴³ Rowntree⁵³⁸ has reported the occurrence of precordial pains in some of his patients. In a series of 35 cases, this observer noted inversion of the T-wave in 2 cases, auricular fibrillation in 1 case, and heart block in another. The heart was normal in all the other cases.

Sampayo and his collaborators⁵⁴³ have recently examined the circulatory system of a large series of patients suffering from Addison's disease. They noted as constant findings a low potential of the electrocardiogram with evidence of conduction defects in the P-Q, Q-R-S, and S-T complexes. The degree of these changes in the electrocardiogram was proportional to the intensity of the disease.

There are, however, no clinical signs of cardiac decompensation. In fact, the above described findings are those one might expect from an asthenic myocardium in fatigue responding to a diminished demand on the part of the organism.

Hypotension is usually considered as one of the most important findings in Addison's disease, but its significance has probably been overestimated. The average blood pressure in Rowntree's⁵³⁸ series was 96/67; with a maximum systolic pressure of 115, diastolic, 85; minimum systolic pressure, 60, diasto-

lic, 40. Values as low as 30 or 40 mms. for the systolic pressure have been reported, but such values are inconceivable in the light of our generally accepted concepts of circulatory hemodynamics.²⁴³

Janneway first called attention to the fact that a low blood pressure is not as constant a finding in this disease as is generally believed. In a quarter of Snell and Rowntree's⁵⁴⁰ cases the systolic pressure was 105 mms. or more and in one case the blood pressure was 145/100 one week before death. The commonly accepted view concerning the indispensability of hypotension in arriving at a clinical diagnosis of Addison's disease is thus erroneous. To attribute the observed hypotension to disease of the medulla and the absence of epinephrine secretion is also untenable, for cases of adrenal atrophy in which the medulla is spared are also accompanied by the usual degree of hypotension.

It may be emphasized that the average blood pressure of normal, healthy individuals in the basal resting condition is only 105/67, which is much lower than the values obtained under usual clinical conditions.²⁴³ The marked asthenia of the patient with Addison's disease will render truly basal values readily obtainable. The reduced metabolism observed in some patients and a vascular hypoactivity comparable to that observed in the rest of the tissues will also tend towards a low blood pressure.²⁴³ All of these factors suffice to explain the observed hypotension without necessitating the assumption that some normal pressor agent is absent from the blood in Addison's disease.

Patients with Addison's disease often suffer vertigo and faintness in changing from the recumbent to the upright position. There is, however, a rise in blood pressure during this change in posture which differentiates this condition from postural hypotension.⁵⁴⁰

The average pulse rate in Rowntree and Snell's series of patients was 94. This elevation of the pulse rate in the presence

of a reduced metabolic activity is similar to that observed in the early stages of experimental adrenal insufficiency. Its significance is still unknown.

Blood picture. Although considered by Addison as a form of "idiopathic anemia," the blood picture in Addison's disease is not usually strikingly abnormal. There is only a slight reduction of hemoglobin to about 85 per cent of normal. Although the leucocyte count is within normal range, there is at times a lymphocytosis, first observed by Neusser.⁴⁶⁸ This lymphocytosis is probably a reflection of the generalized hypertrophy of the lymphoid tissue which is often noticeable clinically by the enlargement of the tonsils, papillae of the tongue, and a persistent thymus. There is often also an increase in the number of the large mononuclear cells while the neutrophilic cells are relatively and absolutely reduced in number.²⁶⁰

Blood chemistry. The marked changes observed in the composition of the blood of adrenalectomized animals in adrenal insufficiency have been described in Chapter XI. In Addison's disease one fails to note these striking abnormalities of the blood chemistry. During a crisis, however, changes very similar to those already described as occurring in experimental animals are encountered. Thus the sodium concentration may be reduced to about 120 milli-equivalents per liter in contrast to its normal value of 140.³⁹⁹ The chloride and bicarbonate ion concentrations are correspondingly decreased while the potassium ion concentration is increased. The non-protein nitrogen and urea nitrogen may assume values twice as great as the normal. The total base of the blood is diminished. Due to the hemoconcentration attendant upon the loss of body fluid, the serum protein concentration becomes elevated. The blood sugar is often reduced to convulsive levels but, in many cases, practically normal values persist despite marked alterations in the concentration of other blood constituents.⁵⁴⁰ Injection of epinephrine does not cause the rise in the blood sugar which it

normally does. The sugar tolerance curve is also often markedly abnormal in Addison's disease.

METABOLISM

Although adrenal insufficiency in mammals, as we have seen (Chapter XIII), is attended regularly by a decrease in the basal metabolic rate, this decrease is not always observed in man. Of 13 cases studied by Rowntree,⁵³⁸ 8 showed a normal metabolic rate, one with active pulmonary tuberculosis showed a B. M. R. of +20, and the remaining 4 cases had a B. M. R. of -30, -20, -13, and -10, respectively. Of the 38 cases studied by Rowntree and Snell,⁵⁴⁰ only 13 showed a low basal metabolic rate. Marañon, Pena, and Florit⁴²⁷ observed a normal metabolic rate in two-thirds of their 52 cases. About a quarter of their cases showed a decreased metabolism while the remainder showed an actual elevation. These findings are in essential agreement with those of other investigators.²⁴⁰

Addison's disease is often complicated by the co-existence of tuberculous infection elsewhere in the body which may account in part for the normal and elevated rates, for the pyrexia of tuberculosis is occasioned by an increased metabolic rate. Marañon's observation of the depressing effect of cortical extracts on the metabolism of his patients is undoubtedly due to the presence of noxious impurities in the extracts. Potent preparations cause a rise in the metabolism to its normal level in the experimental animal.

Although the temperature is usually reduced, it may suddenly rise to hyperpyrexia levels, particularly in the terminal stage of the disease. This hyperpyrexia may possibly be due to a flare-up of the tuberculous infection rather than to any cause related to the adrenal insufficiency. It may, on the other hand, possibly be a reaction analogous to that observed experimentally in an acute partial insufficiency (*cf.* Chapter XIII). The average temperature in Rowntree and Snell's⁵⁴⁰ series of patients was 36.1° to 36.7°C. (97° to 98°F.) with *pre-mortem* rises to 39.4° to 40°C. (103° to 104°F.).

RESPIRATION

The respiration in Addison's disease is usually normal except for dyspnoea on exertion, sighing, and yawning. In crises, the respiration may become irregular with the Biot type of respiration commonly seen in cerebral involvements.⁵⁴⁰

REPRODUCTION

In the late stages of Addison's disease there is loss of the reproductive activities and loss of libido. The failure of the reproductive system in women manifests itself first in irregular menstruation, oligomenorrhea, and amenorrhea. Conception is rare but cases have been reported in which normal pregnancy has proceeded to term.¹¹⁹ During pregnancy the symptoms of the disease are ameliorated, probably for the same reasons suggested (Chapter VIII) for the prolonged survival of adrenalectomized pregnant animals. In men there may be impotence.⁵⁴⁰

LIFE EXPECTANCY

Although there is no fixed order in the appearance of the symptoms, asthenia usually precedes the melanoderma. At times, however, the pigmentation may precede the appearance of the other symptoms. Though there is often a gradual increase in the severity of the symptoms, usually the disease is marked by paroxysmal remissions with intermissions during which the patient may be in fair health.

The "paroxysmal mode of progress" of Addison's disease has been aptly described by Greenhow²⁴² as follows:

"The asthenia, the constitutional symptoms generally, and the change of colour in the skin are all, it is true, progressive, but not steadily so. The course of the disease, on the whole, is slow and chronic; but it is subject to alternate exacerbations and remissions, usually in some degree dependent upon favourable and unfavourable circumstances but sometimes also apparently quite independ-

ent of them. During the remissions, strength is in a great degree recovered, appetite improved, sickness abated; the discolouration becomes paler, and, above all, the patient's whole aspect bespeaks that a heavy weight has been lifted from his head. After each fresh exacerbation, however, the patient remains upon a somewhat lower level than during the previous remission. The recovery of strength and the abatement of other symptoms is less marked, and the skin, though paler than during the last exacerbation is yet visibly darker than before it. Similar alternations may occur several times before the onset of the fatal paroxysm, but on each occasion the patient takes at least one downward step that he never regains."

The duration of Addison's disease is dependent to a large extent on the type of lesion present in the adrenals. The expectancy of life in cases of adrenal atrophy is about three times that of cases due to tuberculosis.²⁶⁰

It has been often noted that patients in whom the pigmentation is an early symptom do better than those in whom the pigmentation is not marked, or absent. Thus in the series of 566 cases analyzed by Guttman,²⁶⁰ the average duration of the disease was 43 months in patients in whom pigmentation was the first symptom and asthenia developed subsequently. In cases in which pigmentation and weakness developed simultaneously, the average duration of life was only 12 months, while in the group of patients in whom weakness was the first and predominant symptom and pigmentation of subsequent onset, the average duration of the disease was only 8 months.

PATHOLOGY

In the earlier reports one finds cases described clinically as Addison's disease which at autopsy failed to reveal lesions of the adrenal glands. Thus in Lewin's³⁹² compilation of 639 cases, 12 per cent showed "normal" glands. It is most likely that the clinical diagnosis in these cases was erroneous for the

diagnosis of Addison's disease based solely on the occurrence of pigmentation and asthenia, as frequently made, is open to error. In the recent compilations only rare cases have been reported of normal glands and the clinical history and autopsy reports in these cases are not entirely convincing.⁴¹⁷ It is possible, of course, that disease of neighboring organs (*e.g.*, malignant involvement of the solar plexus⁵⁴) may lead to an acute suppression of the secretion of the vital hormone without gross anatomical evidence of disease of the adrenals.

The adrenals of patients dying of the disease show almost complete destruction of both cortex and medulla or cortex alone, depending, as shall be described in subsequent sections, on the type of lesion encountered.⁴¹⁷

In Barker's³⁹ series of 73 cases of disease of the adrenals of diverse etiology, in which the cortex remaining comprised approximately 10 to 50 per cent of the normal amount of tissue, no clinical symptoms of Addison's disease were evident during life. It was only when at least 90 per cent of the cortical tissue had been destroyed that clinical symptoms became prominent. Latent symptoms may possibly be present without such extensive destruction but have not been noted clinically. In many cases of adrenal atrophy only the cortex is involved and the medulla remains almost intact.⁶⁶⁸

Accessory interrenal tissue (*cf.* Chapter IV) is rarely encountered except in the intimate neighborhood of the main glands. Hence pathological involvement of the adrenals usually involves these accessories and it is extremely rare for such accessory tissue to prevent the appearance of symptoms of Addison's disease. In the early stages of disease, particularly in cases of so-called adrenal atrophy, the healthy tissue hypertrophies forming adenomatous nodules but these are ultimately involved in the destructive process which caused the initial lesion.

In an analyses of 566 cases of Addison's disease collected from the literature over the period 1900 to 1930, Guttman²⁶⁰

found the causative agent to be: bilateral tuberculosis, in 68.3 per cent; primary atrophy, in 19.4 per cent; amyloid disease, in 1.7 per cent; and neoplasms, in 1.2 per cent of all the cases. Fatty degeneration, pressure atrophy, venous thrombosis, arterial emboli, and syphilis comprised the remaining 9.4 per cent.

Hedinger²⁹¹ found tuberculosis in 14 out of 15 patients with Addison's disease. In Coneybeare and Millis'¹²² 29 autopsied cases, 22 had bilateral fibrocaseous tuberculosis of the adrenals and 6 had simple atrophy. In the remaining case, remnants of the adrenals could not be found. Philpott's⁵⁰⁴ 14 cases comprised 7 cases of tuberculosis, 4 of metastatic carcinoma, and 1 each of mycosis fungoides, simple atrophy, and amyloidosis. Barker³⁹ in his detailed account of the autopsy findings in 28 cases, studied clinically by Rowntree, found tuberculosis in 25 and atrophy in the remaining three.

Tuberculosis. The fibro-caseous lesions of tuberculosis are found in about 90 per cent of all cases of Addison's disease. The greater interest in cases due to other causes results in their being more frequently reported in the literature as isolated case reports. Hence, collections from the literature give an erroneous idea as to the relative frequency of the different lesions.²⁶⁰ Atrophy of the adrenals is found in about 10 per cent of all cases. Vascular defects, malignant growths, syphilis, and other conditions are the cause of relatively few of the cases of Addison's disease.

Tuberculosis affects the adrenals with more or less complete caseation, with softening, fibrosis, or calcification. The glands may be shriveled but most often are enlarged.

Sections of the tuberculous glands show tubercles with endothelial cells, giant cells, fibroblasts, and lymphocytes.²⁹⁶ Acid fast bacilli can be demonstrated.³⁹ The necrosis differs from the caseous necrosis of tuberculosis observed in other glands and tissues in its yellow color and firm consistency.

Some normal cortical tissue was observed by Barker³⁹ in

24 out of his 25 cases. The cells were hypertrophic and hyperchromatic. The amount of this remaining tissue was estimated at less than 5 per cent of the whole normal gland. In only one case was any medulla seen, and this case was the only one in which no pigmentation occurred. In cases in which as much as 80 per cent of the adrenals had been destroyed by tuberculosis there were no symptoms of Addison's disease. The process apparently starts in the medulla or mid-cortical region and spreads centrifugally. Healed processes are not observed, indicating the progressive course of the disease.

Although the adrenals are the chief site of tuberculous infection in many cases, it is doubtful if the lesion in these organs is ever primary. The infection in the adrenals is probably hematogenous in origin.²⁵⁵ As is the case in tuberculous infection of the eye or kidney, the lesion is first unilateral and then extends to the second organ. The fact that 90 per cent of the cortical tissue must be destroyed before symptoms of Addison's disease are evident, explains why (unlike in the kidney or eye) unilateral infection of the adrenals does not manifest itself during life. At autopsy, however, one frequently observes tuberculous lesions of the adrenals from a slight unilateral to a partial bilateral stage of advancement without any clinical symptoms of the disease having manifested themselves during life.

Miliary tuberculosis of the adrenals is not common, but careful examination of the adrenals in generalized miliary tuberculosis will usually reveal some small foci of infection.⁴¹⁷

There is often extensive tuberculous destruction of both adrenal glands with comparatively little disease of other organs. Elsässer⁴¹⁷ found isolated tuberculosis of the adrenals in 17 per cent of 549 cases.

Atrophy. About 10 per cent of all cases of Addison's disease are caused by atrophy, which may involve the cortex only or may affect the entire gland so that no trace of the glands is visible on gross examination at autopsy. However, careful

microscopic examination of the fatty connective tissue will reveal some remnant of islets of cells resembling those of the cortex. These were found by Crosby¹⁴⁰ near the superior poles of the kidneys embedded in retroperitoneal fat. In most cases the adrenals are shrunk to a quarter or less of their normal size.^{84, 130}

Considerable difference of opinion exists in the literature regarding the nature of the atrophy affecting the adrenals. Various descriptive terms have been applied depending upon the author's opinion regarding the nature of the process. The terms idiopathic, primary, or simple atrophy have been frequently used to indicate that the process is of the nature of an aplasia. Others have considered the process, in some cases at least, to be inflammatory in origin but in most cases the histological picture does not conform with that of an inflammation. The assumption that the process is due to a toxic agency led Kovács³⁶⁴ to apply the term "cytotoxic contracted adrenal." Cirrhosis, chronic dystrophy, hypoplasia, *etc.* are other terms which have been frequently used.

The process is not a simple atrophy in the sense that there is no shrinkage due to atrophy of the cellular elements. There is instead a necrosis of the parenchyma which leads to the disappearance of the cells. The expression "primary contracted adrenal gland" suggested by Guttman²⁶⁰ is probably most accurate considering the unknown nature of the etiologic agent causing the lesion. We shall, however, retain the simpler expression "atrophy" in the present volume.

The older view advanced by Neusser⁴⁶³ and Wiesel⁶⁷⁸ that adrenal atrophy is part of a congenital hypoplasia of the chromophil tissue, in general, is no longer tenable. Pathological studies indicate that the process is acquired. Simmonds⁵⁷³ considered the etiology to be tuberculosis or syphilis but his views have not been substantiated by recent observers. The absence of an inflammatory hyperplasia of the interstitial tissue or of any inflammatory reactions around the glands speaks against

the view that adrenal atrophy is due to a chronic inflammation. The pathological picture closely resembles that encountered in yellow atrophy of the liver.⁶⁶⁸ Brenner⁵⁴ considered the atrophy to result from a slow necrosis due to some toxin. The cells which remain unaffected tend to undergo compensatory hypertrophy and form adenomatous nodules which are in turn attacked or become exhausted.

In Barker's³⁹ three cases of adrenal atrophy the medulla was normal while the cortex was reduced to a narrow strip. The remaining cortical cells were large, deeply stained, and pigmented, with numerous lymphocytes interspersed between the cortical layers. In a few places were areas of recent hemorrhage. The diffuse nature of the destruction and the fact that it is bilateral point to a toxic atrophy or a low grade inflammatory process of unknown cause.^{39, 481}

In general, as in the six cases described by Wells,⁶⁶⁸ there is a selective necrosis of the cortical cells with much less visible injury to the elements of the medulla. The other changes observed in the adrenals appear to be secondary to this selective necrosis and consist of attempts at regeneration by proliferation and hypertrophy. According to Wells it is impossible to say if the partial loss of medullary tissue is due to the same agency as is responsible for the cortical injury or if the medullary destruction is the result of the extensive lymphoid infiltration which follows the destruction of the cortex.

A condition closely resembling adrenal atrophy as it occurs clinically is sometimes seen in rats subjected to an incomplete adrenalectomy. In such animals hypertrophy of the remaining fragments of the adrenals maintains them in normal health. In some animals, however, after a variable period of time, the hypertrophied adrenal tissue undergoes atrophy and the animal develops a fatal adrenal insufficiency. On microscopic examination the hypertrophied fragments of cortical tissue, which had maintained the animal in good condition following adrenalectomy, present a picture resembling that observed in adrenal atrophy as it occurs in man.

Amyloid degeneration. Amyloidosis of the adrenals, which, as a rule, involves only the cortex, is an occasional factor in the etiology of Addison's disease. Bronfin and Guttman⁹⁴ in a series of 100 necropsies on patients dying of tuberculosis observed 14 cases of amyloid infiltration of the adrenals. In 5 of these cases a diagnosis of Addison's disease was made during life. In some cases, symptoms suggestive of Addison's disease were present while the remainder showed no symptoms indicative of this disease. Histological study of the adrenals showed that a definite relation existed between the extent of the amyloid involvement of the adrenal cortex and the symptomatology (notably asthenia and gastro-intestinal symptoms).

Amyloid degeneration of the adrenals, as of the spleen or liver, results from chronic suppurations, tuberculosis, or syphilis. The amyloid is deposited chiefly in the fasciculata but may extend throughout the cortex and the medulla, compressing the cells.⁵⁰⁴

Parenchymatous degeneration, with the formation of hyaline or colloid droplets, have been described, but it is questionable if these are not *post-mortem* or agonal changes. These occur usually in the medulla. Fatty degeneration of the adrenal cortex has been noted in several cases of Addison's disease.⁴¹⁷

Malignant growths. Although the adrenals are occasionally the site of primary or metastatic malignant growths, Addison's disease is rarely caused by such invasions.^{163, 241} This is probably to be accounted for by the fact that so large a part of the glands must be destroyed before clinical symptoms of insufficiency are elicited. In most cases death from the malignancy intervenes before such extensive destruction of the adrenals has occurred.⁴¹⁷

Syphilis. There have been a number of clinical reports of cases of Addison's disease attributed to syphilis. These reports have been based on the favorable reaction of the patients to antisyphilitic treatment.^{552, 565}

Syphilitic lesions in the form of gummata with caseation, fibrosis, and infiltration of the surrounding tissues, however,

have been rarely noted at autopsy. The occurrence of spirochaetes in such lesions in a case dying of Addison's disease has been reported.⁴¹⁷

Other findings. Rare changes in Addison's disease are echinococcus cyst, mycosis fungoides, abscess formation following pneumonia, trauma, and vascular lesions. Among the last named, venous thrombosis,⁵⁹ arterial embolism, and hemorrhage into the substance of the adrenal have been reported as causative agents of Addison's disease.^{260, 417}

OTHER AUTOPSY FINDINGS IN ADDISON'S DISEASE

Due to the concomitant presence of tuberculosis, the ravages of this disease will usually be present at autopsy in patients dying of Addison's disease.

Among Barker's³⁹ 75 cases of adrenal tuberculosis, there was active pulmonary tuberculosis in 10, genito-urinary tuberculosis in 4, and both genito-urinary and pulmonary tuberculosis in 2 cases. Of the remaining 8 cases, there was active tuberculosis of the spine, liver, spleen, or abdominal lymph nodes in 5. In only 3 cases could no active tuberculous lesions of other organs be demonstrated. Gsell and Uehlinger,²⁵⁵ on the other hand, report the active tuberculous process to be limited to the adrenals in one-third of their cases.

The body is usually wasted, although extreme emaciation is not present. The heart may show atrophic changes. Its walls are thin causing the coronary vessels to run a tortuous course. The cardiac muscle itself may be distinctly brown. The blood vessels show no distinctive changes except for the hypoplasia of the aorta and large vessels.⁵⁴³

The stomach shows atrophy of the mucosa. The intestines may contain fluid faeces and the lymphoid follicles throughout the alimentary tract are hypertrophied.

As might be anticipated from the renal impairment observed in adrenal insufficiency both experimentally and in Addison's disease, renal lesions are often demonstrable at autopsy. Bar-

ker³⁹ found normal kidneys in only 7 of his 28 cases. Congestion, diffuse tubular atrophy, chronic pyelonephritis, and renal tuberculosis were observed in the other cases. The tubular atrophy had the appearance of a toxic nephrosis and was present in a third of the cases.

The thymus is often persistent (*cf.* Chapter X) and the spleen is enlarged and soft. There is general hyperplasia of all the lymphoid tissues. Hedinger²⁹¹ found a *status thymolymphaticus* in 7 of his 15 cases.

Guttman,²⁶⁰ in his comprehensive analysis of the cases of Addison's disease reported in the literature, found no significant changes in the thyroid. On the other hand, in the six cases of Addison's disease due to adrenal atrophy, described by Wells,⁶⁶⁸ there was a marked infiltration of the thyroid gland with lymphoid cells. This infiltration of the thyroid gland with lymphoid tissue has been described in Addison's disease of tuberculous origin but is less marked and less frequent than it is in cases of non-tuberculous origin.⁶⁶⁸

In a small proportion of cases, extensive alterations occur in the sympathetic system (particularly the semilunar ganglion and the solar plexus) which may extend into the central nervous system.

The hypophysis in some cases of Addison's disease shows a diminution in the number and size of the eosinophil cells and a scarcity of normal, well-granulated, basophilic cells. The significance of these changes has been discussed in Chapter XIV.

The reproductive glands sometimes show atrophic changes.⁴¹⁷

Considerable controversy has always raged regarding the question as to whether the pathological changes in the adrenals were alone responsible for the clinical manifestations of Addison's disease or whether changes in other glands were in part also responsible for these manifestations. A number of workers described changes in the ganglia or the spinal cord to which they attributed the manifestations of the disease rather

than to the comparatively lesser changes demonstrable in the adrenal. Wiesel⁶⁷⁸ attributed Addison's disease to destruction of the medulla rather than to the cortex.

In view of recent developments in our knowledge of the relative functions of the cortex and medulla, we must accept the view that the cortex is responsible for most of the symptoms and for the fatal outcome of Addison's disease. As we have already seen, it is doubtful if the medulla is even responsible for the pigmentation. The symptoms of the disease are identical to those observed in experimental cortical insufficiency. We may, therefore, consider damage to the cortex or to its secretion as the sole responsible agent for the disease. It is possible, however, that a diseased medulla may cause destruction of the cortical hormone; for, as we have seen (Chapter II), the blood from the cortex which presumably carries the hormone is drained into the medulla. Wide involvement of the medulla might easily cause destruction of the hormone as it passes through the diseased tissue. This would explain the existence of cases of Addison's disease in which the adrenal defect was limited to the chromaphil tissue, but the evidence for the existence of such cases is very meager. It is more likely that the clinical diagnoses were in error in these cases.

DIAGNOSIS

It may appear from the preceding sections that diagnosis in Addison's disease is a comparatively simple matter. The pigmentation, asthenia, hypotension, gastric disturbances, *etc.* define a clinical picture to which many cases closely conform. However, in a certain group of cases an error in diagnosis may easily result. Pigmentation closely resembling that of Addison's disease, as has already been noted, is frequent in other conditions. Asthenia is common in protracted debilitating illnesses as are also hypotension and digestive disturbances. Hence the occurrence of this group of symptoms may often,

as in the cases quoted by Coneybeare and Millis,¹²² lead to an erroneous diagnosis of Addison's disease. Cases of pernicious anemia, abdominal tuberculosis, and other conditions may simulate Addison's disease in many of their symptoms.

On the other hand, failure to recognize Addison's disease may often occur, particularly in those cases in which there is no marked pigmentation. Cases of acute insufficiency of the adrenals, to which the name Addison's disease is strictly speaking not applicable, may also fail to be recognized. Thus, cases of acute convulsive seizures, accompanied by low blood pressure and respiratory irregularities, have been described in which autopsy revealed marked adrenal injury as the only finding to which the symptoms might be attributed.⁴⁷³ Such symptoms are obviously to be attributed to adrenal insufficiency. In other cases reported in the literature patients dying suddenly under an anesthetic, for example, or of some unknown cause are found at autopsy to have extensive adrenal disease without having manifested the symptoms of Addison's disease during life. In such cases we are dealing with latent forms of the disease, suddenly aggravated by an operation, or with an adrenal insufficiency which is marked by the co-existence of a second disease. In the absence of pigmentation, one might easily attribute the existence of asthenia, hypotension, *etc.* to an active tuberculous process elsewhere, when in reality they may result from disease of the adrenals.

Calcification, demonstrable by the roentgenogram, is often present in cases due to tuberculosis.³⁰ One must avoid being misled by calcification of a lymph node or other adjacent structure.

The reaction of patients suffering from Addison's disease to a sudden deprivation of salt has recently been suggested as a diagnostic aid in doubtful cases.⁴⁸⁵ Patients suffering from Addison's disease may be thrown into a state of acute crisis by subjecting them to a salt-free diet. This test must, therefore,

be applied cautiously and any untoward reaction promptly counteracted by the administration of liberal amounts of the cortical hormone and salt.³⁵¹

The therapeutic test should prove of value in the future for arriving at a diagnosis in doubtful cases. The results of therapy with the adrenal cortical hormone should indicate whether adrenal insufficiency is the source of the observed clinical manifestations. Such a therapeutic test may also prove to be a means of detecting the disease in its early stages.

TREATMENT

The history of the therapeutic management of Addison's disease has been one of repeated disappointments. False hopes of cure have been inspired by the natural remissions which characterize the disease. Many modes of therapy and reports of cures have been reported, from the case described by Addison as ostensibly cured by brandy to the cases of recent years ostensibly revived by adrenal extracts. Many apparent cures resulted from an original error in diagnosis as in the patient reported cured by an adrenal graft, but who at autopsy was found to have had no disease of the adrenals and no successfully grafted adrenal tissue.

Irradiation of the diseased adrenals has been tried with no success. Removal of a tuberculous gland in the early stage of the disease would be indicated (as in renal tuberculosis) but unfortunately the symptoms of Addison's disease do not manifest themselves until the disease has affected both glands. With a supply of the adrenal cortical hormone available to carry the patient through an operation, it may be that future developments in surgery will make it possible to remove the diseased portion of the glands and leave bits of the remaining healthy tissue to hypertrophy or transplant them to new sites. The absence of any method by which mild degrees of cortical insufficiency can be detected prevents early recognition and treatment of the disease in its early stages. The discovery

of methods for detecting the disease in its initial stages is very desirable.

The emphasis placed on epinephrine as the assumed hormone of the adrenals and its remarkable pharmacological effects led to its early application in Addison's disease. The administration of epinephrine, hypodermically and rectally, and of adrenal substance, by mouth, formed the basis for the so-called Muirhead⁴⁶¹ treatment. Recent studies have demonstrated that lack of epinephrine is certainly not responsible for the symptoms of Addison's disease. Injection of epinephrine into adrenalectomized animals is not only not beneficial but very harmful. It is logical to conclude, therefore, that epinephrine is not only of no value but is to be avoided in patients with Addison's disease. The use of dried glandular materials either in the form of a desiccated powder or as a glycerine extract, as advocated and used in the past, are also to be deprecated. The slight possible usefulness of these preparations is overbalanced by the deleterious effects of the large amounts of epinephrine and its phenolic derivatives which these preparations contain.^{42,249} In the doses in which they are tolerated, no conceivable benefit could be derived, for it would require over a kilogram of most of these preparations to furnish enough of the hormone for the daily need of a patient manifesting symptoms of adrenal insufficiency. Even the use of fresh glands would require an amount of material beyond the digestive capacities of even a normal individual.²⁴⁶ Hence we must utilize concentrates derived from large quantities of glands. The potency of the preparation and its freedom from noxious impurities must be demonstrable by experiments on adrenalectomized animals.

There can be no doubt that the symptoms of Addison's disease are due to an absence of the vital adrenal cortical hormone. Hence the first requirement of successful therapy must consist in supplying an amount of this hormone adequate for relieving the patient of asthenia, gastric disturbances, weak-

ness, *etc.* and permitting him to perform his normal activities without subjective discomfort. Except in rare cases due to syphilis, in which antisyphilitic treatment cautiously administered should prove curative, we must rely upon a replacement therapy to supply the patient with the vital hormone no longer furnished in adequate amounts by the diseased adrenals. Methods for preparing the hormone, its administration, and dosage have already been described in Chapters XV and XVI.

The patient suffering from Addison's disease is particularly sensitive to extraneous influences—physical or mental—which tend to excite or exhaust and must be maintained in as calm and unperturbed an atmosphere as possible. Exhaustive examinations are to be avoided. Transportation over long distances has brought on a severe crisis. Extremes of heat or cold must be avoided. Drugs must be administered very cautiously. Purgation has proven fatal in several cases.⁵³⁸ The operative risk of these patients is remarkable; even the extraction of a tooth has resulted fatally. Only when the patient has received an adequate course of treatment with the cortical hormone and has regained his strength should he be subjected to a surgical operation. The administration of large doses of hormone during and following the operation is desirable. Local anesthesia is preferable to the use of a general anesthetic.⁵⁴⁰

The administration of an adequate supply of the cortical hormone should result in an alleviation of the symptoms due to disease of the adrenals. Certain of the bodily deficiencies due to the disease may be remedied by other means. Thus the diminution in the blood volume which occurs in crises should be corrected by intravenous injections of glucose and saline solutions. Sodium chloride in Addison's disease has been intensively used in recent years since Loeb³⁹⁹ demonstrated the remarkable clinical benefits derived from the use of this substance. The rationale of sodium chloride therapy is well established. The loss of this substance from the body in adrenal

insufficiency leads to a reduction in its concentration in the body. It is to be expected therefore that replacement of the lost sodium chloride will aid in preserving the well-being of the patient. The experiments of Harrop and his collaborators²⁶⁶ would indicate that the use of sodium chloride together with bicarbonate of sodium is advantageous over the use of the former substance alone. The administration of sodium chloride intravenously to patients in crisis has proven life-saving. Ten to twenty grams of sodium chloride administered daily has been of great aid during periods of remission from the more severe stages of the disease. This salt or a mixture of sodium chloride and sodium bicarbonate in the ratio of 2:1 by weight may be administered in enteric coated capsules, in milk, with lemon juice, *etc.*³⁵¹

Since disease of the adrenals is often accompanied by tuberculosis or other diseases it is of course essential to treat these conditions. Even with a replacement therapy available, the presence of the diseased adrenal remains a constant threat to scatter the germs of tuberculosis through the body.⁵⁷⁹

In cases of syphilis, antisyphilitic treatment is indicated although this must be carried out with great caution and only after preliminary and continued treatment of the patient with the cortical hormone.

The problem of adrenal grafts has already been discussed in Chapter XIX. A number of attempts have been made to graft fetal adrenal tissue into the testis, ovary, or abdominal connective tissue. The work in the past was of necessity unsuccessful for the patient could not endure the operative shock of the operation. More recently attempts have been made to transplant cells derived from tissue cultures into the axillary subcutaneous tissue. With a supply of the cortical hormone available to carry the patient through the necessary operative procedures, a new attempt at adrenal transplantation of fetal cortical tissue or tissue cultures is warranted.

Although the results of the therapeutic management of Addi-

son's disease have been exceedingly disappointing, in the past, there is every reason to anticipate better results in the future. The combined use of salt and ample doses of a potent preparation of the adrenal cortex (administered orally in frequent doses)²⁴⁹ should radically improve the poor results hitherto obtained.

CHAPTER XXII

TUMORS OF THE ADRENALS

Primary tumors of the adrenals are relatively rare, but they are of great interest for the light a study of their clinical manifestations throws on the function of the normal gland. Of 46,000 hospital admissions examined by Gibson²²⁰ only four proved to be primary adrenal tumors. Metastatic growths, on the other hand, are relatively frequent. Thus in a survey of 371 malignant tumors which came to autopsy, Burke¹⁰⁰ found that 49 showed metastatic involvement of the adrenals.

Such metastatic growths rarely, however, cause any clinical manifestations, for death results from carcinomatosis before sufficient destruction of cortical tissue has occurred to give symptoms of adrenal insufficiency. The majority of malignant tumors will metastasize to the adrenals but certain types do so more readily than others, namely, tumors of the breast, esophagus, stomach, testicle, penis, Hodgkins disease, and malignant melanomas. Either gland may be involved depending on the location of the primary growth. In many cases metastases are found in the adrenal where there is no general dissemination of the tumor. The medulla is more frequently the site of the metastases than the cortex.

We may subdivide the primary tumors of the adrenal according to their site of origin as medullary, cortical, and androgenic. Previous authors have considered all adrenal tumors as being either medullary or cortical. The assumption made by the present author that a large group of the tumors arising in the cortex are derivatives of the androgenic tissue, although still unproven experimentally, is a useful hypothesis which avoids otherwise irreconcilable inconsistencies and is in accord with all the known facts available at present. We shall defer our

discussion of the tumors of the androgenic zone to the next chapter and consider here only those tumors which originate from the medulla and cortex proper.

TUMORS OF THE MEDULLA

As we have seen in Chapter II, the adrenal chromaphil tissue is derived from the sympathetic nervous system. The original cell which gives rise to both the sympathetic and chromaphil cells is a small, lymphocyte-like structure known as the sympathogone. From these sympathogonic cells arise the sympathoblasts which in turn give rise to the sympathetic ganglion cells, and the chromophiloblasts (also called phaeochromoblasts) which give rise to the chromaphil (or phaeochrome) cells of the medulla. There are thus five types of embryonic and mature cells to be found in the medulla during its development, as indicated in the following schema:

The sympathoblasts and chromophiloblasts, which are the intermediate cell forms between the sympathogone and the sympathetic ganglion cells and chromaphil cells, respectively, are morphologically similar. There are thus in reality only four types of cells in the medulla which can be anatomically differentiated. Each of these may give rise to a tumor conforming to the cell-type from which it is derived. Two of these are immature cell forms: the sympathogone and sympathoblast (or chromophiloblast). The other two are mature forms: the ganglion and paraganglion (or chromaphil) cells. Tumors derived from these cell types are denoted by the names of their cells of origin. The use of the terms glioma, round cell

sarcoma, lymphosarcoma, *et cetera* to describe them is erroneous and fails to indicate their embryological origin.

Symphathogonioma. These tumors (also called symphathoblastoma) originate from the embryonic symphathogone cells and occur in intrauterine life or in earliest infancy, rarely after the first year of life. They are extremely malignant, invade the surrounding tissues, and metastasize to the liver, bones, and retroperitoneal lymph nodes. The tumor is usually bilateral. The fine fibrillar network of the stroma of this tumor resembles glial tissue and has led it to be erroneously designated as an adrenal glioma. Many of the retroperitoneal tumors of infants which have been described as "round cell sarcomas" are, according to Goldzieher,²³⁰ actually symphathogonioma.

The symphathogonioma are large, soft, mottled, yellow-brownish growths composed of small cells resembling lymphocytes. These cells are irregularly scattered, or formed into clusters, or in perfect rosettes which are characteristic.^{230, 335}

Symphathoblastoma. These tumors are variously designated by different authors as neuroblastoma, sympathetic neuroblastoma, neurocytoma, or symphathoblastoma. They arise from the symphathoblasts or chromaphiloblasts and are intermediate as regards the degree of their differentiation between the small celled symphathogonioma, described above, and the mature, large-celled ganglioma and paraganglioma to be described in the next section.

The symphathoblastoma are usually divided clinically into: 1) the Pepper type with metastases to the liver, lungs, or abdominal lymph nodes; 2) the Hutchison type, with metastases to the orbit, skull, and long bones; and 3) the pernicious type characterized by severe anemia. This classification is misleading, however, for the various manifestations and metastatic predilections for certain organs are dependent chiefly on the age of the patient and the site of the initial lesion. Thus due to the different channels of the venous and lymphatic drainage, tumors of the left adrenal are more apt to give rise

to the Hutchison type while those of the right gland give rise to the Pepper type.²¹⁵

The tumors described by Hutchison²²⁷ in 1907 are characterized by the appearance of ecchymosis of one or both eye-lids, followed by unilateral exophthalmus, and an involvement of the auricular and submaxillary lymph nodes. The primary growth in the adrenal often remains small, but may invade the kidney and metastasize to the bones and viscera. Tumors of the orbit in infants are almost pathognomonic of a primary medullary tumor of the adrenal.

In the tumors described by Pepper⁴⁹⁴ in 1901, the first symptom to attract attention is usually a rapidly enlarging abdomen caused by diffuse nodular growths in the liver which may reach an enormous size. The primary growth in the adrenal may remain small.

In the pernicious type of medullary tumor, the chief symptom is an extreme anemia resembling that observed in pernicious anemia.²³⁰

Most abdominal tumors in infants are usually renal or adrenal in origin. The appearance of the symptoms characterizing the above described three types of medullary tumors or of the abnormalities to be described in the next chapter, makes easy the differential diagnosis between renal and adrenal tumors in most cases.

The usual clinical course of sympathoblastoma is progressive, with a rapidly fatal termination. The average duration of symptoms in 17 patients described by Askin and Geschickter²² was 6 months and in half of the cases death followed surgical intervention within a month. In only one patient among the almost 200 cases cited in the literature has surgical removal of the tumor proven effective. Irradiation or other forms of treatment have not proven of value in altering the course of the disease.²²

Ganglioneuroma. The ganglioneuroma are derived from the sympathetic cells. They are exceedingly rare, usually give

no symptoms, and are only discovered as incidental autopsy findings. As found in the adrenal they resemble similar tumors derived from other parts of the sympathetic nervous system. They are sometimes found within the adrenal but more commonly arise from the tissue around the capsule. Although generally benign, the less differentiated type of ganglioneuroma may be malignant and give rise to metastases, in which case they are often spoken of as ganglionic sarcoma.²¹⁵

Microscopically, the ganglioneuroma resemble the paraganglioma to be described next but may be differentiated by the absence of the chromate reaction and by their fibrillar stroma which is interspersed with ganglionic cells.²³⁰

Paraganglioma or chromaffinoma. These tumors are derived from the chromaphil cells of the adrenal medulla or extra-adrenal chromaphil tissue. They are also called phaeochromocytoma or chromaphil cell carcinoma. They form perhaps the rarest of the adrenal tumors.⁵¹⁵

Eisenberg and Wallerstein¹⁷⁶ have collected and analyzed 53 cases of this tumor from reports in the literature. Only 5 of these 53 cases were malignant and all of these 5 were bilateral. The chromaffinomas are most often found in the right gland. Half of all the cases occur during the fourth and fifth decades of life. In some cases they have been associated with tumors in other organs or with neurofibromatosis (von Recklinghausen's disease).

The chromaffinoma vary in size from 1 to 12 centimeters in diameter. The larger tumors are usually cystic and hemorrhagic and are more apt to be benign than the small growths. The latter are greyish bodies of firm consistency, while the larger tumors are usually soft and reddish in appearance. They are characterized microscopically by cells of irregular size, shape, and staining properties. These epithelial-like cells assume an alveolar arrangement. When treated with chromates the typical chromaphil reaction is elicited. The cells are free of fat and glycogen. This differentiates them from similarly appearing tumors of the kidney.²³⁰

The fact that the paraganglioma are derived from epinephrine-producing cells and themselves give all the reactions of epinephrine has led to the belief that they may give rise to intermittent and essential hypertension. Patients with vasomotor instability in whom sudden death follows minor injury (*e.g.*, the extraction of a tooth or the injection of a drug) have been found in several instances to have had such a tumor. The sudden liberation of epinephrine from the tumor has been assumed to be the causative agent of death in these patients.

Labbé, Tinel, and Doumel,³⁷² in 1922, first observed the phenomenon of intermittent paroxysmal hypertension associated with paraganglioma. Mayo,⁴⁴⁶ Shipley,⁵⁶⁶ Kalk,³⁴⁷ and others^{38, 512} have reported similar cases cured by the removal of a palpable tumor. These tumors not only arise from the medulla but more commonly from other chromaphil tissues, particularly from the organ of Zuckerkandl.

The typical case of paroxysmal hypertension, associated with a chromaphil cell tumor, is characterized by periodic attacks which occur at intervals of some hours, or in response to some stimulus, for example, vigorous massage of the palpable tumor. The attacks last for some minutes during which the patient becomes apprehensive and deathly pale. Sweating, mydriasis, hypertension, and hyperglycemia are noted during the paroxysm. Operative removal of the tumor is curative of the condition. In Kalk's³⁴⁷ case, the ablated tumor was found to contain about 460 milligrams of epinephrine.

The association of attacks of paroxysmal hypertension with tumors of the chromaphil tissue, as described above, may be rationally explained by assuming that the tumor when stimulated sets free into the circulation a large amount of epinephrine which gives rise to the observed symptoms. Sudden death in these patients may also be attributed to the toxic effects of the epinephrine secreted by the tumor and released under the stimulus of one of the numerous factors (Chapter VI) which cause an increased secretion of epinephrine.

Although the blood pressure in many cases of chromaffinoma is normal, a group of patients have been described in whom this tumor was associated with hypertension, hypertrophy of the left ventricle, albuminuria, and spasm of the retinal arteries which have lead to the erroneous diagnosis of contracted kidney. At autopsy, however, the renal damage was found to be minimal. As typical of the clinical picture observed in these patients, a case of a chromaphil tumor of Zuckerkandl's body described by Reichardt⁵¹⁷ may be cited. The patient, a 53 year old woman, complained of headache and fatigue with some loss of vision. There was marked pallor, a tendency to sweating, spastic retinitis, dilated pupils, and albuminuria. The blood pressure ranged from 200/115 to 230/140. There was no edema or signs of circulatory insufficiency, and the renal function tests gave normal results. The patient was bled and digitalized but died suddenly on the ninth day of her stay in the hospital. At autopsy a greyish red tumor, the size of an apple, occupied the site of Zuckerkandl's body. The kidneys and circulatory system were found to be entirely normal.

Belt and Powell⁵¹ have suggested the term "adrenal sympathetic syndrome" to denote the clinical condition associated with chromaphil cell tumors. The outstanding symptoms of this syndrome are evidences of instability of the sympathetic nervous system, hypertension or paroxysmal hypertension, glycosuria, periodic attacks of tachycardia, vasoconstriction and vasodilation of the peripheral vessels (as evidenced by pallor followed by flushing), headaches, nausea, vomiting, sensations of constriction in the epigastrium with dyspnoea, and a susceptibility to shock. Operative removal of the chromaffinomomas has proven curative in a number of cases reported in the literature.^{446, 566}

TUMORS OF THE CORTEX

The tumors of the adrenal cortex may be divided into those which originate from the interrenal tissue and those whose

origin, in the author's opinion, is to be sought in the androgenic tissue. The latter group of tumors produce abnormalities of the reproductive system and will be discussed in the next chapter. The tumors of the interrenal tissue are not characterized by any distinctive clinical symptomatology. Unlike the corresponding growths of certain other endocrine organs they do not manifest any symptoms attributable to an overproduction of their specific hormone. It is only by their malignancy which expresses itself by their extension and ready metastasis, or by their destruction of the cortex to such an extent as to produce the symptoms of Addison's disease that their presence in the body is manifested.

Because of certain very superficial similarities, earlier workers often confused tumors of the adrenal cortex with the more commonly occurring hypernephroma of renal origin.⁶⁴² The latter are not derived from the adrenals and should be more accurately denoted as renal or Grawitz tumors. The differentiation between tumors of renal and adrenal origin has been clearly pointed out by Glynn and Guthrie,²²⁷ Goldzieher,²³⁰ and most recent authors.

The growths occurring in the adrenal cortex may be classified as: 1) simple hyperplasia, 2) cortical adenomata, and 3) malignant carcinomata.

Hyperplasia. Simple hyperplasia of the adrenals is a physiological response of the glands to an increased demand for the cortical hormone. Any condition requiring an excessive secretion of the hormone will therefore be expected to lead to hyperplasia. Infections, particularly, result in hypertrophic enlargement of the adrenals. Thus in an infant, 8 weeks old, dying of enteritis, Broster and Vines⁹⁵ found the adrenals to weigh 16.5 grams. Lucien and Parisot⁴⁰⁷ have denoted as chronic hyperplastic adrenalitis, a condition in which the adrenal enlarges as a result of an inflammation of the cortical cells brought about by some toxic irritation.

Cortical hyperplasia may occur either in the form of a diffuse

hyperplasia or as circumscribed nodules ranging in size from microscopic islets of tissue to the size of a hen's egg and are incidental findings in about a third of all autopsies. They may be found in the medulla, in the cortex, or projecting from the surface of the gland, and are difficult to distinguish from accessory interrenal bodies which in a sense they really are.

Microscopically these bodies consist of columns of cells similar to that of the fascicular zone, but at times an arrangement like that of the glomerulosa characterizes the peripheral layers of cells.³³⁵

Adenomata. The cortical adenomata resemble superficially the nodular hyperplasia described in the preceding section. They differ from the latter by their atypical cell structure and their malignant tendencies. The cortical adenomata form yellowish or reddish masses well circumscribed from the rest of the cortex. They often deform or destroy the gland.³³⁵ Although frequently manifesting all the signs of normal functional activity, the adenomata at times are found in varying stages of lipoidal degeneration.

Clinically the cortical adenomata produce no demonstrable symptoms as might be anticipated from the innocuous effects of injections of large amounts of the cortical hormone. Nevertheless, the larger growths appear to be definitely neoplastic.²⁵⁴

Carcinomata. The adenocarcinoma of the adrenal resemble the adenomata from which they probably originate. Unlike the adenomata they contain atypical areas of malignant character and metastasize readily to other organs. In the so-called undifferentiated carcinoma of the adrenals one finds large granular fatty cells arranged around the blood vessels, or rounded polyhedral cells free of fat and glycogen. These also metastasize early and infiltrate the surrounding tissues.^{215, 230, 335}

Malignant tumors of the interrenal tissue are exceedingly rare. Many of the cases described in the literature as carcinoma of the cortex are neoplasms of the androgenic tissue which give rise to the adreno-genital syndrome to be described

in the next chapter. Malignancy of the cortex proper (*i.e.* of the interrenal tissue) does not give rise to virilism or to any abnormality of the reproductive system. These tumors rarely give rise to Addison's disease for the rapidity of their growth and ready metastasis lead to death from carcinomatosis before sufficient destruction of the cortex has taken place to manifest the symptoms of Addison's disease.

OTHER TUMORS

The tumors described in the present and following chapters are derived from tissues specific to the adrenal glands. Other tumors may arise, of course, from the connective tissue, smooth muscle, or blood vessel elements, but such tumors are exceedingly rare. Fibroma, myoma, lipoma, hemangioma, lymphangioma, fibroxanthosarcoma, and sarcoma have been described. Many tumors erroneously described under these terms belong to the groups of tumors described in the preceding sections.²³⁰

CHAPTER XXIII

THE ADRENO-GENITAL SYNDROME

Certain pathological abnormalities involving the reproductive system are regularly accompanied by anatomical changes in the adrenals. Although numerous reports are extant describing obvious examples of the adreno-genital syndrome, the first extensive work, recognizing the relation of certain abnormal developments of the sex organs to an abnormal growth in the adrenal, was that of Bulloch and Sequeira⁹⁹ in 1905. Gal-lais,²¹⁰ in 1912, published a voluminous thesis on the subject and clearly indicated the clinical picture as it occurs in adult life. Glynn²²⁷ in the same year also described at length the condition as it occurs in women and children.¹⁸

In the absence of knowledge of the function of the adrenals, it is not surprising that early considerations of the adreno-genital syndrome should have been rather confusing. This was particularly so since most adrenal anomalies are not accompanied by sexual changes nor are the latter in the majority of cases accompanied by changes in the adrenal. Unless a clear differentiation is made between the androgenic zone and the remainder of the cortex one cannot describe logically the relation of the adrenals to certain changes in the reproductive system and the secondary sex characteristics.

Previous authors have attributed the development of the adreno-genital syndrome to hyperfunction of the adrenal cortex with the elaboration of an excess amount of the hormone specific to the interrenal tissue. This idea, however, is not in accord with the available facts concerning the interrenal tissue. As we have seen in Chapter XIV, the adrenal cortical hormone, although essential for maintaining the normal reproductive function does not control reproductive activity. Nor does the

administration of excessive amounts of this hormone induce changes in the reproductive system analogous to those observed in the adreno-genital syndrome. It is only a specific type of cortical hyperplasia which is associated with disorders of the reproductive system. The tumors of the interrenal system, described in the preceding chapter, are not associated with changes in the sex glands. It is thus erroneous to ascribe the development of the adreno-genital syndrome to overactivity of the interrenal tissue of the adrenal cortex but we must seek some other functionally distinct unit in the adrenal as the source of the observed disorders.

The earliest attempts to explain the relation of the adrenals to sex anomalies was based on a consideration of the common embryological origin of the adrenals and the gonads (Gallais,²¹⁰ Wiesel,⁶⁷⁷ Glynn²²⁷). As we have already noted in Chapter II, the adrenal cortex is developed from the mesentherium of the abdominal hollow contiguous to the point of origin of the testicles and the ovary. There is thus an intimacy between the points of development of the adrenal and sex glands. Moreover, the origin of the ovary is hermaphroditic.³⁵⁹ The ovary passes through a stage in which its cortical portion may be considered female; its deeper medullary part, male. During subsequent development, this male part remains rudimentary. It is this male part which is so intimately related with the beginning stages of the adrenal cortex.³³⁷ If these fetal testicular cells by developmental anomaly are included in the adrenal they might develop into tumors which physiologically would manifest their testicular origin. Hence one might expect virilism to develop in patients in whom these adrenal growths occur.

The above theory was propounded by Krabbe³⁶⁵ and explains to a certain extent the clinical picture associated with the adreno-genital syndrome. However, it is unnecessary to resort to Krabbe's hypothesis to explain the origin of the disorders of the reproductive system which are associated with

adrenal tumors. As we have seen in Chapter IV, the adrenal cortex of man (in whom alone the adreno-genital syndrome has been observed) contains a tissue which is anatomically and functionally distinct from the rest of the cortex. This tissue which we have designated as the androgenic zone is normally only temporary in its existence disappearing in the first years of life, but sometimes remains as a few scattered cells (the juxta-medullary zone) in the adult. It would seem most logical, therefore, to ascribe to this androgenic tissue the occurrence of the disorders of the reproductive system which are associated with tumors of the adrenals. The exact embryological origin of the androgenic zone has not been determined. It may well be that it has a common origin with the "testicular" cells of the ovary, asumed by Krabbe³⁶⁵ to be the cause of the adreno-genital syndrome.

The differentiation of the androgenic tissue as a unit distinct functionally from the remainder of the adrenal cortex would explain the fact that adenoma or carcinoma of the cortex proper do not give rise (Chapter XXII) to abnormalities of the reproductive system. It would also account for the failure of large doses of the cortical hormone to affect the reproductive system. Although Britton and his co-workers¹²⁷ have claimed that extracts of the adrenal cortex of cattle induce "precocious" maturity in rats, all subsequent observers (Castello *et alii*,¹⁰⁹ Cleghorn,¹¹⁵ Howard and Grollman,³²¹ Simpson *et alii*,⁵⁷⁵ and others who have investigated the problem) have disproven Britton's contentions.

The author realizes that the views set forth in the present volume regarding the functional independence and the clinical significance of androgenic tissue require further experimental work to establish them upon a firm basis. However, although theoretical, the view advanced is in accord with all the known facts and permits one to account logically for the adreno-genital relationships. The use of this theory avoids the chaotic confusion otherwise encountered in trying to account for the relation between the adrenals and the reproductive system.

The adreno-genital syndrome is characterized by a masculinization of the female. In children it occurs occasionally in males but in the adult it is limited to the female sex. Several cases have been reported³⁰² in adult males in whom adrenal tumors were associated with some evidences of feminization of the patient. However, the few cases in which this has been described are not convincing and one is inclined to explain the apparent feminization to other causes than to the existence of a tumor of the androgenic tissue. The masculinizing effects induced by a tumor will vary depending upon the age and state of development of the reproductive system in the patient. We can accordingly divide the adreno-genital syndrome into the juvenile type as it occurs in children before puberty and the adult type which gives rise to the condition of adrenal virilism. Besides these two conditions one also finds an hyperplasia of the androgenic tissue in cases of hermaphroditism. These three conditions will now be described in greater detail.

THE JUVENILE FORM OF THE ADRENO-GENITAL SYNDROME

The literature records 24 cases of this condition, 19 of which occurred in girls while only 5 occurred in boys. The adreno-genital syndrome is thus predominantly an affection of the female in childhood. The onset of the disease is usually during the first two years of life. The condition is marked by an hyperplasia of the androgenic zone which although benign in its initial stages degenerates into a malignant process. This over-activity of a masculinizing tissue leads to the development of the secondary male characteristics—muscular development, deepening of the voice, hirsutism, *et cetera*.

The characteristic points of the abnormal sexual developments in female children is the luxurious growth of hair (pubic and sometimes facial), hypertrophy of the *labia majora*, projection of the *labia minora*, and enlargement of the clitoris which may assume a penis-like structure. There is also enlargement, at times, of the ovaries and uterus, but menstrua-

tion is *absent*. In male children, there is also hypertrichiasis, a deep voice, enlargement of the penis, and external genitalia, but the testes remain immature and show no spermatogenesis. There is an unusual muscular development, more marked particularly in the male, so that the child appears as an "infant Hercules." The patients appear matured and have an adult appearance.

A typical case in a male child as described by Gordon and Browder²³² follows: In their patient, at nine months of age, an abnormal development of the external genitalia was apparent. Pubic hair appeared at 12 months. On admission to the hospital, at the age of three, the patient appeared like an "adult dwarf with the musculature and general development of a young Hercules." The voice was deep and gruff. "The scalp was covered with thick hair extending well down over the forehead and posteriorly down on the neck. The eyebrows were heavy, shaggy and continuous across the bridge of the nose." There was a marked growth of hair on the face, upper lip, chin, back, pubic, and perineal regions. The skeleton was markedly enlarged except for the humeri, which were the length expected in a three-year-old child. Dentition and osseous development were that of a child of seven to ten years of age. At no time had there been any priapism, nor evidence of nocturnal emissions or masturbation.

Autopsy of the above described case revealed numerous metastatic tumors in the lung which had been the immediate cause of death. In the region of the left adrenal and extending from the dome of the left diaphragm downward to the upper pole of the right kidney and medially to the midline, was present an irregular nodular tumor lying entirely retroperitoneally. The right adrenal was absent. That part of the adrenal gland not affected by the tumor was normal on gross examination.

An important feature of the juvenile form of the adreno-genital syndrome is the absence of true precocious puberty. Menstruation does not occur in the female at an early age nor

does priapism, spermatogenesis, or other signs of true puberty occur in the male. It is thus a misnomer to refer to the condition as one of precocious puberty as is usually done. True precocious puberty occurs with tumors of the pineal gland (chiefly in males, rarely in the female) and with tumors of the testicles or ovaries. In these cases there is an actual functional precocity of the reproductive organs. In the female, menstruation occurs as early as the first year, while in males, spermatogenesis, priapism, and masturbation regularly occur. This difference should serve as an important clinical sign to differentiate the adreno-genital syndrome from cases of true precocious puberty.³⁶⁵

As regards the treatment for this condition, surgical removal of the affected gland before metastasis has occurred is obviously necessary. Improvement following such removal with retrogression of the abnormal signs of masculinization have been reported.^{120, 228}

In many cases reported in the literature, as in the one cited above, there has been an anomaly or complete absence of the opposite adrenal. Hence the surgeon should ensure himself of the state of the unaffected gland before undertaking the removal of the affected adrenal. Should the adrenal on the unaffected side be absent, removal of the diseased gland would lead to death of the patient from adrenal cortical insufficiency. In such cases it is, therefore, necessary to preserve some of the normal cortical tissue either by allowing it to remain *in situ* or transplanting it to an ovary or testicle. Treatment of the patient postoperatively with the adrenal cortical hormone in minimal doses until regeneration of new cortical tissue has occurred is also necessary.

The juvenile form of the adreno-genital syndrome is probably caused by hyperplasia of the androgenic zone. Instead of disappearing as it does in normal children during the first year of life, this tissue for some unknown reason proliferates and ultimately assumes malignant characteristics.

FIG. 17. THE ADRENO-GENITAL SYNDROME

On the left is shown the appearance of a nine-year-old girl two weeks after the removal of a tumor of the left adrenal. The hirsutism, obesity, and acne of the face are still unaffected by the operation. On the right is shown the same patient, four months later, when the curative effects of the operation are evident. (*Reproduced through the courtesy of Drs. W. Walters, R. M. Wilder, and E. J. Képler, of the Mayo Clinic with the permission of the Publishers of the Annals of Surgery.*⁵³⁵)

Except for the arrhenoblastoma of the ovary, which occur very rarely in children,⁴⁷⁵ the juvenile form of the adrenogenital syndrome should offer no difficulty in diagnosis. Arrhenoblastoma of the ovary give rise to a condition which is identical, as far as one can judge from the few cases in the literature, to the adreno-genital syndrome. This identity in the manifestations of the two disorders supports the view that the ovarian cells which give rise to the arrhenoblastoma and the androgenic tissue are functionally equivalent. The diagnostic errors so frequently encountered in determining the cause of precocious development in children is due to the failure to appreciate the fact that adrenal tumors do not give rise to true sexual precocity and that the occurrence of regular menstruation or spermatogenesis precludes, therefore, the possibility of an adrenal tumor as the cause of the disorder. Failure to appreciate this fact, first pointed out by Krabbe,³⁶⁵ has led to futile operations on the adrenal when the ovary, testicle, or pineal were the true sites of the disorder. In only two cases cited in the literature has menstruation been observed in children suffering from adrenal tumors. In one case⁹⁹ the patient was eleven years of age and in the other⁶⁵⁵ (the patient of Figure 17) the bodily development was that of an adult. In both cases the menstruation occurred at rare intervals and was small in amount, thus differing from the cases of true precocious development in which a normal and regular menstrual flow may begin in infancy.

ADRENAL VIRILISM

By this term, we shall refer to the adreno-genital syndrome as it occurs in adult women. It occurs most often either during the early years of adolescence or at the time of menopause, that is, at those periods in life when the reproductive cycle is undergoing most rapid change. Cases have been reported, however, not infrequently in patients of intermediate ages.^{95,203}

The adreno-genital syndrome as it occurs in women is marked by an appearance of secondary male characters and a retrogression of the female sex characters. Among the former are 1) hypertrichiasis of the male type, 2) changes in bodily contour, 3) changes in the larynx, 4) hypertrophy of the clitoris, and 5) the assumption of the psychological outlook of the male. The retrogression of female characteristics is marked by a cessation of menstruation and the loss of other female characteristics which are replaced by the masculine characters already noted. The above mentioned changes may now be discussed in further detail in the order in which they have been enumerated above.

The development of hair of the masculine pattern is one of the most prominent signs of adrenal virilism and has given rise to the term adrenal hirsutism as descriptive of this syndrome. The degree of this hirsutism is variable. It is often distributed over the entire body. On the face it is commonly a moustache or beard. It may cover the thighs and chest and appear in patches over the dorsum of the feet and proximal phalanges. The axilla and pubis are well covered, the latter assuming the triangular distribution characteristic of the male. In older women baldness of the masculine type has also been described.⁹⁵

The bodily contour assumes a masculine form particularly when the syndrome develops early. The patients are broad-shouldered, narrow-hipped, and muscular. The breasts are usually undeveloped or may retrogress. In certain cases (*cf.* Figure 17) they appear large but these are probably composed of fatty tissue and part of the tendency to increase in weight which is observed in the early stages of virilism. Broster and Vines⁹⁵ found the involutionary changes of later life in one of their cases.

A peculiar striation of the thighs was described by Gallais²¹⁰ and has been noted by subsequent authors as occurring in this condition. This may possibly be explained as analagous to that occurring during pregnancy and due to stretching of the

skin by the imposition of the skin of a small masculine contour over a large female pelvis.

The larynx assumes a masculine form with development of an Adam's apple and a deep voice.⁹⁵

In the early stages of the disease, there is marked adiposity which is limited to the upper half of the body. Later cachexia and progressive asthenia occur, at which time there is a palpable tumor of the adrenal.

The female reproductive system undergoes a change in which there is suppression and atrophy of the female organs and hypertrophy of the male elements. The clitoris, the female analogue of the penis, becomes enlarged and may assume the form of a penile organ with well-developed glans and prepuce. In such cases the undersurface is grooved, as in hypospadias. The vagina, *mons veneris*, and *labia* become atrophied. In a large series of cases in which laparotomy was performed, Broster and Vines⁹⁵ noted constant changes in the ovaries which were atrophic and often cystic and degenerated. The *fundus uteri* and cervix were small.

Amenorrhoea is an important symptom. In the adreno-genital syndrome appearing after puberty, the menses become irregular, intermenstrual periods lengthen, the flow becomes diminished and finally ceases completely.

Certain other clinical symptoms have been described as associated with adrenal virilism—hypertension, pain in the lumbar region, migrainous headaches, *et cetera*. Of greater importance are nausea, vomiting, weakness, and rarely pigmentation on the forehead and border of the axilla. The last named symptoms are those of Addison's disease and are due to the encroachment of the malignant androgenic zone on the true cortical tissue.

Psychologically, the development of male characteristics with suppression of the female may be marked by homosexual tendencies. The psychic disturbances so common and profound in subjects in whom the reproductive system has under-

gone a violent change are commonly observed in patients suffering from adrenal virilism.^{95, 303, 655}

In many of the cases of adrenal virilism described in the literature there has been a unilateral tumor in one adrenal or rarely at the site of a so-called adrenal rest.³⁶³ In the large series of Broster and Vines,⁹⁵ however, the affection was usually bilateral.

The treatment of the adreno-genital syndrome in adults does not differ from that described in children in cases in which a definite tumor has given rise to the condition. Successful operations followed by retrogression of the abnormal signs of masculinization have been recorded.^{95, 303, 655} In patients with a bilateral affection of the androgenic tissue, the removal of one gland with partial removal of the other must lead to an unsatisfactory result, for the hypertrophy of the remaining androgenic tissue will eventually cause a reappearance of the original symptoms. The logical treatment in such patients should consist in the enucleation of the internal portions of the adrenals, thus removing the medulla and androgenic zones while preserving the outer layer of the cortex which is essential for life. Such an operation, performed in two stages to allow regeneration of the cortical tissue left after the first operation, would offer the best prospects for a permanent cure.

A series of cases of adrenal virilism treated surgically with considerable success has been reported by Broster and Vines⁹⁵ in London, and Kepler, Kennedy, Davis, Walters, and Wilder⁶⁵⁵ at the Mayo Clinic. The 10 cases of Broster and Vines showed no untoward signs following unilateral adrenalectomy. In general there was a gratifying return of menstruation following operation and some diminution in the degree of hypertrichiasis. However, as already stated, Broster and Vines found no difference between the two adrenals, both showing the fuchsinophile reaction by which they detected the androgenic tissue. Broster and Vines utilized the trans-thoracic route for their adrenalectomy which they considered superior to the sub-diaphrag-

matic approach. The average age of their patients was 20 years.

The 7 cases of Kepler, *et alii*⁶⁵⁵ differ considerably from those encountered by Broster and Vines. These authors encountered a unilateral tumor in most of their cases. Removal of this growth by a lumbar, extra-peritoneal approach gave remarkably good results in some of their patients. In figure 17 is reproduced a photograph of one of the patients of Walters and his co-workers⁶⁵⁵ showing the curative effects of removal of the affected adrenal.

THE ADRENALS AND HERMAPHRODITISM

Gallais²¹⁰ in his monograph collected 11 cases of hermaphroditism in which marked anomalies of the adrenals were present. In these cases the secondary sexual characters were masculine, the anomalous development having occurred during embryonic development. The adrenals were hyperplastic and there were supplementary aberrant cortical tissues or tumors of cortical origin. Gallais considered the hermaphroditism to be of adrenal origin. However, there is considerable question as to the rôle of the adrenals in these cases. Further work is necessary to decide if the adrenal hyperplasia (probably of the androgenic tissue) is the cause of the sexual anomalies or whether the changes in the two organs are part of the same underlying cause and due to a more fundamental anomaly of the germ plasm.

In embryonic life, the internal genital organs are hermaphroditic containing both masculine and feminine generative potentialities. This embryonic bisexuality is manifested in the coexistence of the Müllerian and Wolffian ducts. Later in embryonic development, in the female, the Müllerian ducts persist while the Wolffian are partially resorbed. Their persistence results in a partial (tubular) hermaphroditism. The external genital organs are in reality only degrees of the same ascending evolution, each organ of one sex having its counter-

part in the other, *e.g.*, the penis and clitoris, or the prostate and para-urethral glands of Skene.

Hermaphroditism may involve a true co-existence of male and female generative organs in the same individual. A lesser degree of hermaphroditism exists in cases in which the secondary characteristics of one sex are present in individuals with the anatomical generative organs of the opposite sex. These secondary characteristics in man are hypertrichiasis, muscular development, low pitch of the voice, and virility of character; in the female, they are menstruation, soft voice, adiposity, gynecomasty, and a feminine psychological outlook.

The cases reviewed by Gallais²¹⁰ showed for the most part secondary male characteristics with female generative organs. One might consider the male characteristics as results of the accompanying hyperplasia of the androgenic zone of the adrenals. Whether this hyperplasia is primary or merely a compensatory mechanism due to deficiency in the female characters is still open to question. The most logical interpretation of the observed facts would be that the adrenals are not in themselves responsible for the development of hermaphroditism. If by some anomalous development hermaphroditism of the female type occurs, there is a tendency for the androgenic zone to become overactive and thus accentuate the secondary male characters present in these primary female hermaphrodites. In accord with this view we find that bilateral hyperplasia of the adrenal gland is found in about 15 per cent of female but in only 0.7 per cent of male pseudo-hermaphrodites.²²⁷ The so-called adrenal cortical rests so commonly found in female pseudo-hermaphrodites¹⁹⁵ are composed of androgenic tissue and not true cortical tissue as has previously been believed. They have often been mistaken for a displaced testicle or ovary.

According to the theory outlined in the preceding paragraph, the adrenal hyperplasia observed in hermaphroditism may be looked upon as a process analogous to the enlargement of the

androgenic zone in male mice following castration. In both cases there is a compensatory hypertrophy of a masculinizing tissue (the androgenic zone) as a consequence of a congenital or induced dysfunction of the normal reproductive system.

OTHER CONDITIONS RESEMBLING THE ADRENO-GENITAL SYNDROME

Two other clinical conditions occurring in women are characterized by a symptom-complex which is practically identical to the adrenal virilism described in the preceding section. These conditions are the "diabetes of bearded women" described by Achard and Thiers⁶ and Cushing's syndrome.¹⁴² According to most observers these two syndromes are identical. Achard and Thier's symptom-complex is marked by hypertrichosis of the face, obesity, glycosuria with decreased carbohydrate tolerance, hypertension, and usually amenorrhea. At autopsy, a pronounced hyperplasia of the adrenals has been noted. It is probable that this adrenal hyperplasia represents an hypertrophy of the androgenic tissue which is in part at least responsible for the observed masculinization.

Cushing's syndrome is characterized by a rapidly acquired and usually painful adiposity, confined to the face, neck, and trunk, the extremities being spared; a tendency to become round shouldered, associated with lumbo-spinal pains; sexual dystrophy, shown by early amenorrhea and sterility; hypertrichosis of the face and trunk; a dusky or plethoric appearance of the skin with purplish *striae distensae* of the abdomen and legs; vascular hypertension; acrocyanosis; and osteoporosis.

In Cushing's earlier reports,¹⁴² he attributed the above described disorders to basophilic adenomata of the pituitary which were present in his patients. However, more recently, cases have been reported which were clinically indistinguishable from Cushing's syndrome, but in which at autopsy no disorders of the pituitary were found. Instead, an adrenal tumor was

found as the only pathologically observable source of the disorder.^{102, 1388, 411, 662}

The question, therefore, arises as to what part the adrenals play in producing Cushing's syndrome. Broster and Vines⁹⁵ have suggested that all cases of the syndrome may be associated with a change in the adrenals manifested by a different staining reaction of the juxta-medullary tissue. If this suggestion of Broster and Vines is substantiated, one might consider Cushing's syndrome as a manifestation of adrenal virilism, primarily induced perhaps by the pituitary basophilism. Basophilic adenomata of the pituitary are frequently found at autopsy in patients who in life manifested no clinical symptoms.³⁵¹ Crookes¹³⁹ has suggested that it is only such basophilic cells as are characterized by a distinctive hyalinization of their cytoplasm that are associated with Cushing's syndrome.

Further investigation is obviously necessary before one can decide the relative importance of the pituitary and the adrenal in causing Cushing's syndrome. It may be that the pituitary basophilism initiates the observed clinical picture by suppressing the ovarian function which in turn elicits a reaction of the androgenic zone. As we have seen in Chapter IV, castration in mice also causes an hypertrophy of the androgenic tissue. The observed disorder might according to this view be caused either by a primary hyperplasia of the androgenic tissue of the adrenal or by an hyperplasia of this tissue induced primarily by a disorder of the pituitary.

Finally, attention may be called to those cases in which sex changes occur which are identical to those observed in adrenal virilism but in which, at operation or autopsy, no discoverable cortical abnormalities are evident on superficial examination. In Broster and Vines'⁹⁵ patients the adrenals in these cases showed a staining reaction of the juxta-medullary tissue which differentiated it from normal cortical tissue. This juxta-medullary tissue, in the author's opinion, is identical with what we have denoted as the androgenic zone and is probably the

cause of the observed disorder. What appears by the ordinary methods of staining as interrenal tissue is in reality androgenic tissue. Further investigation of the adrenals in these cases as well as those of Cushing's syndrome and other forms of virilism are desirable to elucidate the rôle of the adrenals in the genesis of these disorders.

CHAPTER XXIV

OTHER PATHOLOGICAL ABNORMALITIES OF THE ADRENALS

Besides the destructive lesions of the adrenals which give rise to Addison's disease and the tumors of the adrenals discussed in previous chapters, a number of pathological conditions are accompanied by abnormalities of the adrenals. The significance of some of these abnormalities is not clear at present and many of them are probably of no clinical importance. The adrenals rapidly undergo autolytic changes after death and many of the histological pictures described as characteristic of certain diseases are in reality *post-mortem* changes. It would appear, however, that these *post-mortem* changes are more prone to appear in individuals dying of infectious or toxic processes for the observed abnormalities are more striking in these subjects.¹⁵⁷ The gland subjected to toxic influences apparently becomes subject to easier attack by autolysis than the normal gland. Fissures are also often noted between the medulla and cortex but it is questionable if they are due to *post-mortem* changes or are the results of a necrosis which has occurred during life.¹⁷⁵

The pharmacodynamic effects of epinephrine have led authors to assume that certain diseases of the vascular system are resultants of abnormalities of the medulla, but there is little to substantiate this clinical hypothesis. Except for the rare cases of paroxysmal hypertension due to chromaphil cell tumors, there is no disease which has been definitely shown to be caused by hyper- or hypo- activity of the medulla or other chromaphil tissues.

Many diseases have from time to time been suggested as associated with dysfunction of the adrenals.²³⁰ Glaucoma,

paralytic ileus, thyrotoxicosis, diabetes, obesity, progeria, osteomalacia, rickets, peptic ulcer, bronchial asthma, cancer, *etc.* have been attributed to adrenal insufficiency or hyperactivity. The evidence for this association is so unconvincing and in many cases so obviously fallacious that we need not consider it here. In the following sections shall be discussed a number of clinical conditions in which the involvement of the adrenals is unquestionable, although the significance of the adrenals in the clinical condition is in many cases unknown.

ANENCEPHALY

The remarkable hypoplasia of the adrenals in anencephalic monsters was observed as early as 1723 by Morgagni.⁵⁵ Meckel⁴⁴⁷ and Nagel⁴⁶⁴ claimed that the adrenals were absent in these monsters and that their absence was responsible for the maldevelopment of the reproductive organs. Although one still finds these views quoted in recent texts it has been repeatedly shown that they are erroneous. As shown by Meyer,⁴⁵⁴ in the five-month anencephalic monster the adrenals may be completely normal. At birth they appear much smaller than the normal glands but this hypoplasia as Elliott and Armour¹⁸² first showed is due to the absence of what they termed the "fetal" cortex or what we have designated as the androgenic zone. The other two components of the adrenal—the medulla and the cortex proper—are normally developed so that the glands really resemble a miniature adrenal of a one-year old child. Microscopically too the adrenals appear normal except for this absence of the androgenic zone of the new-born.

The absence of the androgenic zone in anencephaly might be anticipated if we accept the view that the androgenic zone is associated with the development of the reproductive system. It is possible, however, that the maldevelopment of the hypophysis which occurs in anencephaly is the primary cause of the failure of the androgenic zone for this gland, as we have seen, and the androgenic zone may be closely related.

As noted by Zander,⁶⁹⁶ the ganglia of the sympathetic system are fully developed in cases of hemicephaly. In spina-bifida and in hydrocephalus, no abnormality of the adrenals is noted.

STATUS LYMPHATICUS

The adrenal cortex is often hypoplastic in cases of so-called "status lymphaticus."²³⁰ In adrenal insufficiency, conversely, there is often hyperplasia of the thymus and other lymphoid tissue.³³⁵ Unfortunately, our ignorance of the exact function of the thymus and lymphatic system renders any discussion of the probable significance of this adrenal-lymphatic relationship futile. The lymphatic system responds by hypertrophy or atrophy to so many clinical conditions that it is unjustifiable to attribute any more fundamental relation between the adrenals and the lymphoid tissue than exists between the latter and the thyroid, for example. As regards the relation of the adrenals to *status lymphaticus*, there is no longer a tendency to regard this condition as a disease entity. As Groll¹⁵⁷ showed, the hyperplasia of the thymus and other lymphoid tissues which characterize *status lymphaticus* were frequently observed in healthy normal individuals killed during the Great War. It would, therefore, be too far-fetched to attribute the sudden deaths, presumably due to a questionable pathological condition (*status lymphaticus*), to an acute adrenal insufficiency.

Instances of sudden death following a prolonged exposure to heat, narcosis, muscular exertion, or certain poisons have also been attributed to an acute adrenal insufficiency. Autopsy in such cases has revealed a marked decrease in the epinephrine content of the medulla and vacuolar degenerative changes in the cortex with no changes in other organs to account for the sudden death. Since the influences cited above are accompanied by an increased utilization of the cortical hormone, it is not irrational to assume that they might by exhausting the supply of the hormone in the body

cause collapse and death from cortical insufficiency. Further studies are needed, however, to substantiate these assumptions as to the rôle of the adrenal in these conditions.

OTHER ANOMALIES

Defects of the adrenal have been reported in other developmental anomalies but it is questionable if any very important significance can be attributed to these findings. Thus in Schridde's disease in which edema of the newborn is accompanied by maldevelopment of the erythropoietic system, Yasukawa⁶⁹⁴ noted fatty infiltration of the innermost layers of the cortex. This slight anomaly certainly appears to merit no great attention.

There is also a reduction in the lipid content of the adrenals and hypoplasia of the adrenals and thymus in exfoliation of the skin of the newborn (Ritter's disease). These associations too are of doubtful significance.²³⁰

Reports of absence of the adrenals reported by earlier workers¹⁴⁰ are now generally discounted. As we have seen (Chapter XXI), in Addison's disease due to atrophy, the remnant of adrenal tissue may be so small as to escape notice unless carefully sought. The reports of absence of one adrenal may be accepted as due to destruction by unilateral atrophy. The congenital absence of one gland has been reported in a patient in whom some of the other abdominal viscera on the same side were also absent and in whom a number of other congenital anomalies were found.⁴¹⁷ The adrenal has been described as malformed by an unnaturally projected fissure through the entire gland at the hilum. In some cases the gland on one side is doubled or the chromaphil tissue has failed to occupy its normal position within the cortex but occupies a position on the surface of the gland¹⁵⁷ as in the reptilian adrenal.

HEMORRHAGE AND OTHER VASCULAR LESIONS

The adrenals are sometimes the site of hemorrhages which if extensive may induce adrenal insufficiency.⁵⁷² Massive bi-

lateral hemorrhages following acute septic conditions have resulted in symptoms simulating peritonitis and rapid death from acute adrenal insufficiency. Such hemorrhages are apt to occur in leukemia or other conditions in which there is an hemorrhagic diathesis.²³⁰

Death in the new born has been ascribed in some instances to hemorrhage into the adrenals. The hemorrhage observed in the androgenic zone of the new born has, however, been mistakenly attributed to injuries suffered during delivery, whereas in reality, as Landau³⁷⁶ showed, such hemorrhages are part of a physiological process, and are observed in cases of infants delivered by Caesarian section. It is possible that the normal hemorrhage and diapedesis of the androgenic zone might result in an unnatural extravasation of blood particularly when aggravated by the trauma of a difficult delivery. Such hemorrhages may, if extensive, cause death by interference with the normal cortical function. Large cystic hematoma have been produced which in rare cases have ruptured through the capsule and filled the retroperitoneal space. Adrenal hemorrhages of sufficient magnitude to cause death are rare in adults but are found more frequently in infants dying of unknown cause about one day after birth.³⁹⁰ Traumatic hemorrhages of the newborn are acquired during passage through the birth canal (particularly in breech presentation), or by slapping the lumbar region in attempts at resuscitation.⁴⁰⁸

Small hemorrhages are frequently observed in general infections, intoxications, burns, and diseases in which there is an hemorrhagic diathesis. Such hemorrhages are replaced by pigmented scar tissue, calcified nodules, or the formation of bone.^{230, 432}

The adrenals are also occasionally subject to other vascular lesions, passive congestion, thrombosis, infarction, or embolism. Passive congestion of the adrenals has been observed in obstruction of the portal circulation, in cirrhosis of the liver, and in conditions of general circulatory insufficiency. Throm-

bosis of the adrenal veins with death from acute adrenal insufficiency has been reported. Such thrombi have been considered as due in most cases to an extension from smaller thrombi formed in the capillaries of the cortex in acute infections or other processes causing degeneration of the parenchymal tissues. Thrombosis may also arise in the adrenal veins as a result of conditions in the circulation. Infarction of the adrenals results in a rapid destruction of the medullary tissue. The cortical tissue is less apt to be irreversibly damaged.²³⁰

The clinical symptoms attributed to extensive hemorrhage in the adrenals are convulsions, vomiting, diarrhea, abdominal pains, and hyperpyrexia.²³⁰ As we have noted in previous chapters, these symptoms may occur in experimental acute adrenal insufficiency and it is thus not irrational to attribute their occurrence in man to the same cause. An elevated temperature is not uncommonly seen in animals for a day or two following bilateral adrenalectomy (Chapter XIII).

HYPERFUNCTION OF THE ADRENALS

In considering hyperactivity of the adrenals we must differentiate between the effects of hyperfunction of the three elements of the gland 1) the medullary tissue, 2) the androgenic tissue, and 3) the cortical or interrenal tissue.

The only condition definitely associated with over-activity of the medulla is the occurrence of paroxysmal hypertension in cases of paraganglioma which we have described in Chapter XXII. There is no valid evidence for linking other diseases of the circulatory, lymphatic, or endocrine systems with the adrenal medulla.

The results of overactivity of the androgenic zone have been discussed in Chapter XXIII.

No effects have been noted clinically which might be linked with overactivity of the cortex proper. The hormone elaborated by this tissue is of a relatively small molecular weight

and is probably rapidly secreted or destroyed when introduced in great excess into the organism. Over-dosages of this hormone have also failed to demonstrate any changes in the organism. Hence we might anticipate that physiological hyperplasia of the adrenal cortical tissue with the production of an abnormally great amount of its hormone would lead to no pathological condition such as follows the elaboration of an over-abundance of certain other endocrine products such as insulin or the growth hormone of the pituitary.

Hyperplasia of the adrenals, as noted in Chapter XXII, is not infrequently found at autopsy. Such hypertrophy is probably the result of an increased demand for the secretory product of the cortex and can be induced in animals by subjecting them for an extended period to any of the influences (cold, heat, drugs, toxins, inanition, *etc.*) which cause an increase in the rate of utilization of the cortical hormone. Although it can not be denied that an actual overproduction of the hormone beyond the needs of the organism occurs in certain cases (as evidenced by adenomatous growths) no clinical symptoms have been noted which might be attributed to this cortical overactivity.

HYPERTENSION

The fact that epinephrine produces so marked a pressor effect when injected has led authors from time to time to ascribe the condition of essential hypertension to over-activity of the adrenal medulla. Neusser⁴⁶⁸ in 1898 first pointed out the association of tumors of the adrenal gland with hypertension and his theory as to the causal relation existing between these two conditions has been revived periodically. Even within recent years unilateral adrenalectomy has been performed for the relief of essential hypertension. As has been indicated in Chapter VI, there is no justification for assuming that hypertension is in any way related to overactivity of the adrenal medulla. Excision of a gland or cutting the splanchnic nerves

is, therefore, to be deprecated in these conditions as a procedure involving great hazard for the patient with no reason for assuming that any therapeutic benefit will be derived.

Aside from Neusser's findings, an association between hypertension and hyperactivity of the adrenals has been claimed on the basis of the frequency with which cortical adenomata and hyperplasia are found in cases of hypertension. Thus Aubertin and Ambard²⁴ in eight cases of hypertension found that four showed a diffuse cortical hyperplasia, three had adenomata of the cortex, and only one had normal adrenal glands. Hypertrophy of the cortical tissue is, however, a common finding at autopsy and any observed hyperplasia is more apt to be secondary to rather than the primary cause of the hypertension.

It has repeatedly been shown that there is no increase in the epinephrine content of the blood in cases of hypertension.⁶³⁹ The hyperglycemia observed in hypertension can not, therefore, be attributed to an assumed increased secretion of epinephrine, but is probably due to a deficient pancreatic activity resulting from arterio-sclerosis.⁴⁸³ It is extremely difficult to estimate the size of the medulla by simple examination at autopsy. Attempts to arrive at an accurate estimate have been made and would seem to indicate that there is some hypertrophy of the medulla in patients dying of hypertensive cardio-vascular-renal disease. These enlargements are, however, inconstant, and best explained as the result rather than as the cause of the disease.⁴⁸³

HYPOFUNCTION OF THE ADRENALS

There is no clear-cut evidence to associate hypofunction of the adrenals with any clinical condition other than Addison's disease. This disease, however, is the result of marked destruction of the adrenal cortex and it would seem most logical to suppose that lesser degrees of injury or temporary conditions which call for an excessive utilization of the hormone should also result in deficiency symptoms of a minor degree. Un-

fortunately we are at present ignorant of these conditions and can only surmise as to their nature and causative agents. Further clinical investigation and a study of the therapeutic effects of administration of the cortical hormone should elucidate these problems.

One would anticipate that hypofunction of the adrenals should result in some of the symptoms which characterize insufficiency in experimental animals or patients with Addison's disease, *viz.*, asthenia, gastro-intestinal disturbances, a tendency to infection, *etc.* Unfortunately these symptoms are also characteristic of other pathological conditions as well, and hence, to attribute indiscriminately any condition characterized by one or more of these symptoms to adrenal insufficiency is unjustified. On the other hand, one is justified in our present state of ignorance to apply the therapeutic test in any given case in which there is a suspicion of adrenal hypofunction. For example, animals subjected to excessive heat require an excessive amount of the cortical hormone. It would seem logical to hypothecate, therefore, that the denervating effects of heat may in part at least be a result of a mild degree of adrenal cortical insufficiency. The experimental administration of the hormone to a statistically significant number of cases with proper controls and avoidance of vitiating factors would determine the validity of the above hypothesis.

A number of acute and chronic diseases may cause degenerative changes in the adrenals and it is logical to conclude that such changes may induce a degree of adrenal insufficiency which contributes to the observed clinical picture. Thus in uncomplicated cases of superficial burns the most characteristic changes occur in the adrenals.⁶⁶⁵ These organs are swollen and deep red. On section one may find extensive hemorrhage. The cells are pale and swollen and in a process of hydropic degeneration. The cause of death in extensive burns has not been definitely proven. It is generally assumed that absorption of toxins from the injured skin and from bacterial infec-

tions of the burned area is the prime cause of a fatal outcome. In view of the demonstrable lesions in the adrenals, it would seem worth while to investigate the therapeutic effects of the adrenal cortical hormone in these cases.

INFECTIONS

In acute bacterial toxemias, focal necroses are often observed in the *zona fasciculata* which resemble those occurring in the spleen and other viscera. Regeneration may occur in these areas as noted by mitotic activity. Cloudy swelling and edema of the adrenals also occur and are most prominent in the inner cortical zone. Such edema is common in patients dying of acute infections. Congestion and small focal hemorrhages are also frequently found and there is a disappearance of the cholesterol bodies from the cortex.¹⁵⁷

Changes in the adrenals, of the nature described above, were observed by Klotz in 14 of 32 patients dying of influenza.¹³² Paiseau and Lemaire described similar changes in pernicious malaria and others have found them in diphtheria, bronchopneumonia and other toxemias of bacterial origin.⁴¹⁷

One might anticipate that bacterial toxins will affect the adrenals as they do other parenchymatous glands. The questions of prime importance are: Does this injury to the adrenal cortex as observed pathologically contribute to the clinical symptoms of these diseases? Are the epigastric pain, the vomiting, asthenia, hypothermia, and hypotension due to an acute cortical insufficiency induced by the disease? It is impossible to answer these questions at present. Clinical investigation of the effects of therapy with the adrenal cortical hormone is obviously necessary for elucidation of the problem.

The symptoms of the nitroid crises following injections of arsphenamine and preventive vaccination have also been attributed to adrenal hypofunction. However, the nature of these symptoms and their acute onset speaks against an adrenal involvement.

Diseases other than those cited in the preceding sections have been associated with the adrenals. The evidence for this association has usually been so flimsy and in violation of so many well authenticated facts as to warrant little credence. Thus glaucoma has recently been considered as a disease due to adrenal cortical insufficiency. The basis for this view is the assumption that the intra-ocular tension which characterizes glaucoma is caused by an increased permeability of the capillary endothelium. Administration of a commercial preparation of the cortical hormone is claimed to have relieved the glaucoma. As we have seen (Chapter IX), there is no basis for the view that the capillaries become more permeable in adrenal insufficiency. Hence the conception of glaucoma as a disease due to cortical insufficiency is not justified. Moreover, the preparation used in "curing" the glaucoma contains so little of the adrenal cortical hormone that one could scarce attribute any therapeutic effect to its use.

The adrenal has been cited by several authors as in some way implicated in the causation of cancer. The claimed curative effects of concoctions derived from the adrenals have been shown to be unfounded. Joannovics³⁴⁰ found that partial adrenalectomy did not affect the percentage of successful implants of virulent tumors into mice. The slight retardation of growth of the tumors in the adrenalectomized animals is attributable to their general poor condition and does not imply any specific relation of the adrenals to the development of carcinoma.³⁷⁴

There is a natural tendency to apply any new therapeutic agent to all the sundry human ailments for which satisfactory methods of treatment are lacking, and to rationalize these attempts by a line of reasoning which is often fallacious, as exemplified by the cases cited above. Besides such ill-advised attempts to apply the newer knowledge, there may emerge, however, genuine cases in which the cortical hormone shall prove to be really efficacious and shall demonstrate relationships between the adrenals and pathological processes concerning which we are still in ignorance.

EPILOGUE

Despite the gradual advance in our knowledge of the adrenal glands, we are still unable to answer satisfactorily the question proposed by the Academy of Bordeaux in 1716—“*Quel est l'usage de glandes surrénales?*” Neither chance observation, which Montesquieu suggested might some day answer this question, nor the devoted zeal of the many workers who have applied themselves to its solution have answered satisfactorily this fundamental question. However, the labor of these investigators has not been in vain. The past work has made possible the formulation of certain definite views concerning the adrenals and has divested our minds of some of the early confusing ideas concerning these glands.

The fundamental problems relating to the adrenals which await future solution are clearly definable. The chemical study of the cortical hormone is of paramount importance. A knowledge of its structure will perhaps throw light on its action in the organism, and make possible an attempt to synthesize it. The synthesis of the hormone is extremely desirable because of the comparatively difficult and expensive task of obtaining it in pure form from natural sources.

Any physiological effects of the cortical hormone when injected into normal animals has not been demonstrated. The claims that such effects have been obtained are to be attributed to the presence of sundry impurities. Future workers must ensure themselves that they are actually dealing with relatively pure preparations of the hormone and are not being misled by extraneous effects. The same criticism is to be applied to the past work on the effects of the hormone in disease. As we have seen, the extant work claiming therapeutic effects in surgical shock, infections, glaucoma, *etc.* is scarcely to be taken seriously. The worker in the future should be less naive in accepting the claims of potency as stated on the label of his

purchased extract or the arrant claims of his predecessors. He must convince himself of the potency of his preparations, be certain that he is actually administering an adequate amount of the hormone, and refrain from drawing conclusions from results based on inadequately controlled work.

The fields for the clinical application of the cortical hormone are too obvious to require specific enumeration. With a potent preparation available one should certainly be able to produce worthy therapeutic effects in Addison's disease. The pessimistic reports of recent workers in this field should not be taken too seriously. The use of impotent and impure extracts explains why such poor results have hitherto been obtained. With the administration of potent extracts in sufficiently large doses, there is every reason to believe that the cortical hormone should actually result in the beneficent effects which many writers, rather prematurely and on insufficient grounds, claimed to have obtained.

The availability of the adrenal cortical hormone permits a more thorough and reliable study of the physiological and pathological results of adrenal insufficiency. The effects of the hormone on isolated tissues and on tissue cultures should also be studied providing one has available a highly purified solution. Adrenal cortical extracts are noteworthy for the ease with which they may be contaminated with highly active pharmacological agents, such as histamine, choline, epinephrine, *etc.* and the complete removal of these substances is imperative before one can rely on the results of many types of experiments.

The tripartite nature of the adrenal gland has been suggested in the present volume in the hope that it may stimulate experimental studies on the androgenic zone and its relation to the adreno-genital syndrome. Pathological studies of the tumors which give rise to this syndrome and efforts to differentiate such tumors histologically from true cortical tumors are important problems for future investigations. The extraction and isolation of the active principle of the androgenic tissue also remain as problems for the future.

BIBLIOGRAPHY

1. **Abel, J. J.** Method of preparing epinephrine and its compounds. *Bull. Johns Hopkins Hosp.* **13**: 29 (1902).
2. ——— and **Macht, D. I.** Two crystalline pharmacological agents obtained from the tropical toad. *J. Pharm. Exptl. Ther.* **3**: 319 (1912).
3. **Abelous, J. E.** and **Langlois, P.** La mort des grenouilles après la destruction des deux capsules surrénales. *C. r. soc. biol.* **43**: 855 (1891).
4. ——— ——— Essais de greffe de capsules surrénales sur le grenouille. *Ibid.* **44**: 864 (1892).
5. ——— ——— Sur la fonctions des capsules surrénales. *Ibid.* **47**: 334 (1895).
6. **Achard, C.** and **Thiers, J.** Diabète des femmes à barbe. *Bull. de l'acad. de méd.* **86**: 58 (1921).
7. **Addison, T.** "On the Constitutional and Local Effects of Disease of the Suprarenal Capsules." *London* (1855).
8. **Aichel, O.** Ueber die Nebennierenentwicklung der Säuger und die Entstehung der accessorischen Nebennieren des Menschens. *Anat. Anz.* **17**: 30 (1900).
9. **Aldrich, T. B.** Adrenalin, the active principle of the adrenals. *J. Am. Chem. Soc.* **27**: 1074 (1905).
10. **Allen, W. F.** Pulse changes from the injection of epinephrine in rabbits. *J. Pharm. Exptl. Ther.* **50**: 70 (1934).
11. **Alsterberg, G.** "Bau und Funktion der Nebennierenrinde." *Lund.* (1928).
12. **Altenberger, H.** Kastration und Nebennieren. *Arch. ges. Physiol.* **202**: 668 (1924).
13. **Amberg, S.** Ueber die Toxizität des Suprarenins. *Arch. int. Pharmacodyn.* **11**: 57 (1902).
14. **Andersen, D. H.** and **Kennedy, H. S.** Changes in the adrenal of the female rat associated with the oestrus cycle. *J. Physiol.* **76**: 247 (1932).
15. ——— ——— The adrenal cortex in pregnancy and lactation. *Ibid.* **77**: 159 (1933).
16. ——— ——— The effect of gonadectomy on the adrenal, thyroid and pituitary. *Ibid.* **79**: 1 (1933).
17. **Anderson, H. B.** Tumors of the adrenal with fatal hypoglycemia. *Am. J. Med. Sci.* **180**: 71 (1930).
18. **Apert, E.** Dystrophies en relation avec des lésions des capsules surrénales, hirsutisme et progeria. *Bull. soc. de pédiat. de Paris* **12**: 501 (1910).
19. **Arnold, J.** Der feineren Struktur und das Chemismus der Nebennieren. *Arch. path. Anat.* **35**: 64 (1866).

20. Artundo, A. La glycémie, le glycogène et l'action de l'insuline chez les rats décapsulés. *C. r. soc. biol.* **97**: 411 (1927).
21. Aschoff, L. "Lectures on Pathology." *New York* (1924).
22. Askin, J. A. and Geschickter, C. F. Neuroblastoma of the adrenal in children. *J. Pediatrics* **7**: 157 (1935).
23. Aub, J. C., Forman, J. and Bright, E. M. Effect of adrenalectomy upon the metabolism of the cat. *Am. J. Physiol.* **61**: 326, 349 (1922).
24. Aubertin, C. and Ambard, L. Lésions des capsules surrénales dans les néphrites avec hypertension. *Bull. et mem. soc. méd. de hôp. de Paris* **21**: 175 (1904).
25. Austmann, K. J., Halliday, C. W. and Vincent, S. The relations of the adrenal medulla to the blood pressure. *Trans. Roy. Soc. Canada* **11**: 123 (1918).
26. Bailey, C. H. Atheroma and other lesions produced in rabbits by cholesterol feeding. *J. Exptl. Med.* **23**: 69 (1916).
27. Bainbridge, F. A. and Parkinson, P. R. Morbid histology of the adrenals. *Lancet* **1**: 1296 (1907).
28. Balfour, F. M. "Monograph on the development of Elasmobranch fishes." *London* (1878).
29. Ball, E. G. and Chen, T. T. Oxidation-reduction potential of epinephrin. *J. Biol. Chem.* **102**: 691 (1933).
30. ——— R. G., Greene, C. H., Camp, J. D. and Rowntree, L. G. Calcification in tuberculosis of the adrenals. *J. Am. Med. Ass.* **98**: 954 (1932).
31. Banting, F. G. and Gairns, S. Adrenal insufficiency in dogs. *Am. J. Physiol.* **77**: 100 (1926).
32. Barbour, H. G. and Prince, A. L. The influence of epinephrin on the coronary circulation of the monkey. *J. Exptl. Med.* **21**: 330 (1915).
33. Bard, P. A diencephalic mechanism for the expression of rage. *Am. J. Physiol.* **84**: 490 (1928).
34. Barger, G. "The Simpler Natural Bases." *London* (1914).
35. ——— "Some Applications of Organic Chemistry to Biology and Medicine." *New York* (1930).
36. ——— and Dale, H. H. Chemical structure and sympathomimetic action of amines. *J. Physiol.* **41**: 19 (1910).
37. Barker, J. H., Eastland, C. J. and Evers, N. Colorimetric determination of adrenalin. *Biochem. J.* **26**: 2129 (1933).
38. ——— L. F. Paraganglioma of adrenal. *Med. Clinics North Am.* **14**: 265 (1930).
39. ——— N. W. Pathologic anatomy in 28 cases of Addison's disease. *Arch. of Path.* **8**: 432 (1929).
40. Barlow, O. W. and Ellis, M. M. Blood coagulation time after adrenalectomy. *Am. J. Physiol.* **70**: 58 (1924).
41. ——— and Sollmann, T. Deceptive effects of adrenal extracts. *Ibid.* **72**: 343 (1925).
42. Baumann, E. J. Epinephrin content of commercial adrenal "cortex" preparations. *Endocrinol.* **7**: 81 (1923).

43. **Baumann, E. J.** and **Holly, O. M.** Cholesterol and lipid phosphorus contents of the blood of rabbits before and after adrenalectomy. *J. Biol. Chem.* **55**: 457 (1923).
44. ——— and **Kurland, S.** Changes in the inorganic constituents of the blood in adrenalectomized cats and rabbits. *Ibid.* **71**: 281 (1927).
45. **Bayer, G.** Die Nebennieren. *Handb. der inn. Sekretion* **2**: 467 (1929).
46. ——— and **Lang, F. J.** Macrosomia interrenalis congenita. *Endokrinol.* **14**: 225 (1934).
47. **Bazett, H. C.** Blood pressure changes after adrenalectomy. *J. Physiol.* **53**: 320 (1920).
48. ——— and **Quinby, W. C.** The nature of pressor reflexes. *Quart. J. Exptl. Physiol.* **12**: 199 (1920).
49. **Beer, E.** and **Oppenheimer, B. S.** Transplantation of adrenals for Addison's disease. *Ann. Surg.* **100**: 689 (1934).
50. **Belding, D. L.** and **Wyman, L. C.** Rôle of the adrenal in the natural resistance of the rat to diphtheria toxin. *Am. J. Physiol.* **78**: 50 (1926).
51. **Belt, A. E.** and **Powell, T. O.** Chromaffin cell tumors. *Surg. Gyn. and Obstet.* **59**: 9 (1934).
52. **Bernard, L.** and **Bigart.** Étude des capsules surrénales dans quelques intoxications expérimentales. *J. de physiol.* **4**: 1014 (1902).
53. **Bertrand, G.** La composition chimique et la formule de l'adrénaline. *C. r. acad. sci. Paris.* **139**: 502 (1904).
54. **Bicknell, F.** Addison's disease due to malignant involvement of the solar plexus. *Brit. Med. J.* **2**: 206 (1934).
55. **Biedl, A.** Die geschichtliche Entwicklung der Kenntnisse über die Nebenniere bis Addison. *Janus* **15**: 193, 294 (1910).
56. ——— "Innere Sekretion." *Berlin* (1913, 1916).
57. ——— and **Wiesel, J.** Ueber die funktionelle Bedeutung der Nebenorgane des Sympathicus. *Arch. ges. Physiol.* **91**: 434 (1902).
58. **Bierry, H.** and **Malloizel, L.** Hypoglycémie après décapsulation. *C. r. soc. biol.* **65**: 232 (1908).
59. **Bittorf, A.** Zur Kenntnis der Nebennierenerkrankungen. *Münch. med. Wochenschr.* **73**: 1928 (1926).
60. ——— "Die Pathologie der Nebennieren und der Morbus Addisonii." *Jena*, (1908).
61. **Blanchard, E. W.** Relation of opsonins of the blood to adrenal function. *Physiol. Zool.* **7**: 493 (1934).
62. **Blanchetière, A., Binet, L.** and **Arnaudet, A.** Capsules surrénales et glutathion. *J. de physiol.* **28**: 816 (1930).
63. **Bloch, B.** Die Dopaoxydase. *Zeit. physiol. Chem.* **98**: 226 (1917).
64. **Blum, F.** Ueber Nebennierendiabetes. *Deut. Arch. klin. Med.* **71**: 146 (1901).
65. **Bøggild, D. H.** Die Bedeutung der Nebennieren für die Blutzuckerregulierung. *Acta Path. Scand.* **2**: 68 (1925).
66. **Boeminghaus, H.** Geschlechtsverändernde Geschwülste der Nebennieren. *Zeit. f. Urol.* **28**: 798 (1934).

67. **Borberg, N. C.** Adrenalin und der Nachweiss desselben. *Skand. Arch. f. Physiol.* 27: 341 (1912).
68. ——— Das chromaffine Gewebe. *Ibid.* 28: 91 (1913).
69. **Borchardt, W.** Ueber die Bedeutung der Nebennieren beim Zustandekommen der neutrophilen Reiz-Leukocytose. *Arch. exper. Path. u. Pharm.* 139: 47 (1929).
70. **Bornstein, A. and Gremels, H.** Ueber den Anteil von Mark und Rinde an den Ausfallerscheinungen nach Nebennierenexstirpation. *Arch. path. Anat.* 254: 409 (1925).
71. ——— and **Holm, K.** Ueber die Ausfallerscheinungen nach Nebennierenexstirpation. *Zeit. ges. exper. Med.* 37: 1 (1923).
72. ——— and **Hornemann, C.** Die Beziehungen der Nebennieren zum Zuckerstoffwechsel. *Ibid.* p. 24.
73. **Borutta, H.** Erfahrungen über die Nebennieren. *Arch. ges. Physiol.* 78: 97 (1899).
74. **Bouckaert, J.** L'influence de l'adrénaline sur la respiration. *Arch. neerl. de. physiol.* 7: 285 (1922).
75. ——— and **Heymans, C.** Hyperthermie et hyperglycemia par la betate-trahydro-naphtylamine. *Arch. internat. pharmacodyn.* 35: 153 (1929).
76. **Bourne, A. and Burn, J. H.** Action of adrenalin on the uterus in labour. *J. Obstet.* 34: 249 (1927).
77. **Bourne, G.** Unique structure in the adrenal of the female opossum. *Nature* 134: 664 (1934).
78. ——— The Golgi apparatus of the adrenals. The adrenals and infections. *Australian J. Exper. Biol. and Med. Sci.*, 12: 123 (1934); 13: 133 (1935).
79. **Brandt, A.** Ueber den Zusammenhang der Glandula suprarenalis mit dem Parovarium resp. der Epididymis bei Hühnern. *Biol. Centralbl.* 9: 522 (1890).
80. **Brauer, A.** Der Entwicklung und Anatomie der Gymnophionen. *Zool. Jahrbücher (Abth. f. Anat. u. Ontogenie)* 16: 1 (1902).
81. **Braun, M.** Ueber Bau und Entwicklung der Nebennieren bei Reptilien. *Arb. aus dem Zool-zoot. Inst. der Univ. in Würzburg* 5: 1 (1882).
82. **Brem, J. and Wechsler, H. F.** Myasthenia gravis with thymoma. *Arch. Int. Med.* 54: 901 (1934).
83. **Brems, A.** Ueber die perorale Adrenalinwirkung. *Acta med. scand.* 63: 431 (1926).
84. **Brenner, O.** Addison's disease with atrophy of the adrenal cortex. *Quart. J. Med.* 22: 121 (1928).
85. **Britton, S. W.** The adrenals. *Physiol. Revs.* 10: 617 (1930).
86. ——— Adrenalectomy in marsupial, hibernating and higher mammalian types. *Am. J. Physiol.* 99: 9 (1931).
87. ——— **Flippin, J. C. and Silvette, H.** Oral administration of cortical extract. *Ibid.* 99: 44 (1931).
88. ——— **Geiling, E. M. K. and Calvery, H. O.** Medulliadrenal secretion and carbohydrate metabolism. *Ibid.* 84: 141 (1928).

89. Britton, S. W. and Silvette, H. Effects of cortical extract on carbohydrate metabolism in normal animals. *Ibid.* 100: 693 (1932).
90. ——— Function of the adrenals. *Ibid.* 107: 190 (1934).
91. ——— Adrenal insufficiency in the marmot and opossum. *Science* 82: 230 (1935).
92. Bröking, E. and Trendelenburg, P. Adrenalin-nachweis und Gehalt des menschlichen Blutes. *Deut. Arch. klin. Med.* 103: 168 (1911).
93. Brodie, T. G. and Dixon, W. E. Action of adrenalin on the pulmonary vessels. *J. Physiol.* 30: 476 (1904).
94. Bronfin, I. D. and Guttman, P. H. Amyloid degeneration of adrenals in chronic tuberculosis. *Am. Rev. Tuberc.* 31: 1 (1935).
95. Broster, L. R. and Vines, H. W. C. "The Adrenal Cortex." London (1933).
96. Brown-Séquard, E. La physiologie et la pathologie des capsules surrénales. *Arch. gén. de méd.* 8: 385, 572 (1856).
97. ——— L'importances des fonctions des capsules surrénales. *J. de physiol.* 1: 160 (1858).
98. ——— Influence de l'extrait aqueux des capsules surrénales sur des cobayes presque mourants à la suite de l'ablation de ces organes. *C. r. soc. biol.* 44: 410 (1892).
99. Bulloch, W. and Sequeira, J. H. The relation of the adrenals to the sex organs. *Trans. Path. Soc. London* 66: 189 (1905).
100. Burke, E. M. Tumors of the adrenals. *Am. J. Cancer* 20: 338 (1934).
101. Calder, R. M. and Porro, F. W. Adenoma of adrenal cortex simulating pituitary basophilism. *Bull. Johns Hopkins Hosp.* 57: 99 (1935).
102. Cannon, W. B. The emergency function of the medulla. *Endocrinol. and Metab.* 2: 171 (1922).
103. ——— Evidence that medulliadrenal secretion is not continuous. *Am. J. Physiol.* 98: 447 (1931).
104. ——— and Gray, H. The hastening or retarding of coagulation by adrenalin injections. *Ibid.* 34: 232 (1914).
105. ——— and Hoskins, R. G. Effects of asphyxia, hyperpnoea, and sensory stimulation on adrenal secretion. *Ibid.* 29: 274 (1911).
106. ——— McIver, M. A., and Bliss, S. W. Adrenal mechanism for mobilizing sugar in hypoglycemia. *Ibid.* 69: 46 (1924).
107. ——— Querido, A., Britton, S. W., and Bright, E. M. The rôle of adrenal secretion in the chemical control of body temperature. *Ibid.* 79: 466 (1927).
108. Castaldi, L. Accrescimento delle sostanze corticale e midollare della ghiandola surrenale e loro rapporti volumetrici. *Arch. di fisiol.* 20: 33 (1922).
109. Castillo, E. B., Leloir, L. F., and Novelli, A. Action de l'extrait cortico-surrénal sur les glands endocrines. *C. r. soc. biol.* 115: 338 (1933).
110. Chen, K. K. and Meek, W. J. Comparative study of ephedrine, tyramine, and epinephrine on the circulation. *J. Pharm. Exptl. Ther.* 28: 59 (1926).

111. Chiari, H. Zur Kenntniss des accessorischen Nebennieren des Menschen. *Zeit. f. Heilk.* 5: 449 (1884).
112. Christian, H. A. Epinephrine in internal medicine. *J. Am. Med. Assoc.* 83: 1588 (1924).
113. Clark, G. A. Vaso-dilator action of adrenaline. *J. Physiol.* 80: 429 (1934).
114. — J. H. and Rowntree, L. G. Diseases of the adrenals as revealed in 25,000 autopsies. *Endocrinol.* 18: 256 (1934).
115. Cleghorn, R. A. Effects of cortical extracts on the sex functions of the mouse. *J. Physiol.* 76: 193 (1932).
116. Clevers. Le cortex surrénal au cours de la vaccination anti-diphtérique. *Bull. Acad. roy. méd. Belgique.* 5 me série 2: 425 (1922).
117. Clough, P. W. Epinephrin sensitiveness in patients with hypertension. *Bull. Johns Hopkins Hosp.* 31: 266 (1920).
118. Cohen, M. B., Rudolph, J. A., Wasserman, P. and Rogoff, J. M. Output of epinephrine during anaphylactic shock. *Am. J. Physiol.* 106: 414 (1933).
119. Cohoe, B. A. Clinical syndromes due to adrenal diseases. *Endocrinol. and Metab.* 2: 277 (1922).
120. Collett, A. Genito-adrenal syndrome in a girl with successful operation. *Am. J. Dis. Child.* 27: 204 (1924).
121. Collip, J. B., Anderson, E. M. and Thomson, D. L. The adrenotropic hormone of the pituitary. *Lancet* 2: 347 (1933).
122. Conybeare, J. J. and Millis, G. C. 29 cases of Addison's disease treated in Guy's Hospital between 1904 and 1923. *Guy's Hosp. Rep.* 74: 369 (1924).
123. Cooper, E. R. A. "The Histology of the more Important Human Endocrine Organs at Various Ages." London (1925).
124. Cope, O. and Marks, H. P. Relation of the pituitary to the action of insulin and adrenalin. *J. Physiol.* 83: 157 (1934).
125. Corey, E. L. Survival of pregnant and lactating cats after adrenalectomy. *Physiol. Zool.* 1: 147 (1928).
126. — and Britton, S. W. Blood cellular changes in adrenal insufficiency. *Am. J. Physiol.* 102: 699 (1932).
127. — — The ovarian cycle and the adrenals. *Ibid.* 107: 207 (1934).
128. Cori, C. F. Mammalian carbohydrate metabolism. *Physiol. Revs.* 11: 143 (1931).
129. Corkill, A. B. and Marks, H. P. Effects of adrenalin on muscle glycogen. *J. Physiol.* 70: 67 (1930).
130. Coupland, S. Atrophy of adrenals. *Trans. path. Soc. London* 36: 423 (1885).
131. Cow, D. The adrenals and diuresis. *J. Physiol.* 48: 443 (1914).
132. Cowie, D. M. and Beaven, P. W. Involvement of the adrenals in influenza. *Arch. Int. Med.* 24: 78 (1919).
133. Coxe, J. R. Functions of the capsulae renales. *Am. J. Med Sci.* 1: 40 (1828).

134. Cramer, W. "Fever, Heat Regulation, Climate and the Thyroid Adrenal Apparatus." *London* (1928).
135. Crawford, A. C. Chemistry of the adrenals. *Endocrinol. and Metab.* 2: 77 (1922).
136. Crile, G. Indications and contra-indications for denervation of the adrenals. *Ann. Surg.* 100: 664 (1934).
137. Cristiani, H. and Cristiani, A. Les capsules surrénales et leurs greffes. *Jour. de physiol.* 4: 837, 982 (1902).
138. Crivellari, C. A. Sensitiveness of adrenalectomized rats to toxic substances. *Am. J. Physiol.* 81: 414 (1927).
139. Crooke, A. C. A change in the basophil cells of the pituitary common to conditions exhibiting the syndrome attributed to basophilic adenoma. *J. Path. and Bact.* 41: 339 (1935).
140. Crosby, E. H. Addison's disease associated with congenital absence of the adrenals. *Arch. of Path.* 10: 38 (1930).
141. Crowe, S. J. and Wislocki, G. B. The functions of the interrenal portion of the adrenals. *Bull. Johns Hopkins Hosp.* 25: 287 (1914); *Beitr. zur klin. chir.* 95: 8 (1914).
142. Cushing, H. Basophil adenomas of the pituitary. *Bull. Johns Hopkins Hosp.* 50: 137 (1932); *J. Am. Med. Ass.* 99: 281 (1932).
143. Cushny, A. R. Action of the optical isomers of epinephrine. *J. Physiol.* 37: 130 (1908).
144. Cuvier, G. "Leçons d'anatomie comparée." 2^{me} éd. *Paris* (1846).
145. D'Abreu, F. Transplantation of adrenals in Addison's disease. *Lancet* 2: 1478 (1933).
146. Dakin, H. D. The physiological activity of substances indirectly related to adrenalin. *Proc. Roy. Soc. London B*, 76: 498 (1905).
147. Dale, H. H. Conditions conducive to the production of shock by histamine. *Brit. J. Exptl. Path.* 1: 103 (1920).
148. ——— and Laidlow, P. P. Significance of the adrenals in the action of certain alkaloids. *J. Physiol.* 45: 1 (1912).
149. Daly, I. B. Reactions of the pulmonary and bronchial blood vessels. *Physiol. Revs.* 13: 149 (1933).
150. Dambrosi, R. G., Leloir, L. F. and Novelli, A. Rôle des surrénales dans la resynthèse du glycogène. *C. r. soc. biol.* 114: 1219 (1933).
151. Daoud, K. M. and Gohar, H. A. F. Correlation of the action of insulin and adrenalin upon muscle and liver glycogen. *J. Physiol.* 80: 314 (1933).
152. Davis, B. L. and Van Winkle, W. Effect of insulin and epinephrine on the amino-acid content of the blood. *J. Biol. Chem.* 104: 207 (1934).
153. Deanesly, R. The adrenal cortex of the mouse and its relation to the gonads. *Proc. Roy. Soc. London B*, 103: 523 (1928).
154. ——— The histology of adrenal enlargement under experimental conditions. *Am. J. Anat.* 47: 475 (1931).
155. Degti, T. Resistance of adrenalectomized rats to acetonitrile. *Tohoku J. Exptl. Med.* 24: 468 (1934).

156. Dietrich, A. Die Nebennieren bei den Wundinfektionskrankheiten. *Centralbl. allg. Path.* 29: 169 (1918).
157. ——— and Siegmund, H. Die Nebenniere und das chromaffine System. *Handb. path. Anat.* 8: 951 (1926).
158. Dill, D. B., Edwards, H. T. and de Meio, R. H. Effects of adrenaline in work. *Am. J. Physiol.* 111: 9 (1935).
159. Dixon, W. E. and Halliburton, W. D. Action of drugs on the cerebral vessels. *Quart. J. Exptl. Physiol.* 3: 315 (1910).
160. ——— and Ransom, F. Broncho-dilator nerves. *J. Physiol.* 45: 413 (1912).
161. Donaldson, J. C. Adrenals in pregnancy. *Anat. Rec.* 38: 239 (1928).
162. ——— Adrenals in wild and albino rats. *Proc. Soc. Exptl. Biol. & Med.* 25: 300 (1928).
163. ——— S. W. and Howard, S. C. Primary carcinoma of the adrenal. *Am. J. Cancer* 24: 75 (1935).
164. Dragonet, J. Beiträge zur pathologischen Anatomie der Nebennieren. *Zeit. f. Heilk.* 6: 1 (1885).
165. Dragstedt, C. A. and Huffmann, J. W. Comparative sensitiveness to epinephrine of blood pressure and intestinal motility in man. *Am. J. Physiol.* 85: 363 (1928).
166. D'Silva, J. L. Action of adrenalin on serum potassium. *J. Physiol.* 82: 393 (1934).
167. Durrant, E. P. Effect of adrenalectomy on activity of the rat. *Am. J. Physiol.* 70: 344 (1924).
168. Eagle, E. Choline in adrenal extracts. *Proc. Soc. Exptl. Biol.* 30: 1094 (1933).
169. Ecker, A. "Der feinere Bau der Nebennieren beim Menschen und den vier Wirbelthierklassen." *Braunschweig* (1846).
170. Edmunds, C. W. Vasomotor reactions of the liver. *J. Pharm. Exptl. Ther.* 6: 569 (1915).
171. ——— The importance of the adrenals in the action of pilocarpine, physostigmine and strychnine. *Ibid.* 20: 405 (1923).
172. ——— and Johnston, F. D. Relation of the adrenals to the circulatory collapse of diphtheria. *Am. Heart J.* 4: 3 (1928).
173. ——— and Smith, R. G. Experimental adrenal exhaustion. *J. Lab. Clin. Med.* 17: 399 (1932).
174. Ehrmann, R. Die Wertbestimmung des Adrenalins in Blut. *Arch. exptl. Path.* 53: 97 (1905).
175. ——— and Dinkin, L. Klinische Pathologie der Nebennieren. *Handb. inn. Sekret.* 3: 283 (1928).
176. Eisenberg, A. A. and Wallerstein, H. Pheochromocytoma of the adrenal. *Arch. Path.* 14: 818 (1932).
177. Elliott, T. R. The action of adrenalin. *J. Physiol.* 32: 401 (1905).
178. ——— The control of the adrenals by the splanchnics. *Ibid.* 44: 374 (1912).
179. ——— The innervation of the adrenals. *Ibid.* 46: 285 (1913).

180. Elliott, T. R. Some results of excision of the adrenal glands. *Ibid.* **49**: 38 (1914).
181. ——— Pathological changes in the adrenals. *Quart. J. Med.* **8**: 47 (1914).
182. ——— and Armour, G. The development of the adrenal cortex and its condition in hemicephaly. *J. Path. and Bact.* **15**: 481 (1911).
183. ——— and Tuckett, I. Cortex and medulla in the adrenal. *J. Physiol.* **34**: 332 (1906).
184. Elman, R. and Rothman, P. Insufficiency following venous obstruction of the adrenals. *Bull. Johns Hopkins Hosp.* **35**: 54 (1924).
185. Emery, F. E. and Winter, C. A. The adrenotropic substance of the hypophysis. *Anat. Rec.* **60**: 381 (1934).
186. Eskin, J. A. Ueber den Einfluss der Nebenniere auf die Metamorphose bei Amphibien. *Endokrinol.* **11**: 249 (1932).
187. Euler, U. S. Ueber Hyperadrenalinämie bei Fieberzuständen. *Arch. ges. Physiol.* **217**: 699 (1927).
188. ——— Adrenalinbestimmung. *Biochem. Zeit.* **260**: 18 (1933); *Arch. f. expul. Path.* **171**: 186 (1933).
189. ——— and Liljestrand, G. Einwirkung der Sinusentlastung auf Adrenalin-, Zucker- und Kalziumgehalt des Blutes. *Skand. Arch. Physiol.* **71**: 73 (1934).
190. Eustachius, B. "Opuscula Anatomica." Venice (1563).
191. Everse, J. W. R. and De Fremery, P. Measurement of fatigue in rats and its application for testing cortical extracts. *Acta brevia neerl.* **2**: 152 (1932).
192. Feldberg, W., Minz, B., and Tsudzimura, H. The mechanism of the nervous discharge of adrenaline. *J. Physiol.* **81**: 286 (1934).
193. ——— and Schild, H. Distribution of choline and acetylcholine in adrenals. *Ibid.* **81**: 37P (1934).
194. Ferguson, J. S. The veins of the adrenal. *Am. J. Anat.* **5**: 63 (1906).
195. Fibiger, J. Beiträge zur Kenntniss der weiblichen Scheinzwittertums. *Arch. path. Anat.* **181**: 1 (1905).
196. Findlay, G. M. Pigments of the adrenal. *J. Path. and Bact.* **23**: 482 (1920).
197. Finzi, O. Ueber Veränderungen des Magenschleimhaut bei Tieren nach Nebennierenexstirpation. *Arch. path. Anat.* **214**: 413 (1913).
198. Firor, W. M. and Grollman, A. Adrenalectomy in mammals with particular reference to the white rat. *Am. J. Physiol.* **103**: 686 (1933).
199. Flächen, F. Ueber die Spaltung des synthetischen *dl*-suprarenins in seine Komponenten. *Zeit. physiol. Chem.* **58**: 189 (1908).
200. Flashman, D. H. The effect of adrenalectomy on anaphylactic shock in the rat. *J. Infect. Dis.* **38**: 461 (1926).
201. Flint, J. M. The blood vessels, angiogenesis, etc., of the adrenal. *Johns Hopkins Hosp. Rep.* **9**: 153 (1900).
202. Folin, O., Cannon, W. B., and Denis, W. A colorimetric method for determining epinephrine. *J. Biol. Chem.* **13**: 477 (1912).
203. Fordyce, A. D. and Evans, W. H. Adrenal virilism. *Quart. J. Med.* **22**: 557 (1929).

204. Fourneau, E. "Organic Medicaments and their Preparation." *Philadelphia* (1925).
205. Freed, S. C., Brownfield, B., and Evans, H. M. Effect of adrenalectomy on the testes of the rat. *Proc. Soc. Exptl. Biol. and Med.* **29**: 1 (1931).
206. Freeman, H., Linder, F. E., and Hoskins, R. G. Glycerin extract of adrenal potent by mouth. *Endocrinol.* **17**: 677 (1933).
207. Friedmann, E. Die Konstitution des Adrenalins. *Beitr. chem. Physiol.* **8**: 95 (1906).
208. Fürth, O. Zur Kenntnis des Suprarenins. *Ibid.* **1**: 243 (1901).
209. Fulk, M. E. and MacLeod, J. J. R. Evidence that the active principle of the retroperitoneal chromophil tissue contains epinephrine. *Am. J. Physiol.* **40**: 21 (1916).
210. Gallais, A. "Le syndrome génito-surrénal." *Paris* (1912).
211. Gaskell, W. H. The origin of the vertebrate body cavity and the excretory organs. *J. Anat. and Physiol.* **27**: 168 (1903).
212. Gaunt, R., Tobin, C. E., and Gaunt, J. H. Survival of salt-treated adrenalectomized rats. *Am. J. Physiol.* **111**: 321 (1935).
213. Gérard, G. Etude morphologique des artères des capsules surrénales de l'homme. *J. de l'anat. et physiol.* **49**: 269 (1913).
214. Gerstel, G. and Nagel, W. Die bösartigen Nebennierenrindengeschwülste. *Endokrinol.* **16**: 41 (1935).
215. Geschickter, C. F. Tumors of the adrenal. *Arch. of Path.*; **15**: 775 (1933); *Am. J. Cancer* **23**: 104 (1935).
216. Giacomini, E. Sulle capsule surrenali dei Petromizonti. *Monit. zool. ital.* **13**: 1 (1902).
217. ——— Sulla esistenza della sostanza midollare nelle capsule surrenali dei Teleostei. *Ibid.* p. 182.
218. ——— Sulla disposizione del sistema interrenale e del sistema feocromo nelle anguille adulte, nelle cieche e nei leptocefali. *Rendiconto d. R. Acad. d. Sci. de Bologna* **12**: 172 (1908).
219. Giaja, J. and Chahovitch, X. Le métabolisme de sommet et des capsules surrénales. *C. r. acad. Sci.* **181**: 885 (1925).
220. Gibson, T. E. The diagnosis of adrenal tumors. *J. Urology* **18**: 33 (1927).
221. Gilman, A. Experimental sodium loss analogous to adrenal insufficiency. *Am. J. Physiol.* **108**: 662 (1934).
222. Gley, E. The problem of the adrenals. *N. Y. Med. J.* **114**: 9 (1921).
223. ——— La physiologie des surrénales. *Rev. de méd.* **40**: 193 (1923).
224. ——— and Quinquaud, A. La sécrétion surrénale d'adrénaline n'est pas nécessaire au maintien de la pression artérielle. *C. r. soc. biol.* **82**: 1175 (1919).
225. Glick, D. and Biskind, G. R. The quantitative distribution of vitamin C. *J. Biol. Chem.* **110**: 1 (1935).
226. ——— The distribution of lipolytic enzymes in the adrenal. *Ibid.* p. 575.
227. Glynn, E. E. The adrenal cortex, its rests, and tumors, and its relation to sex. *Quart. J. Med.* **5**: 157 (1911).

228. Glynn, E. E. and Hewetson, J. T. Adrenal hypernephroma associated with sex changes. *J. Path. and Bact.* 18:81 (1913).
229. Gohar, H. Effect of diet, insulin, and thyroxin on the adrenalin content of the adrenals. *J. Physiol.* 80:305 (1933).
230. Goldzieher, M. A. "The Adrenals." *New York* (1929).
231. Goormaghtigh, N. "Le cortex surrénal humain dans les plaies de l'abdomen et aux périodes intéressantes de la vie sexuelle." *Liege* (1922).
232. Gordon, M. B. and Browder, E. J. Adrenal carcinoma with precocity in a boy. *Endocrinol.* 11:265 (1927).
233. Gottesmann, J. M. and Gottesmann, J. Histamine as a standard test for diminished resistance in adrenalectomized rats. *J. Exptl. Med.* 47:503 (1928).
234. Gottschau, M. Structur und Entwicklung der Nebennieren. *Arch. Anat. u. Physiol. Anat. Abt.* p.412 (1883).
235. Gourfein, D. Sur la fonction des glandes surrénales. *Rev. méd. de la Suisse romande* 16:113 (1896).
236. Gradinescu, A. V. Der Einfluss der Nebennieren auf den Blutkreislauf und den Stoffwechsel. *Arch. ges. Physiol.* 152:187 (1913).
237. Graham, G. S. Lesions of the adrenal and their repair. *J. Med. Res.* 34:241 (1916).
238. Gratiolet, P. Les effets qui suivent l'ablation des capsules surrénales. *C. r. acad. sci.* 43:468 (1856).
239. Gray, H. The development of the ductless glands in the chick. *Phil. Trans. Roy. Soc. London* 142:295 (1852).
240. Green, C. H., Rowntree, L. G., Swingle, W. W., and Piffner, J. J. Metabolic studies in Addison's disease. *Am. J. Med. Sci.* 183:1 (1932).
241. Greenhow, E. H. Cancer of the adrenals. *Trans. Path. Soc. London* 24:238 (1873).
242. ——— Addison's disease. *Lancet* 1:327, 361, 395, 429, 463, 532 (1875).
243. Grollman, A. "The Cardiac Output of Man in Health and Disease." *Springfield, Ill.* (1932).
244. ——— and Firor, W. M. Extraction of cortical hormone from urine. *Proc. Soc. Exptl. Biol. and Med.* 30:669 (1933).
245. ——— Preparation of an active extract of the adrenal cortex. *J. Biol. Chem.* 100:429 (1933).
246. ——— Oral administration of the adrenal cortical hormone and the use of fresh glands therapeutically. *Bull. Johns Hopkins Hosp.* 54:216 (1934).
247. ——— Relation of the adrenals to the vitamins. *J. Nutrition* 8:569 (1934).
248. ——— The rôle of the hypophysis in experimental chronic adrenal insufficiency. *Am. J. Physiol.* 112:310 (1935).
249. ——— Experimental studies on replacement therapy in adrenal insufficiency. *Bull. Johns Hopkins Hosp.* 57:281 (1935).
250. ——— and Grollman, E. Extraction of the adrenal cortical hormone from the interrenal body of fishes. *Am. J. Physiol.* 108:237 (1934).

251. Grollman, A., Firor, W. M. and Grollman, E. A simple preparation of the adrenal cortical hormone suitable for oral administration. *J. Biol. Chem.* **109**:189 (1935).
252. Gruber, C. M. Significance of epinephrine in muscular activity. *Endocrinol.* **3**: 149 (1919).
253. Grynfeldt, E. "Recherches anatomiques et histologiques sur les organes surrénaux des Plagiostomes." *Paris* (1903).
254. ——— and Rimbaud, P. Adénome cortical des capsules surrénales. *Bull. de l'assoc. franc. pour l'étude du cancer.* **21**: 614 (1932).
255. Gsell, O. and Uehlinger, C. Tuberkulöser Morbus Addison. *Beitr. z. Klin. der Tuberk.* **83**: 121 (1933).
256. Guieysse, A. La capsule surrénale du cobaye. *C. r. soc. biol.* **51**: 898 (1899); *J. de l'anat. et physiol.* **37**: 312, 435 (1901).
257. Gunn, J. A. Antagonism between adrenin and chloroform, chloral, etc. on the heart. *Quart. J. Exptl. Physiol.* **7**: 75 (1914).
258. ——— and Chavasse, F. B. The action of adrenin on veins. *Proc. Roy. Soc. London B* **86**: 192 (1913).
259. Gutowski, B. La relation du corps actif des ganglions étoilés avec l'adrénaline. *C. r. soc. biol.* **90**: 1469 (1924).
260. Guttman, P. H. A statistical analysis of 566 cases of Addison's disease. *Arch. Path.* **10**: 742, 895 (1930).
261. Haberer, H. Die gestielte Nebennierentransplantation. *Arch. klin. Chirur.* **94**: 606 (1911).
262. Habersang. Die Wirkung und Anwendung des Suprarenins beim Pferde. *Monats. f. prakt. Tierheilk.* **32**: 127 (1921).
263. Haller, A. "Elementa Physiologiae Corporis Humani." *Lusanne* (1765).
264. Harrop, G. A., Pfiffner, J. J., Weinstein, A., and Swingle, W. W. A biological method of assay of the adrenal cortical hormone. *Proc. Soc. Exptl. Biol. and Med.* **29**: 449 (1932).
265. ——— Soffer, L. J., Ellsworth, R., and Trescher, J. H. Plasma electrolytes and their excretion in adrenal insufficiency in the dog. *J. Exptl. Med.* **58**: 17 (1933).
266. ——— ——— Nicholson, W. M., and Strauss, M. Effect of sodium salts in sustaining the adrenalectomized dog. *Ibid.* **61**: 839 (1935).
267. ——— and Weinstein, A. Adrenal insufficiency and the action of the cortical hormone in normal and adrenalectomized dogs. *Ibid.* **57**: 305 (1933).
268. ——— ——— Soffer, L. J., and Trescher, J. H. Metabolism, circulation, and blood concentration during adrenal insufficiency in the dog. *Ibid.* **58**: 1 (1933).
269. Hartman, F. A. General physiology and pathology of the adrenals. *Endocrinol. and Metab.* **2**: 101 (1922).
270. ——— Pharmacology of the adrenals. *Ibid.* p. 237.
271. ——— Function and clinical use of cortin. *Ann. Int. Med.* **7**: 6 (1933).
272. ——— Chronic adrenal insufficiency produced by cautery. *Endocrinol.* **17**: 43 (1933).

273. **Hartman, F. A. and Blatz, W. E.** Death produced by tying the adrenal veins. *Ibid.* 3: 137 (1919).
274. ——— and **Brownell, K. A.** Preparation of cortin. *Science* 73: 620 (1931).
275. ——— ——— **Hartman, W. E., Dean, G. A., and MacArthur, C. G.** The hormone of the adrenal cortex. *Am. J. Physiol.* 86: 353 (1928).
276. ——— ——— and **Lockwood, J. E.** The function of cortin. *Endocrinol.* 16: 521 (1932).
277. ——— and **Hartman, W. B.** Influence of temperature changes on the secretion of epinephrin. *Am. J. Physiol.* 65: 612 (1923).
278. ——— ——— The production of epinephrine by the adrenal cortex. *Ibid.* 65: 623 (1923).
279. ——— **Lockwood, J. E., and Lockie, L. M.** Lowered resistance to heat in adrenal insufficiency. *Proc. Soc. Exptl. Biol.* 29: 409 (1932).
280. ——— **MacArthur, C. G., Gunn, F. D., Hartmann, W. E., and McDonald, J. J.** Kidney function in adrenal insufficiency. *Am. J. Physiol.* 81: 244 (1927).
281. ——— **McCordock, H. A., and Loder, M. M.** Conditions determining adrenal secretion. *Ibid.* 64: 1 (1923).
282. ——— and **Scott, W. J.** Protection of adrenalectomized animals against bacterial intoxication. *J. Exptl. Med.* 55: 63 (1932).
283. ——— and **Winter, C. A.** Irreversibility in adrenal insufficiency. *Endocrinol.* 17: 180 (1933).
284. **Hartung, W. H.** Epinephrine and related compounds. *Chem. Revs.* 9: 389 (1931).
285. **Hastings, A. B. and Compere, E. L.** Effect of adrenalectomy on certain constituents of the blood of dogs. *Proc. Soc. Exptl. Biol.* 28: 376 (1931).
286. **Hatai, S.** Weight of the ductless glands of the rat. *Anat. Rec.* 8: 511 (1914).
287. ——— Growth of the organs of the rat after gonadectomy. *J. Exptl. Zool.* 18: 1 (1915).
288. **Hays, V. J.** Development of the adrenals in birds. *Anat. Rec.* 8: 451 (1914).
289. **Hazard, R.** Le potassium élément adrénalinogène. *C. r. acad. sci. Paris* 197: 1455 (1933).
290. **Heard, R. D. H. and Raper, H. S.** The oxidation of 3:4 dihydroxyphenyl-N-methylalanine and its possible function as a precursor of adrenalin. *Biochem. J.* 27: 36 (1933).
291. **Hedinger, E.** Die Kombination von Morbus addisonii mit Status lymphaticus. *Frankfurter Zeit. f. Path.* 1: 527 (1907).
292. **Heinekamp, W. J. R.** Action of adrenalin on the heart. *J. Pharm. Exptl. Therap.* 16: 247 (1921); 26: 385 (1926).
293. **Henle, J.** Das Gewebe der Nebenniere und der Hypophyse. *Zeit. f. rat. Med., Dritte Reihe* 24: 143 (1865).
294. **Herring, P. T.** Adrenalin content of the rat's adrenal after thyroid feeding and other conditions. *Quart. J. Exptl. Physiol.* 12: 115 (1920).

295. Heuer, G. J. and Andrus, W. D. Adrenal extract in controlling shock. *Ann. of Surg.* 100: 734 (1934).
296. Hewer, E. E. Histological conditions in a case of Addison's disease. *Brit. Med. J.* 1: 235 (1923).
297. Heymans, C. Le sinus carotidien, zone réflexogène régulatrice de l'adrénalsecretion. *Arch. internat. Pharmacodyn.* 35: 269 (1929).
298. ——— J. F. and Heymans, C. Sur les modifications directes et sur la régulation réflexe de l'activité du centre respiratoire. *Ibid.* 33: 273 (1927).
299. Himwich, H. E., Fazikas, J. F., Barker, S. B., and Hurlburt, M. H. Metabolism of tissues excised from adrenalectomized rats. *Am. J. Physiol.* 110: 348 (1934).
300. Hirassee, K. Der Einfluss der Nebennierenexstirpation auf die Arbeitsfähigkeit des Muskels. *Arch. ges. Physiol.* 212: 582 (1926).
301. Hoerr, N. The cells of the adrenal in the guinea-pig; their reaction to injury and their replacement. *Am. J. Anat.* 48: 139 (1931).
302. Holl, G. Zwei männliche Fälle von Nebennierenrindentumoren. *Deut. Zeit. f. Chir.* 226: 277 (1930).
303. Holmes, G. A case of virilism associated with an adrenal tumor with recovery after its removal. *Quart. J. Med.* 18: 143 (1925).
304. Hoskins, E. R. The growth of the body and organs of the rat after feeding various ductless glands. *J. Exptl. Zool.* 21: 295 (1916).
305. ——— R. G. Relation of the adrenals to the circulation. *Endocrinol. and Metab.* 2: 187 (1922).
306. ——— and Freeman, H. Effects of glycerine extracts of the adrenal potent by mouth. *Endocrinol.* 17: 29 (1933).
307. ——— and McClure, C. W. The adrenals and blood pressure. *Arch. Int. Med.* 10: 343 (1912).
308. ——— ——— The comparative sensitiveness of blood pressure and intestinal peristalsis to epinephrine. *Am. J. Physiol.* 31: 59 (1912).
309. Houssay, B. A. Relaciones entre la hipofisis y las suprarrenales. *La prensa méd. Argentina* 20: 1563 (1933).
310. ——— Biasotti, A., Mazzocco, P., and Sammartino, R. Action de l'extrait antéro-hypophysaire sur les surrénales. *C. r. soc. biol.* 114: 737 (1933).
311. ——— and Lewis, J. T. Relative importance to life of cortex and medulla of the adrenals. *Am. J. Physiol.* 64: 512 (1923).
312. ——— ——— and Molinelli, E. A. Rôle de la sécrétion d'adrénaline produite par l'insuline et par la morphine. *C. r. soc. biol.* 91: 1011, 1013 (1924).
313. ——— and Marenzi, A. D. L'extrait cortico-surrénale protège les rats surrénalectomisés contre les toxiques. *Ibid.* 107: 1199 (1931).
314. ——— and Mazzocco, P. Le glutathion et les propriétés réductrices des tissus des rats decapsulés. *Ibid.* 97: 417 (1927).
315. ——— and Molinelli, E. A. Adrenal secretion during anaphylactic shock and after the injection of drugs. *Am. J. Physiol.* 77: 181, 184 (1926).

316. Houssa, P. Action de l'adrénaline sur la glycémie des animaux phlorhizinés. *C. r. soc. biol.* 118: 1214 (1935).
317. Howard, E. Development of epinephrine in the early stages of the mouse. *Am. J. Physiol.* 75: 267 (1926).
318. ——— A transitory zone in the adrenal which shows age and sex relationships. *Am. J. Anat.* 40: 251 (1927).
319. ——— Development of the mouse adrenal. *Science* 69: 406 (1929).
320. ——— The x-zone of the adrenal in relation to gonadal maturation in monkeys and mice. *Anat. Rec.* 46: 93 (1930).
321. ——— and Grollman, A. Effect of adrenal extracts on growth and the reproductive system of normal rats with particular reference to intersexuality. *Am. J. Physiol.* 107: 480 (1934).
322. Howell, S. C. Action of adrenaline on the human eye. *Arch. Ophthalm.* 12: 833 (1934).
323. Hultgren, E. O. and Andersson, O. A. Die Physiologie und Anatomie der Nebennieren. *Skand. Arch. Physiol.* 9: 73 (1899).
324. Hunt, R. Blood pressure lowering bodies in extracts of the adrenals. *Am. J. Physiol.* 5: Pvi (1901).
325. Huot, A. Les poissons lophobranches. *Ann. des sci. nat.* 14: 197 (1902).
326. Huszák, I. Zur Chemie der Nebennierenmarkes. *Zeit. physiol. Chem.* 222: 229 (1933).
327. Hutchison, R. Adrenal sarcoma in children with metastases in the skull. *Quart. J. Med.* 1: 33 (1907).
328. Hyman, A. S. Resuscitation of the stopped heart by intracardiac therapy. *Arch. Int. Med.* 46: 553 (1930).
329. Ingier, A. and Schmorl, G. Der Adrenalinhalt der Nebennieren. *Deut. Arch. klin. Med.* 104: 125 (1911).
330. Innes, J. R. M. Nebennierenveränderungen bei experimentellen Skorbut. *Endokrinol.* 14: 128 (1934).
331. Jaffe, H. L. Influence of the adrenal on the thymus. *J. Exptl. Med.* 40: 325, 619, 753 (1924).
332. ——— Effects of adrenalectomy on the life of rats. *Am. J. Physiol.* 78: 453 (1926).
333. ——— The diminished resistance following adrenalectomy in the rat and the protection afforded by autoplasmic transplants. *Am. J. Path.* 2: 421 (1926).
334. ——— The transplantation of the guinea-pig adrenal. *J. Exptl. Med.* 45: 587 (1927).
335. ——— The adrenals. *Arch. of Path.* 3: 414 (1927).
336. ——— and Marine, D. Effect of adrenalectomy on agglutinin formation. *J. Infect. Dis.* 35: 334 (1924).
337. Janošik, J. Die Entwicklung des Genitalsystems. *Sitzb. Akad. Wiss. Wien., Math. Naturwiss. Kl.* 99: 260 (1890).
338. ——— Die Entwicklung der Nebennieren. *Arch. mikr. Anat.* 22: 738 (1883).
339. Jensen, H. and Chen, K. K. Isolation of epinephrine from the chinese toad. *J. Biol. Chem.* 82: 397 (1929).

340. Joannovics, G. G. Das Wachstum der transplantablen Mäusetumoren in kastrierten und in epinephrektierten Tieren. *Beitr. path. Anat. und Path.* 62: 194 (1916).
341. Johannessohn, F. Der Suprareninegehalt handelsüblicher Präparate. *Biochem. Zeit.* 76: 377 (1916).
342. Jowett, H. A. D. The constitution of epinephrine. *J. Chem. Soc.* 85: 192 (1904).
343. Kahn. Der Morbus addisonii und seine Beziehungen zur Hyperplasie der lymphatischen Apparate und der Thymusdrüse. *Arch. path. Anat.* 200: 399 (1910).
344. ———, R. H. Studien an Paraganglien. *Arch. ges. Physiol.* 147: 445 (1912).
345. ——— Die nach zentraler Reizung zur Störungen des Kohlehydratstoffwechselsführenden Vorgänge. *Ibid.* 169: 326 (1917).
346. Kaiwa, T. and Wada, M. Effect of piqûre upon the blood sugar and the blood pressure of dogs with the adrenals or medullae removed. *Tohoku J. Exptl. Med.* 23: 536 (1934).
347. Kalk, H. Krankheitsbild der paroxysmalen Hypertension. *Verhandl. der. Deut. Ges. inn. Med.* 46: 351 (1934).
348. Kellaway, C. H. and Cowell, S. J. The concentration of the blood and the effects of histamine in adrenal insufficiency. *J. Physiol.* 57: 82 (1922).
349. Kendall, E. C. Adrenal cortex extract. *J. Am. Med. Assoc.* 105: 1486 (1935).
350. Kepinow, L. Surrénales et anaphylaxie. *C. r. soc. biol.* 87: 327 (1922).
351. Kepler, E. J. Diseases of the adrenals. *Arch. Int. Med.* 56: 105 (1935).
352. Kern, H. Der Umbau der Nebennieren im extrauterinen Leben. *Deut. med. Wochschr.* 37: 971 (1911).
353. Kisch, B. Die Funktion des Interrenalgewebes bei Torpedo. *Arch. ges. Physiol.* 219: 426 (1928).
354. Koehlsche, G. A. and Kendall, E. C. Relation of the cortical hormone to nitrogen metabolism in hyperthyroidism. *Am. J. Physiol.* 113: 335 (1935).
355. Kohn, A. Die Nebenniere der Selachier. *Arch. mikr. Anat.* 53: 281 (1899).
356. ——— Bau und Entwicklung der Carotisdrüse. *Ibid.* 56: 81 (1900).
357. ——— Das chromaffine Gewebe. *Ergeb. Anat. und Entwicklungsges.* 12: 235 (1902).
358. ——— Die Paraganglien. *Arch. mikr. Anat.* 62: 263 (1903).
359. ——— Der Bauplan der Keimdrüsen. *Arch. f. Entwicklungsmech.* 47: 95 (1921).
360. Kojima, T. and Saito, S. A comparison of the colorimetric determination of epinephrine with the rabbit intestinal method. *Tohoku J. Exptl. Med.* 10: 528 (1928).
361. Kolde, W. Veränderungen der Nebenniere bei Schwangerschaft und nach Kastration. *Arch. f. Gyn.* 99: 272 (1913).

362. Kolmer, W. Zur vergleichenden Histologie, Zytologie und Entwicklungsgeschichte der Säugernebenniere. *Arch. f. mikr. Anat.* 91: 1 (1918).
363. Kolodny, A. Adrenal virilism in a woman. *J. Am. Med. Ass.* 102: 925 (1934).
364. Kovacs, W. Zur Nebennierenpathologie. *Beitr. path. Anat.* 79: 213 (1928).
365. Krabbe, K. H. The relation of the adrenal to sexual development. *New York Med. J.* 114: 4 (1921).
366. Kraus, E. J. Zur Pathologie des Morbus addisoni. *Beitr. path. Anat.* 78: 283 (1927).
367. Krawkow, N. P. Die Eigenschaften der Blutgefäße isolierten Organe. *Zeit. ges. exper. Med.* 27: 127 (1922).
368. Kroc, R. L. and Martin, S. J. Effect of adrenalectomy on the body weight and oestral cycle of the rat. *Am. J. Physiol.* 108: 438 (1934).
369. Kuriyama, S. The adrenals in relation to carbohydrate metabolism. *J. Biol. Chem.* 34: 269 (1918).
370. Kutz, R. L. A method of assay of adrenal extracts. *Proc. Soc. Exper. Biol.* 29: 91 (1931).
371. La Barre, J. and Houssa, P. Les effets d'adrénaline sur la glycémie des chiens hépatéctomisés. *C. r. soc. biol.* 118: 1217 (1935).
372. Labbé, M., Tinel, J., and Doumer, E. Crises solaires et hypertension paroxystique en rapport avec une tumeur surrénale. *Bull. et mém. soc. méd. de hôp. de Paris.* 46: 982 (1922).
373. Lacassagne, A. and Samssonow, N. Destruction des capsules surrénales par le rayonnement radioactifs. *C. r. soc. biol.* 89: 72 (1923).
374. Laclau, N. C. La croissance des tumeurs chez les rats privés de capsules surrénales. *Ibid.* 106: 146 (1930).
375. Låwen, A. Die Gefäßwirkung von Suprarenin. *Arch. exper. Path.* 51: 415 (1904).
376. Landau, M. "Die Nebennierenrinde." *Jena* (1915).
377. Lang, K. Nebennierenrinde und Muskelchemismus. *Arch. ges. Physiol.* 229: 60 (1931).
378. Langley, J. N. The physiological action of adrenal extracts. *J. Physiol.* 27: 237 (1901).
379. ——— Reaction of cells and nerve-endings to certain poisons. *Ibid.* 33: 374 (1905).
380. Langlois, P. L'alteration fonctionnelle des capsules surrénales. *Arch. de physiol.* 9: 152 (1897).
381. ——— L'identité physiologique des corps surrénaux chez les batraciens et les mammifères. *Ibid.* 10: 104 (1898).
382. ——— Le mécanisme de destruction du principe actif des capsules surrénales. *Ibid.* p. 124.
383. Langlois, J. P. and Garrelon, L. L'apnée adrénalinique. *J. de physiol.* 14: 960 (1912).

384. Lanzillotta, R. Sulla funzione della ghiandola intercarotica. *Arch. di fisiol.* 11:447 (1913).
385. Lascano-Gonzalez, J. M. Les surrénales accessoires chez le rat blancs. *C. r. soc. biol.* 116: 451 (1934).
386. Leloir, L. F. "Suprarrenales y Metabolismo de los Hidratos de Carbono." *Buenos Aires* (1934).
387. ——— and Novelli, A. Titration de l'hormone cortico-surrénale par la résistance à la morphine. *C. r. soc. biol.* 114: 798 (1933).
388. Lescher, F. G. Comparison of pituitary basophilism and the adrogenital syndrome. *Quart. J. Med.* 4: 23 (1935).
389. Levi della Vida, M. Sierri tossici specifici per le capsule surrenali. *Lo Sperimentale* 58: 919 (1904).
390. Levinson, S. A. Adrenal hemorrhages in the new-born. *Am. J. Surg.* 29: 94 (1935).
391. Lewandowsky, M. Die Wirkung des Nebenninerextractes auf die glatten Muskeln. *Arch. Anat. u. Physiol., Physiol. Abt.* p. 360 (1899).
392. Lewin, G. Ueber Morbus Addisonii. *Charité-Annalen.* 17: 536 (1892).
393. Lewis, J. H. The presence of epinephrine in human fetal adrenals. *J. Biol. Chem.* 24: 249 (1916).
394. ———, J. T. Sensibility to intoxication in albino rats after adrenalectomy. *Am. J. Physiol.* 64: 506 (1923).
395. ———, R. W. and Pappenheimer, A. M. The involutional changes of the adrenal during infancy. *J. Med. Res.* 34: 81 (1916).
396. Leydig, F. "Anatomisch-histologische Untersuchungen ueber Fische und Reptilien." *Berlin* (1853).
397. Livingston, A. E. Effect of castration on the endocrine glands of the rabbit. *Am. J. Physiol.* 40: 153 (1916).
398. Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J. Electrolyte balance in adrenalectomized dogs. *J. Exptl. Med.* 57: 775 (1933).
399. ——— ——— and Stahl, J. Rôle of sodium in adrenal insufficiency. *J. Am. Med. Ass.* 104: 2149 (1935).
400. Loeser, A. Die Wirkung der thyreotropen Substanz auf die Nebennieren. *Arch. exptl. Path.* 173: 62 (1933).
401. Loewi, O. and Gettwert, W. Die Folgen der Nebennierenexstirpation. *Arch. ges. Physiol.* 158: 29 (1914).
402. Long, C. N. H. and Lukens, F. D. W. Observations on adrenalectomized depancreatized cats. *Science* 79: 569 (1934).
403. Loos, H. O. and Rittmann, R. Nebennierenrinde und Geschlechtsentwicklung. *Endokrin.* 13: 82 (1933).
404. Lucas, G. H. W. Blood and urine findings in adrenalectomized dogs. *Am. J. Physiol.* 77: 114 (1926).
405. Luckhardt, A. B. and Carlson, A. J. The presence of vasomotor fibers in the vagus to the pulmonary vessels of the amphibian and reptilian lung. *Ibid.* 56: 72 (1921).
406. ——— and Koppányi, T. Hemodynamic action of subcutaneously injected epinephrine. *Ibid.* 81: 436 (1927).

407. Lucien, M. and Parisot, J. "Glandes surrénales et organes chromaffines." *Paris* (1913).
408. Lundsgaard, C. Nebennierenblutungen bei Neugeborenen. *Arch. path. Anat.* 210: 164 (1912).
409. Lutz, B. R. and Wyman, L. C. The chromophil tissue and interrenal bodies of Elasmobranchs. *J. Exptl. Zool.* 47: 295 (1927).
410. Lyon, D. M. Does the reaction to adrenalin obey Weber's law? *J. Pharm. Exptl. Ther.* 21: 229 (1923).
411. McCallum, W. G., Fitcher, T. B., Duff, G. L., and Ellsworth, R. Relation of Cushing's syndrome to the hypophysis. *Bull. Johns Hopkins Hosp.* 56: 350 (1935).
412. McEuen, C. S. and Selye, H. Histologic changes in the adrenals of tumor-bearing rats. *Am. J. Med. Sci.* 189: 423 (1935).
413. MacKay, E. M. and MacKay, L. L. Compensatory hypertrophy of the adrenal. *J. Exptl. Med.* 43: 395 (1926).
414. ——— Enlargement of the adrenal in uremia. *Ibid.* 46: 429 (1927).
415. ——— Susceptibility of adrenalectomized rats to morphine intoxication. *J. Pharm. and Exptl. Ther.* 35: 67 (1929).
416. McKenna, C. M. and Hines, L. E. Paraglioma of the adrenal. *J. of Urol.* 34: 93 (1935).
417. MacKenzie, J. J. Pathological anatomy of the adrenals. *Endocrinol. and Metab.* 2: 257 (1922).
418. McKinley, E. B. and Fisher, N. F. Effects of feeding adrenals to rats. *Am. J. Physiol.* 76: 268 (1926).
419. MacLeod, J. J. R. Control of carbohydrate metabolism. *Bull. Johns Hopkins Hosp.* 54: 79 (1934).
420. MacMahon, H. E. and Zwemer, R. L. Pathological histology of the adrenalectomized cat. *Am. J. Path.* 5: 491 (1929).
421. Mahorner, H. R. Attempts to produce hyperplasia of the thyroid by adrenal injury. *Proc. Soc. Exptl. Biol.* 30: 770 (1933).
422. Maiweg, H. Die Auswertung Adrenalinlösungen. *Biochem. Zeit.* 134: 292 (1923).
423. Mann, F. C. Gastric ulcers following adrenalectomy. *J. Exptl. Med.* 23: 203 (1916).
424. Mann, F. C. and Drips, D. Relation of the adrenals to the pancreas. *Arch. Int. Med.* 16: 681 (1915).
425. ——— and Magath, T. B. Die Wirkungen der totalen Leberexstirpation. *Ergeb. der Physiol.* 23: 212 (1924).
426. Marañón, G. El caso más antiguo conocido de enfermedad de Addison. *El siglo med.* 70: 605 (1922).
427. ——— Calvo Pena, L., and Florit, E. O. Le métabolisme basal dans l'insuffisance surrénale. *Ann. de méd.* 37: 168 (1935).
428. ——— and Collazo, J. A. Die Störungen des Wasser- und Mineralstoffwechsels in der Addisonschen Krankheit. *Klin. Wochschr.* 14: 1107 (1935).

429. **Marañon, G., Sala, P. and Arguelles, G.** Digestive symptoms in Addison's disease. *Endocrinol.* 18: 497 (1934).
430. **Marchand, F.** Accessorische Nebennieren im Ligamentum latum. *Arch. path. Anat.* 92: 11 (1883).
431. **Marchetti, S.** Die Nebennieren. *Ibid.* 172: 472 (1903).
432. **Marine, D.** Calcification of the cat's adrenals. *J. Exptl. Med.* 43: 495 (1926).
433. ——— Studies on the etiology of goiter. *Ann. Int. Med.* 4: 423 (1930).
434. ——— and **Baumann, E. J.** Effects of adrenal insufficiency in rabbits. *Am. J. Physiol.* 57: 135 (1921); 59: 353 (1922).
435. ——— Survival of adrenalectomized cats and attempts to prolong life with solutions containing sodium salts, glucose and glycerol. *Ibid.* 81: 86 (1927).
436. ——— and **Lenhart, C. H.** Effect of adrenalin on normal and thyroidectomized rabbits. *Ibid.* 54: 248 (1920).
437. **Marino-Zucco, F. and Marino-Zucco, S.** Ricerche sul morbo di Addison. *La riforma med.* 1: 759 (1892).
438. **Marshall, E. K. and Davis, D. M.** Influence of the adrenals on the kidneys. *J. Pharm. Exptl. Ther.* 8: 525 (1916).
439. **Martin, S. J.** Effects of adrenalectomy in rats. *Am. J. Physiol.* 100: 180 (1932).
440. ——— and **Maresh, F.** Temperature of adrenalectomized rats. *Ibid.* 105: 273 (1933).
441. **Martinotti, C.** Les alterations des capsules surrénales consécutives à l'occlusion des veines centrales. *Arch. ital. de biol.* 49: 236 (1908).
442. **Marval, L. de.** Sensibilité au froid des rats décapsulés. *C. r. soc. biol.* 95: 1087 (1926).
443. **Masui, K.** Einfluss der Entfernung der Nebennieren auf die weiblichen Genitalorgane bei Mäusen. *Endokrin.* 2: 19 (1928).
444. **Materna, A.** Das Gewicht der Nebennieren. *Zeit. f. Konstitut.* 9: 1 (1924).
445. ——— and **Januschke, E.** Gewicht, Wasser- und Lipoidgehalt der Nebennieren. *Arch. path. Anat.* 263: 537 (1927).
446. **Mayo, C. H.** Paroxysmal hypertension with tumor of retroperitoneal nerve. *J. Am. Med. Ass.* 89: 1047 (1927).
447. **Meckel, J. F.** Beschreibung dreier kopfloser Missgeburten. *Beitr. vergl. Anat.* 1: 136 (1809).
448. ——— "Handbuch des Menschlichen Anatomie." Berlin (1815).
449. **Meio, R. H. de.** Le soufre non-protéique du sang et les relations avec les surrénales. *C. r. soc. biol.* 114: 807 (1933).
450. **Mellanby, J. and Huggett, A. S.** Adrenalin and vagal types of apnoea. *J. Physiol.* 57: 395 (1923).
451. **Meltzer, S. J. and Auer, C. M.** The paradoxical pupil dilatation caused by adrenalin. *Am. J. Physiol.* 11: 28, 37, 40, 449 (1904).
452. ——— and **Meltzer, C.** Effects of adrenalin on the blood vessels of the rabbit's ear. *Ibid.* 9: 252 (1903).

453. Meyer, R. Die subserösen Epithelknötchen an Tuben, Ligamentum latum, Hoden und Nebenhoden. *Arch. path. Anat.* 171: 443 (1903).
454. ———. Nebennieren beim Anenzephalie. *Ibid.* 210: 158 (1912).
455. Mira, F. de. Les extraits cortico-surrénaux. *J. Lisboa méd.* 11: 581 (1934).
456. Mitzkewitsch, M. S. Der Zustand der endokrinen Drüsen während der A-Avitaminose bei Ratten. *Arch. exptl. Path.* 174: 339 (1934).
457. Molinelli, E. A. "La secrezion de adrenalina." *Buenos Aires* (1926).
458. Montgomery, H. and O'Leary, P. A. Pigmentation in Addison's disease, acanthosis, and hemochromatosis. *Arch. Derm. and Syph.* 21: 970 (1930).
459. Moore, B. and Purinton, C. O. Effects of adrenalectomy in goats. *Am. J. Physiol.* 5: 182 (1901).
460. ——— and Vincent, S. Comparative chemistry of the adrenals. *Proc. Roy. Soc. London.* 62: 280 (1897).
461. Muirhead, A. L. An autograph history of a case of Addison's disease. *J. Am. Med. Assoc.* 76: 652 (1921); 79: 556 (1922).
462. Muto, K. Die Wirkung des Adrenalins auf die Schweißsekretion. *Mitt. med. Fak. der. Kais. Univ. zu Tokyo.* 15: 365 (1916).
463. Nachmansohn, D., Wajzer, J., and Lippmann, R. L'effet de l'adrénaline sur le métabolisme du muscle isolé. *C. r. acad. sci. Paris.* 200: 1981 (1935).
464. Nagel, M. Die Struktur der Nebennieren. *Arch. Anat. Physiol. und wiss. Med.* p. 365, 408 (1836).
465. ——— W. "Zur Klinik der Nebennierenerkrankungen. *Deut. Arch. klin. Med.* 177: 600 (1935).
466. Neuberg, C. Enzymatische Umwandlung von Adrenalin. *Biochem. Zeit.* 8: 383 (1908).
467. Neumann, O. Nebennierenrinden-Blastome und Interrenalismus. *Endokrin.* 15: 41 (1934).
468. Neusser, E. Die Erkrankungen der Nebennieren. *Nothnagel's Handb.* 18: 1 (1897).
469. Nice, L. B. and Fishman, D. Effects of emotional excitement on the viscosity of the blood in adrenalectomized animals. *Am. J. Physiol.* 107: 113 (1934).
470. ——— Rock, J. L. and Courtwright, R. O. Influence of adrenalin on respiration. *Ibid.* 34: 326 (1914).
471. Nicholson, W. M. and Soffer, L. J. Cardiac arrhythmia in adrenal insufficiency in dogs. *Bull. Johns Hopkins Hosp.* 56: 236 (1935).
472. Nikolaeff, M. P. Die Wirkung verschiedener Gifte auf die isolierten Nebenniere. *Zeit. exptl. Med.* 42: 213 (1924).
473. Norris, J. C. Adrenal disease in relation to hypoglycemia and death. *Am. J. Clin. Path.* 5: 120 (1935).
474. Nothnagel, H. Untersuchungen ueber die Addison'sche Krankheit. *Zeit. klin. Med.* 1: 77 (1880).
475. Novak, E. and Long, J. H. Ovarian tumors associated with secondary sex changes. *J. Am. Med. Ass.* 101: 1057 (1933).

476. **Novak, J.** Der Einfluss der Nebennierenausschaltung auf das Genitale. *Arch. für Gyn.* 101: 36 (1914).
477. **Oberling, C. and Jung, G.** Paragangliome de la surrénale avec hypertension paroxystique. *Bull. et mém. soc. méd. de hôp. de Paris* 51: 366 (1927).
478. **Oberndorfer, S.** Keimversprengung von Nebennieren in die Leber. *Centralbl. allg. Path.* 11: 145 (1900).
479. **Oikawa, K.** Susceptibility of adrenalectomized rabbits to various drugs. *Tohoku J. Exptl. Med.* 18: 1 (1931).
480. **Oliver, G. and Schaefer, E. A.** Physiological effects of adrenal extracts. *J. Physiol.* 18: 230 (1895).
481. **Omelskyj, E.** Cytotoxische Schrumpfnebenniere bei hypophysärer Kachexie. *Arch. path. Anat.* 271: 377 (1929).
482. **Oppenheim, R. and Loeper, M.** Insuffisance surrénale expérimentale par injections intracapsulaires des poisons du bacille tuberculeux humain et par lésions directes des capsules. *C. r. soc. biol.* 55: 330, 332 (1903).
483. **Oppenheimer, B. S. and Fishberg, A. M.** Association of hypertension with adrenal tumors. *Arch. Int. Med.* 34: 631 (1924).
484. **Orahovats, D. P. and Gotsev, T.** Blutgefäße, Blutdruck und Adrenalin. *Arch. ges. Physiol.* 235: 367 (1935).
485. **Parker, R. L.** Use of a salt-poor diet in the diagnosis of Addison's disease. *Proc. Staff Meetings, Mayo Clinic* 10: 344 (1935).
486. **Parkins, W. M., Taylor, A. R., and Swingle, W. W.** Sodium chloride and blood pressure changes induced by adrenal insufficiency, trauma, and intraperitoneal glucose. *Am. J. Physiol.* 112: 581 (1935).
487. **Pauly, H.** Das Adrenalin. *Ber. deut. chem. Gesell.* 37: 1388 (1904).
488. **Pearlman, I. and Vincent, S.** Function of the chromophil tissues. *Endocrinol.* 3: 121 (1919).
489. **Peiper, H.** Methodik der Exstirpation der Nebennieren. *Abderhalden's Handb. Abt. V. Teil 3 B* p. 149 (1924).
490. **Peiser, B.** Störungen der Adrenalinbildung unter äusserer Einflüssen. *Zeit. ges. exptl. Med.* 27: 234 (1922).
491. **Pencharz, R. I., Olmsted, J. M. D., and Giragossintz, G.** Survival of rats after adrenalectomy. *Physiol. Zool.* 4: 501 (1931).
492. **Pende, N.** "Endocrinologia patologia e clinica degli organi a secrezione interna." *Milano* (1920).
493. ——— Has adrenin any physiological value in the organism? *New York Med. J.* 118: 469 (1923).
494. **Pepper, W.** Congenital sarcoma of the liver and adrenal. *Am. J. Med. Sci.* 121: 287 (1901).
495. **Perla, D.** Hemorrhagic changes in the adrenals after pituitarectomy. *Proc. Soc. Exptl. Biol.* 32: 655 (1935).
496. ——— and **Gottesman, J. M.** Hemolysin formation in adrenalectomized rats. *J. Exptl. Med.*, 47: 723 (1928).
497. ——— Effect of cortin on resistance of adrenalectomized rats to histamine poisoning. *Proc. Soc. Exptl. Biol.* 28: 650 (1931).

498. Pettit, A. Les capsules surrénales. *J. de l'Anat.* 32: 301, 369 (1896).
499. Piffner, J. J. and Swingle, W. W. Revival of cats from adrenal insufficiency with an aqueous extract of the cortex. *Am. J. Physiol.* 96: 180 (1931).
500. ——— and Vars, H. M. Hormone requirement of the adrenalectomized dog. *J. Biol. Chem.* 104: 701 (1934).
501. ——— Vars, H. M., and Taylor, A. R. Extraction studies on the adrenal cortical hormone. *Ibid.* 106: 625, 639, 645 (1934).
502. ——— Wintersteiner, O., and Vars, H. M. Fractionation of adrenal hormone concentrates. *Ibid.* 111: 585 (1935).
503. Philpot, A. Histology of the adrenals in conditions associated with hypertension. *Quart. J. Med.* 3: 34 (1909).
504. Philpott, N. W. Addison's disease and amyloidosis. *Ann. Int. Med.* 1: 613 (1928).
505. Pick, L. Die Marchand'schen Nebennieren und ihre Neoplasmen. *Arch. für Gynäk.* 64: 670 (1901).
506. Pico-Estrada, O. Sensibilité des rats privés de surrénales à l'avitaminose B. *C. r. soc. biol.* 97: 1031 (1927).
507. Pilliet, A. H. Capsule surrénale dans le plexus solaire et sous la capsule du rein. *Bull. soc. anat. Paris* 66: 267 (1891); 68: 478 (1893).
508. Poll, H. Die vergleichende Entwicklungsgeschichte der Nebennierensysteme der Wirbeltiere. *Hertwig's Handb.* 3: 443 (1905).
509. ——— and Sommer, A. Phäochrome Zellen im Centralnervensystem des Blutegeles. *Verhand. physiol. Gesel. Berlin.* p. 77 (1902).
510. Porak, R. L'hypertrophie des surrénales dans les infections. *J. de physiol.* 18: 95, 1194 (1919).
511. Porges, O. Hypoglykämie bei Morbus Addison sowie bei nebennierenlosen Hunden. *Zeit. klin. Med.* 69: 341 (1910).
512. Porter, M. F. and Porter, M. F., Jr. Paroxysmal hypertension cured by removal of an adrenal tumor. *Surg. Gyn. and Obstet.* 50: 160 (1930).
513. Preston, M. I. Effects of thyroxin on the mouse adrenal. *Endocrinol.* 12: 323 (1928).
514. Quinan, C. and Berger, A. A. Weight of human adrenals. *Ann. Int. Med.* 6: 1180 (1933).
515. Rabin, C. B. Chromaffin cell tumor of medulla. *Arch. of Path.* 7: 228 (1929).
516. Rabl, H. Die Entwicklung und Struktur der Nebennieren bei den Vögeln. *Arch. mikr. Anat.* 38: 492 (1891).
517. Reichardt, R. Chromaffiner Tumor des Zuckerkanldischen Organes. *Endokrin.* 14: 180 (1934).
518. Reiss, M. Die Funktion der Nebennierenrinde. *Endokrin.* 6: 321 (1930).
519. Rich, A. R. Relation of adrenals to shock. *Bull. Johns Hopkins Hosp.* 33: 79 (1922).
520. Richardson, E. P. Surgical application of epinephrine. *J. Am. Med. Ass.*, 83: 1587 (1924).
521. Riddle, O. Adrenal hypertrophy coincident with ovulation. *Am. J. Physiol.* 66: 322 (1923).

522. **Rogoff, J. M.** Addison's disease. *J. Am. Med. Ass.* **99**: 1309 (1932).
523. ——— A commercial adrenal extract. *Ibid.* **103**: 1764 (1934).
524. ——— Relation between adrenals and parathyroids. *Science* **80**: 319 (1934).
525. ——— The adrenal medulla. *J. Am. Med. Ass.* **104**: 2088 (1935).
526. ——— and **Dominguez, R.** Effects of adrenalectomy on the blood pressure of the rabbit. *J. Metab. Res.* **6**: 141 (1924).
527. ——— and **Ecker, E. E.** Susceptibility of adrenalectomized rats to tetanus toxin. *Am. J. Physiol.* **80**: 200 (1927).
528. ——— and **Stewart, G. N.** Adrenal insufficiency in dogs. *Ibid.* **78**: 683 (1926).
529. ——— ——— Influence of pregnancy upon the survival of adrenalectomized dogs. *Ibid.* **79**: 509 (1927).
530. ——— ——— Blood studies in adrenal insufficiency. *Ibid.* **78**: 711 (1926); **86**: 25 (1928).
531. ——— ——— Influence of intravenous injections of Ringer's solution on the survival of adrenalectomized dogs. *Ibid.* **84**: 649 (1928).
532. ——— ——— Influence of adrenal extracts on survival of adrenalectomized dogs. *Ibid.* p. 660.
533. ——— ——— Influence of heat on survival of dogs after adrenalectomy. *Ibid.* **86**: 20 (1928).
534. ——— ——— Survival period of adrenalectomized cats. *Ibid.* **88**: 162 (1929).
535. **Rokitansky, C.** "Lehrbuch der pathologischen Anatomie," *Wien* (1861).
536. **Rolleston, H. D.** Lectures on the adrenals. *Brit. Med. J.*, p. 629, 687, 745 (1895).
537. **Rosenblueth, A.** Mode of action of adrenin and its quantitation by biological methods. *Am. J. Physiol.* **101**: 149 (1932).
538. **Rowntree, L. G.** Addison's disease. *J. Am. Med. Ass.* **84**: 327 (1925).
539. ——— and **Ball, R. G.** Diseases of the adrenals. *Endocrinol.* **17**: 263 (1933).
540. ——— and **Snell, A. M.** "A Clinical Study of Addison's Disease," *Philadelphia* (1931).
541. **Rubin, M. I.** and **Krick, E. T.** Effect of adrenalectomy on salt metabolism in rats. *Proc. Soc. Exptl. Biol.* **31**: 228 (1933).
542. **Saito, S.** Effects of hemorrhage, cold, and heat on the epinephrine output. *Tohoku J. Exptl. Med.* **11**: 79, 544 (1928).
543. **Sampayo, A. D., Morales, J. M. L., and Lafuente, A.** Der Zustand des Herzens bei der Nebenniereninsuffizienz. *Endocrinol.* **14**: 22 (1934).
544. **Sandiford, I.** Effect of adrenalin on heat production, blood pressure, and pulse rate in man. *Am. J. Physiol.* **51**: 407 (1920).
545. **Sataké, Y., Sugawara, T., and Watanabé, M.** A method for collecting the blood from the dog's adrenals without fastening, narcotizing, laparotomy or provoking any pain. *Tohoku J. Exptl. Med.* **8**: 501 (1927).
546. ——— ——— Effect of fastening and of sensory stimulation upon the rate of epinephrine output from the adrenal. *Ibid.* **9**: 1 (1927).

547. Sato, H., Ohguri, M., and Wada, M. Epinephrine discharge, blood sugar, and blood pressure in anaphylactic shock of non-anesthetized, non-fasted dogs. *Ibid.* 25: 504 (1935).
548. ——— Satow, Y. and Degti, T. Epinephrine secretion and blood sugar in dogs anesthetized with avertin. *Ibid.* 24: 485 (1934).
549. Schäfer, W. Die Nierenfunktion nach Nebennierexstirpation. *Zeit. ges. expil. Med.* 90: 552 (1933).
550. Schafer, E. S. "The Endocrine Organs." London (1924).
551. ——— and Lim, R. K. S. Effects of adrenalin on the pulmonary circulation. *Quart. J. Exptl. Physiol.* 12: 157 (1920).
552. Schaffner, P. M. and Howard, T. Addison's disease of syphilitic origin. *New York Med. J.* 103: 1026 (1916).
553. Schmalfuss, H., Spitzer, K., and Brandes, H.: Kolorimetrische Bestimmung von o-dioxybenzolen. *Biochem. Zeit.* 189: 226 (1927).
554. Schmidt, H. Der suprarenal-genitale Syndrom. *Arch. path. Anat.* 251: 8 (1924).
555. Schmitz, E. and Kühnau, J. Die innere Sekretion der Nebennierenrinde. *Biochem Zeit.* 259: 301 (1933).
556. Schmorl, S. Die akzessorischen Nebennieren. *Beit. path. Anat.* 9: 523 (1891).
557. Schuler, W. and Weidemann, A. Die Adrenalinsynthese im Reagenzglas unter physiologischen Bedingungen. *Zeit. physiol. Chem.* 233: 235 (1935).
558. Schultz, P. Mortality of adrenalectomized rats. *J. Physiol.* 84: 70 (1935).
559. Schwartz, O. Stoffwechselstörungen nach Exstirpation der Nebennieren. *Arch. ges. Physiol.* 134: 259 (1910).
560. Schwarz, H. Einwirkung des Adrenalins auf dem Kaliumgehalt des Blutserums. *Arch. expil. Path.* 177: 628 (1935).
561. Scott, W. J. M. Effect of adrenalectomy in cats on the respiratory exchange. *J. Exptl. Med.* 36: 199 (1922).
562. ——— Influence of the adrenals on resistance. *Ibid.* 38: 543 (1923); 39: 457 (1924).
563. ———, Bradford, W. L., Hartman, F. A., and McCoy, O. R. Effect of adrenal cortical hormone on resistance to infections and intoxications. *Endocrinol.* 17: 529 (1933).
564. Scoville, W. L. Colorimetric estimation of adrenalin. *J. Ind. Eng. Chem.* 12: 769 (1920).
565. Sézary, A. Syphilis et glandes surrénales. *Gaz. de hôp.* 87: 1317 (1914).
566. Shipley, A. M. Paroxysmal hypertension associated with a tumor of the adrenal. *Ann. Surg.* 90: 742 (1929).
- 566a. Shumacker, H. B. A history of studies on the adrenal before Addison. *Bull. Inst. History Med.* 4: 39 (1936).
567. ——— and Firor, W. M. Interrelation of the adrenal and the hypophysis. *Endocrinol.* 18: 676 (1934).
568. Silberstein, F., Wachstein, M. and Gottdenker, F. Stoffwechselstudien an epinephrektomierten Katzen. *Zeit. ges. expil. Med.* 81: 133 (1932).

569. Silvette, H. Effects of adrenal extract on glycolysis in vitro. *Am. J. Physiol.* **102**: 693 (1932).
570. ——— and Britton, S. W. Blood chemistry changes in adrenalectomy. *Ibid.* **111**: 305 (1935).
571. Simmonds, M. Kompensatorische Hypertrophie der Nebenniere. *Arch. path. Anat.* **153**: 138 (1898).
572. ——— Nebennierenblutungen. *Ibid.* **170**: 242 (1902).
573. ——— Nebennierenschrumpfungen bei Morbus Addisonii. *Ibid.* **172**: 480 (1903).
574. Simpson, S. L. Clinical and pathological aspects of the adrenals. *Proc. Roy. Soc. Med.* **27**: 383 (1934).
575. ——— Kohn, A. S., and Korenchevsky, V. Adrenal cortex and sex. *Lancet* **1**: 1194 (1933).
576. Singer, E. and Zwemer, R. L. Microscopic observations of the adrenals of the living frog. *Anat. Rec.* **60**: 183 (1934).
577. Sisson, E. D. and March, B. Survival of rats following adrenalectomy. *Endocrinol.* **19**: 389 (1935).
578. Sjöstrand, T. Capillary circulation of the blood in the adrenal of mice under physiological conditions and the influence of drugs. *Skand. Arch. Physiol.* **71**: 85 (1934).
579. Snell, A. M. The changing relations of tuberculosis and Addison's disease. *Proc. Staff Meet. Mayo Clinic* **10**: 337 (1935).
580. Soddu, L. Intorno agli effetti della estirpazione delle capsule surrenali nel cane. *Lo Sperimentale* p. 87 (1898).
581. Soulié, A. H. Le développement des capsules surrénales chez les vertébrés supérieurs. *J. de l'Anat.* **39**: 197, 390, 492, 634 (1903).
582. Squier, T. L. and Grabfield, G. P. Adrenal enlargement in rabbits. *Endocrinol.* **6**: 85 (1922).
583. Srdinko, O. V. Bau und Entwicklung der Stannius'schen Körperchen der Lophobranchier. *Arch. mikr. Anat.* **62**: 773 (1903).
584. Stannius, H. Die Nebennieren bei Knochenfischen. *Arch. für Anat., Physiol. und wiss. Med.* p. 97 (1839).
585. Star, P. Unusual case of Addison's disease with sudden death. *Lancet* **1**: 284 (1895).
586. Stella, G. Action of adrenalin on the cardiac vagus centres. *J. Physiol.* **77**: 68 (1932).
587. Stewart, G. N. The adrenals in relation to the vital processes. *Endocrinol. and Met.* **2**: 127 (1922).
588. ——— The adrenals. *Arch. Int. Med.* **43**: 733 (1929).
589. ——— and Rogoff, J. M. Demonstration that the spontaneously liberated epinephrine can exert an action on the heart. *J. Pharm. Exptl. Ther.* **13**: 397 (1919).
590. ——— Action of drugs upon the output of epinephrine from the adrenals. *Ibid.* **13**: 95, 167, 183, 361 (1919); **16**: 71 (1920); **17**: 227 (1921).

591. Stewart, G. N. and Rogoff, J. M. Effect of stimulation of afferent nerves upon the rate of liberation of epinephrine from the adrenals. *Am. J. Physiol.* **69**: 605 (1924).
592. ——— Influence of adrenal extracts on survival of adrenalectomized cats and dogs. *Ibid.* **91**: 254 (1929).
593. Stewart, H. A. Relations between lipid substances and the adrenals. *Int. Congress Med.* **17**: 173 (1912).
594. Stilling, H. Die kompensatorische Hypertrophie der Nebennieren. *Arch. path. Anat.* **118**: 569 (1889).
595. ——— Zur Anatomie der Nebenniere. *Arch. mikr. Anat.* **52**: 176 (1898).
596. ——— Die chromaphilen Zellen des Sympathicus. *Anat. Anz.* **15**: 229 (1898).
597. ——— Die Transplantation von Nebennierengewebe. *Beitr. path. Anat.* **37**: 480 (1905).
598. Stoerk, O. Die Chromreaktion der Glandula cecygea. *Arch. mikr. Anat.* **69**: 322 (1907).
599. Stolz, F. Adrenalin und Alkylaminoacetobrenzcatechin. *Ber. deut. chem. Gesell.* **37**: 4149 (1904).
600. Strehl, H. and Weiss, O. Zur Physiologie der Nebenniere. *Arch. ges. Physiol.* **86**: 107 (1901).
601. Sugawara, T. Comparative assays of epinephrine. *Tohoku J. Exptl. Med.* **11**: 410 (1928).
602. ——— Einfluss des β -tetrahydronaphtylamins auf die Epinephrinabgabe der Nebennieren. *Ibid.* **9**: 368 (1927).
603. ———, Saito, S. and Nemoto, M. Effect of adrenalin on the epinephrine output. *Ibid.* p. 149.
604. ——— and Tada, H. Effect of guanidine upon the epinephrine output and blood sugar. *Ibid.* p. 295.
605. Sun, T. P. Effect of thyroid deficiency on the rat's adrenal. *J. of Morph.* **48**: 45 (1929).
606. Sundberg, G. A. "Studien über die Blutzuckerregulation beim epinephrektomierten Tieren. *Stockholm* (1925).
607. Svirbely, J. L. Vitamin C content of adrenals and liver. *Biochem. J.* **27**: 960 (1933).
608. Swanson, E. E. Action of epinephrine on the bronchioles. *J. Pharm. Exptl. Ther.* **36**: 541 (1929).
609. Swingle, W. W. Blood changes after adrenalectomy in cats. *Am. J. Physiol.* **79**: 666 (1927).
610. ——— and Eisenman, A. J. Acid-base equilibrium of adrenalectomized cats. *Ibid.* p. 679.
611. ——— and Parkins, W. M. Effects of trauma on dogs with and without adrenals. *Ibid.* **111**: 426 (1935).
612. ——— and Piffner, J. J. Preparation and properties of the cortical hormone. *Ibid.* **98**: 144 (1931).
613. ——— The adrenal cortical hormone. *Medicine* **11**: 371 (1932).

614. Swingle, W. W., Pflüger, J. J., Vars, H. M. and Parkins, W. M. Effect of hemorrhage on the normal and adrenalectomized dog. *Am. J. Physiol.* 107: 259 (1934).
615. Szent-Györgyi, A. L'acide ascorbique. *Bull. soc. chim. biol.* 15: 694 (1933).
616. Szymonowicz, L. Die Funktion der Nebennieren. *Arch. ges. Physiol.* 64: 97 (1896).
617. Tachi, H. and Hirayama, S. Urethanhyperglykämie beim nebennierenlosen Kaninchen. *Tohoku J. Exptl. Med.* 10: 191, 307 (1928).
618. Takamine, J. Adrenalin, the active principle of the adrenals. *Am. J. Pharm.* 73: 523 (1901).
619. Takáts, G. and Cuthbert, F. P. Effect of adrenal denervation on the sugar tolerance. *Arch. of Surg.* 30: 151 (1935).
620. Také, N. M. and Marine, D. Effect of adrenalectomy on hemolysin formation. *J. Infect. Dis.* 33: 217 (1923).
621. Takéda, Y. Sur l'érythroïse surrénale expérimentale par injection de lécithine. *C. r. soc. biol.* 116: 1164 (1934).
622. Tamura, Y. Changes in the adrenal of the mouse during pregnancy. *Brit. J. Exptl. Biol.* 4: 81 (1926).
623. Targett, J. H. Accessory adrenals in broad ligaments. *Trans. Obstet. Soc. London* 39: 157 (1898).
624. Tatum, A. L. PicROTOXIN hyperglycemia. *J. Pharm. Exptl. Ther.* 20: 385 (1922).
625. Taylor, N. B. and Caven, W. R. Serum calcium after adrenalectomy. *Am. J. Physiol.* 81: 511 (1927).
626. Thomas E. Die Nebenniere des Kindes und ihre Veränderungen. *Ziegler's Beitr. zur path. Anat.* 50: 283 (1911).
627. Tiffeneau, M. Nécessité du contrôle physiologique de l'adrénaline et des préparations de surrénals. *J. de pharm. et de chim.* 23: 313, 366 (1921).
628. Tizzoni, G. Ablation des capsules surrénals chez le chien. *Arch. ital. de biol.* 10: 372 (1888).
629. Torino, A. and Lewis, J. T. Morphin intoxication in adrenalectomized rats. *Am. J. Physiol.* 81: 405 (1927).
630. Tournade, A. L'adrénalino-sécrétion paralytique se renforce-t-elle à l'occasion d'une hypotension artérielle? *C. r. soc. biol.* 106: 642 (1931).
631. ——— and Chabrol, M. L'adrénalinémie. *Rev. de méd.* 40: 222 (1923).
632. ——— ——— L'hyperadrénalinémie par excitation du nerf splanchnique. *C. r. soc. biol.* 91: 176 (1924).
633. ——— ——— La vaso-constriction observée au cours de l'asphyxie. *Ibid.* p. 873.
634. ——— ——— Réalité de l'adrénalinémie physiologique. *Ibid.* 92: 587 (1925).
635. ——— ——— L'adrénalino-sécrétion après la injection d'adrénaline. *Ibid.* 94: 535 (1926).

636. **Trendelenburg, P.** Bestimmung des Adrenalingehaltes des Blutes. *Arch. exptl. Path.* **63**: 161 (1910).
637. ——— Die Adrenalinsekretion unter normalen und gestörter Bedingungen. *Ergeb. Physiol.* **21**: 500 (1923).
638. ——— Adrenalin und verwandte Substanzen. *Heffter's Handb.* **2**: 1130 (1924).
639. ——— "Die Hormone." *Berlin* (1929).
640. **Trousseau.** Maladie bronzée d'Addison. *Arch. gén. de méd.* **8**: 478 (1856).
641. **Tsuji, K.** Etiology of exophthalmic goitre. *Acta scholae med. Kioto* **5**: 329 (1923).
642. **Ulrich, A.** Accessorische Nebennieren und Nierengeschwülste. *Beitr. path. Anat.* **18**: 589 (1895).
643. **Verdozzi, C.** Capsules surrénales et allaitement. *Arch. ital. biol.* **66**: 121 (1917).
644. **Viale, G.** La funzione della ghiandola surrenale. *Rivista di Biol.* **10**: 99 (1928).
645. **Vincent, S.** Adrenals of fishes and their relation to the head kidney. *Trans. Zool. Soc.* **14**: 44 (1895).
646. ——— The comparative physiology of the adrenals. *Proc. Roy. Soc. London* **62**: 280 (1897); **64**: 176 (1897).
647. ——— Effect of extirpating the adrenals of the eel. *J. Physiol.* **22**: 48 (1898).
648. ——— The chromophil tissues and the adrenal medulla. *Proc. Roy. Soc. London. B* **82**: 502 (1910).
649. ——— "Internal Secretion and the Ductless Glands." *London* (1924).
650. **Virchow, R.** Zur Chemie der Nebennieren. *Arch. path. Anat.* **12**: 481 (1857).
651. **Vosburgh, C. H. and Richards, A. N.** Sugar content and coagulation of the blood after adrenalin administration. *Am. J. Physiol.* **9**: 35 (1903).
652. **Vulpian, A.** Quelques réactions propres à la substance des capsules surrénales. *C. r. acad. sci. Paris* **43**: 663 (1856).
653. **Wacker, L. and Hueck, W.** Die Bedeutung des Cholesterins im Organismus. *Arch. exptl. Path.* **71**: 373 (1913); **74**: 416 (1913).
654. **Wakefield, E. G.** Serum sulphates in Addison's disease. *Ann. Int. Med.* **3**: 662 (1930).
655. **Walters, W., Wilder, R. M., and Kepler, E. J.** The adrenogenital syndrome with presentation of 10 cases. *Ann. of Surg.* **100**: 670 (1934).
656. **Warthin, A. S.** Accessory adrenal in the broad ligament. *Am. J. Obstet.* **42**: 797 (1900).
657. **Watanabé, M.** Effect of strychnine and peptone poisoning upon the epinephrine output. *Tohoku J. Exptl. Med.* **9**: 251, 412 (1927).
658. ——— Effect of tetanus toxin, diphtheria and drugs of the caffeine group upon epinephrine secretion. *Ibid.* **10**: 26, 29, 177 (1928).
659. ——— and **Sato, H.** Die kolorimetrischen und biologischen Auswertungen des Epinephringehaltes der Nebennieren. *Ibid.* **11**: 433 (1928).

660. Watrin, J. L'hypertrophie des capsules surrénales chez la lapine gestante. *C. r. soc. biol.* 82: 1405 (1919).
661. Wearn, J. T. Pharmacology of epinephrine. *J. Am. Med. Ass.* 83: 1508 (1924).
662. Weber, F. P. Cortical tumors of the adrenals. *Brit. J. Dermat. and Syph.* 38: 1 (1926).
663. Webster, B., Piffner, J. J., and Swingle, W. W. Effects of adrenal cortical hormone on the respiratory metabolism. *Am. J. Physiol.* 99: 710 (1932).
664. Weinstein, A. and Marlow, A. Use of adrenal cortex in the treatment of thyroid disorders. *Bull. Johns Hopkins Hosp.* 52: 408 (1933).
665. Weiskotten, H. G. Fatal burns and the adrenals. *J. Am. Med. Ass.* 69: 776 (1917).
666. Welch, A. D. Oxidation and Stabilization of epinephrine. *Am. J. Physiol.* 108: 360 (1934).
667. Weldon, W. F. R. The origin of the adrenals of *Bdellostoma*. *Quart. J. Micr. Soc.* 24: 171 (1884).
668. Wells, H. G. Addison's disease with selective destruction of the cortex. *Arch. of Path.* 10: 499 (1930).
669. Weymann, M. F. Development of function in the adrenal medulla of pig embryos. *Anat. Rec.* 24: 299 (1922).
670. Wheeler, T. D. and Vincent, S. The relative importance to life of the cortex and medulla. *Trans. Roy. Soc. Canada* 11: 125 (1917).
671. Whitehead, R. Variations in structure and lipid of the mouse adrenal. *Brit. J. Exptl. Path.* 12: 305 (1931).
672. ———. Involution of the transitory cortex of the mouse adrenal. *J. of Anat.* 67: 387 (1933).
673. ———. Involution of permanent cortex of rabbit by fatty change. *J. Path. and Bact.* 41: 305 (1935).
674. ———. R. H. Histogenesis of the adrenal in the pig. *Am. J. Anat.* 2: 349 (1903).
675. Whitehorn, J. C. A method for estimating epinephrine in the blood. *J. Biol. Chem.* 108: 633 (1935).
676. Wiesel, J. Accessorische Nebennieren am Nebenhoden. *Sitz. der Akad. der Wiss. in Wien.-Math. Natur. Kl.* 108: 257 (1899).
677. ———. Anatomie und Entwicklung der menschlichen Nebenniere. *Anat. Hefte. Erste. Abt.* 19: 481 (1902).
678. ———. Pathologischen Anatomie der Addison'schen Krankheit. *Zeit. f. Heilk.* 24: 257 (1903).
679. ———. Zur Pathologie des chromaffinen Systems. *Arch. path. Anat.* 176: 103 (1904).
680. Wiggers, C. J. Action of adrenalin on pulmonary vessels. *J. Pharm. Exptl. Ther.* 1: 341 (1909).
681. Wilks, S. Morbus Addisonii. *Guy's Hosp. Rep.* 23: 1 (1862); 26: 23 (1865).

682. Wintersteiner, O. and Piffner, J. J. Isolation of several physiologically inactive crystalline compounds from active adrenal cortical extracts. *J. Biol. Chem.* **111**: 599 (1935).
683. Wislocki, G. B. Modification of the chromaffin reaction. *Bull. Johns Hopkins Hosp.* **33**: 359 (1922).
684. ——— and Crowe, S. J. The abdominal chromaffin body in dogs. *Ibid.* p. 377.
685. ——— The adrenals and the chromaffin system. *Ibid.* **35**: 187 (1924).
686. Wolfram, J. and Zwemer, R. L. Cortin protection against anaphylactic shock. *J. Exptl. Med.* **61**: 9 (1935).
687. Wright, S. Action of adrenaline on the respiration. *J. Physiol.* **69**: 493 (1930).
688. Wyman, L. C. Effect of adrenal insufficiency on reproduction and the oestrus cycle of the rat. *Am. J. Physiol.* **86**: 528 (1928).
689. ——— Studies on adrenal insufficiency. *Ibid.* **87**: 29 (1928); **89**: 356 (1929).
690. ——— and Suden, C. T. Adrenal insufficiency in rats. *Ibid.* **94**: 579 (1930).
691. ——— Response to histamine of normal and adrenalectomized rats. *Ibid.* **99**: 285 (1932).
692. ——— Growth of transplanted cortical tissue in the rat. *Ibid.* **101**: 662 (1932).
693. ——— and Walker, B. S. Blood changes in adrenalectomized rats. *Ibid.* **89**: 215, 349 (1929).
694. Yasukawa, Y. Die Nebenniereninvolution. *Endokrinol.* **14**: 161 (1934).
695. Zalesky, M. Seasonal changes in the adrenal of the ground-squirrel. *Anat. Rec.* **60**: 291 (1934).
696. Zander, R. Functionelle und genetische Beziehungen der Nebennieren zum Grosshirn. *Beitr. path. Anat.* **7**: 439 (1890).
697. Zuckerkandl, E. Nebenorgane des Sympathicus im Retroperitonealraum des Menschen. *Anat. Anz. Ergänzungsheft* **19**: 95 (1901).
698. Zueltzer, G. Das Nebennierendiabetes. *Berl. klin. Wochschr.* **38**: 1209 (1901).
699. Zunz, E., Vesselovsky, O. and Iagnov, S. L'action de l'adrénaline sur la diurèse. *Arch. int. pharmacodyn.* **46**: 315 (1933).
700. Zwarenstein, H. and Schrire, I. The adrenal of *Xenopus laevis*. *Proc. Roy. Soc. Edinburgh.* **52**: 323 (1932).
701. Zwemer, R. L. An adrenal cortex extract. *Endocrinol.* **15**: 382 (1931).
702. ——— The adrenal cortex and electrolyte metabolism. *Ibid.* **18**: 161 (1934).
703. ——— and Lyons, C. Leucocyte changes after adrenalectomy. *Am. J. Physiol.* **86**: 545 (1928).
704. ——— and Sullivan, R. C. Blood chemistry of adrenal insufficiency in cats. *Endocrinol.* **18**: 97 (1934).

INDEX

- Abscess, 320
- Accessory, androgenic tissue, 58
 - bodies, 32
 - rôle of, after adrenalectomy, 157
- Acetonitrile, 267
- Acetylcholine, 97, 291, 296
 - effect of, on blood pressure, 89
 - on epinephrine secretion, 103
- Acid-base equilibrium, 187
- Acromegaly, 210
- Addison, 5, 299
- Addison's disease, 299
 - clinical course, 301, 312
 - diagnosis, 322
 - etiology, 300
 - incidence, 300
 - latent, 323
 - life expectancy in, 312
 - pathology of, 313
 - symptomatology, 302
 - treatment of, 324, 368
- Adenocarcinoma, 337
- Adenoma, 337
- Adrenal, anatomy of, 12
 - discovery of, 1
 - diseases of, 297
 - nomenclature, 2
 - synonyms for, 1
 - weights, 19, 23
 - see* specific items
- Adrenalectomy, effects of, 161, 172, 179, 205, 227, 267
 - history of, 141
 - survival following, 143
 - technique for, 149
 - in rats, 252
- Adrenalin, *see* epinephrine
- Adrenalitis, 336
- Adrenalone, 83
- Adreno-genital syndrome, 223, 339
 - juvenile form, 342
- Adrenotropic hormone, 218
- Agglutinins, 268
- Aldehydase, 296
- Amyloidosis, 319
- Analeptic, epinephrine as, 134
- Anaphylaxis, 268
- Anatomy, comparative, 12
 - gross, 9
 - microscopic, 37
- Androgenic tissue, 32, 56, 281, 341, 368
 - accessory, 59
 - in man, 58
 - in mice, 63
 - in other species, 64
 - involution of, 60
 - pathology of, 339
- Androtin, 228
- Anemia, 290
- Anencephaly, 357
- Anesthesia, effect of, on epinephrine, 99
 - epinephrine in, 134, 136
 - in Addison's disease, 326
 - for adrenalectomy, 144
- Anomalies, of adrenals, 359
- Anorexia, 163, 307
- Ant-eater, 39, 69
- Antibodies, 268
- Antidote, for epinephrine, 134
- Apes, anthropoid, 39, 69
- Apnea, epinephrine, 122
- Apocodeine, 118
- Arecoline, 103
- Armadillo, 39, 47, 69
- Arrhenoblastoma, 347
- Arsenic, 279
- Arterenol, 86

- Arteries, adrenal, 24
 effect of epinephrine on, 119
Ascorbic acid, 81, 293, 305
Asphyxia, 99
Assay, of cortical hormone, 248
 of epinephrine, 80, 88
Asthenia, 163, 302
Asthma, 135, 357
Atrophy, 310, 318
Atropin, 103, 134, 197
Auto-assay method, 90
Avitaminosis, 292, 295
- Bacterial injections, 279
Base, of blood, 310
Basophilic adenoma, 217, 223, 353
Bat, 43, 53
Bdellostoma myxinidae, 13
Bear, 47
Beef, *see* cow
Bensley-Helly fixative, 37
Bible, 1
Bicarbonate, 187, 310
Bichromate reaction, 71
Bile, 128
 ducts, 126
Biot respiration, 312
Birds, 16, 38
 adrenalectomy in, 150
Bladder, 126
Blood, in Addison's disease, 310
 in insufficiency, 166, 172
 chemistry, 179
 pressure, in Addison's disease, 309
 effect of epinephrine on, 117, 121
 in insufficiency, 166
 method for assay of epinephrine, 88
 sugar, *see* glucose
 supply, *see* circulation
 vessels, 177
 volume, 167
Body temperature, 164, 311
Bos taurus, 21
Boundary-zone, 57
Bronchioles, 123
- Bufo aqua*, 74
Burns, 54, 105, 364
- Caffeine, 102, 116
Calcification, 323
Calcium, 185, 223
Camelopardalis, 43
Camphor, 101, 102
Cancer, 56, 357, 366
Canis familiaris, *see* dog
Cannaubic acid, 290
Capillaries, action of epinephrine on, 120
Capsule, of adrenal, 23
Carbohydrate metabolism, 131, 197
 effect of, cortical hormone on, 203
 epinephrine, 198
 in insufficiency, 203
 utilization, 130
Carcinoma, 328, 337
Cardiac output, effect of epinephrine on, 120, 121
 in insufficiency, 166
Cardio-vascular disease, 290
Carotene, 292, 296
Carotid gland, 27
 epinephrine content of, 74
 exclusion of, 101
Castration, effect of, on adrenals, 226
 on survival after adrenalectomy, 148
Cat, adrenals of, 21, 28, 32, 47
 circulation in, 25
 adrenalectomy in, 156
Cauterization, 279
Cavia cobaya, *see* guinea-pig
Cebus, 43
Center, for epinephrine, 97
Cevitamic acid, *see* ascorbic acid
Charcoal, as adsorbant for cortical hormone, 234
 hormone preparation, 234
 activity of, 239
 dosage of, 243
 elution of hormone from, 240

- Chelonia*, 16
Chicken, embryology of, 30
 see bird
Chimpanzee, 39
Chiroptera, 43
Chloral, 116
Chloreton, 113
Chlorides, blood, in Addison's disease, 193, 310
 in cortical insufficiency, 187
Chloroform, 55, 101, 265, 279
Cholesterol, 54, 228
 blood, 289
 in insufficiency, 179
Choline, *see* acetylcholine
Chromaffinoma, 333
Chromaphil bodies, 11, 26, 48, 54
 abdominal, 28
 accessory, 28
 diseases of, 297
 staining of, 27
 tumors of, 330
Chromophiloblast, 330
Chronic insufficiency, 279
Circulation, of adrenals, 24, 94, 111
 in Addison's disease, 308
 after adrenalectomy, 166
 effect of epinephrine on, 116
Coagulation, of blood, 133
Coccygeal body, 27
Cold, effect of, on epinephrine secretion, 100
 in cortical insufficiency, 168
Conductivity, of blood, 188
Coniine, 103
Connective tissue, 40, 55
Coronary arteries, 120
Cortex, extracts of, 231
 function of, 139
 injection of, 232
 oral administration of, 232
Cortin, 233
Cow, 21, 33, 38
Creatinine, 182
Cricetus, 53
Curare, 48, 103, 267
Cushing's syndrome, 217, 223, 353
Cuttle fish, 86
Cyclostomata, 12
Cysteine, 80
Cystisine, 103
Cytology, 47
Dasyus, *see* armadillo
Decerebration, 198, 274
Degeneration, of adrenal cells, 55
Dementia praecox, 54
Diabetes, 54, 357
 adrenalectomy in, 199
Diagnosis, of Addison's disease, 322
 by use of epinephrine, 136
Diarrhea, 163, 307
Diastase, 296
Diet, for assay, 256
Digestive disturbances, 306
Dioxyindole, 85
Diphtheria, 54, 55, 279, 365
 toxin, 105, 265, 267
 for assay of cortical hormone, 249
Dipnoi fishes, 15
Diuresis, 128, 182
Dog, adrenals of, 21, 28, 35
 circulation in, 25
 adrenalectomy in, 154
 assay method, 249
Dogfish, 29
Dopa, methyl ether of, 83
 reaction, 85
Dopase, 85
Duckbill, 39
Echidna aculeata, 39
Echinococcus cyst, 320
Elasmobranchs, interrenals of, 13, 28, 29
 extracts of, 14
 interrenalectomy in, 14, 149
Electrocardiogram, in Addison's disease, 308
 in cortical insufficiency, 166
Electrolytes, of blood, 191, 192
Elephant, 39, 47, 69

- Embolism, 320, 360
Embryology, of adrenals, 28
 in amphibia, 30
 in birds, 30
 in mammals, 31
Emergency function, of adrenals, 109
Emu, 47
Endocrines, 209
Enzymes, 296
Ephedrine, 86
Epilepsy, 54
Epinephrine, in Addison's disease, 325
 administration of, 134
 adsorption of, 82
 analysis of, 80
 antidote for, 134
 assay of, 87
 benzoyl derivative of, 80
 borate, 80
 center, 97
 clinical significance of, 298
 content, of adrenals, 73, 105, 106
 of blood, 95, 105
 of extra-medullary tissues, 74
 of extracts, 232
 diagnostic use of, 136
 fate of, in body, 138
 function of, 106
 in histamine poisoning, 268
 optical activity of, 76
 origin of, 82
 oxidation-reduction system, 80
 parasympathetic action of, 114
 pharmacology of, 113
 physical properties of, 79
 physiology of, 87
 piqûre, 97
 preparation of, 77
 related compounds, 86
 secretion, rate of, 94
 control of, 96
 factors affecting, 98, 101, 104
 stability of, 79
 structural formula of, 76
 sympathomimetic action of, 113
 synthesis of, 76
Equus caballus, 43, 53
Erinaceus, 43, 53
Erythrocytes, 183
Estrus, effect of epinephrine on, 225
 effect of, on survival after adrenal-ectomy, 147
Ether, 101, 144
Exercise, muscular, 130
Extracts, cortical adrenal, 231
 charcoal, 234
 dosage of, 243
 effects of, on carbohydrate metabolism, 203
 in normal animals, 367
 on reproductive system, 228
 efficacy of, 242
 glandular sources of, 235
 glycerine, 233
 mode of administration of, 245
 for parenteral use, 240
 preparation of, 234
 see Hormone
Eye, excised, 90
 effect of epinephrine on, 124

Fasciculata, 40
Fatty degeneration, 319
Felis catus, see cat
Femmes à barbe, 353
Fetal cortex, 57
Fever, 100, 105
Fibroma, 338
Fibroxanthosarcoma, 338
Fishes, elasmobranch, 14, 29
 interrenalectomy in, 149
 selachian, 15
 teleost, 15
Fixative, 37
Flying-fox, 39
Folin-Denis reagent, 81
Freezing-point, of blood, 188
Frog, adrenal of, 15, 17, 48
 adrenalectomy in, 149
 grafts in, 283
Function, of adrenals, 3
 of epinephrine, 106
 of interrenal tissue, 138

- Gall bladder, 126
Ganglioneuroma, 332
Gastro-intestinal disturbances, 163, 173
Gelsemin, 103
Giraffe, 43
Glands, feeding of fresh, 231
 source of, 235
Glaucoma, 356, 366, 367
Glioma, 331
Glomerulosa, 40
Glucose, blood, in Addison's disease, 192, 310
 in experimental insufficiency, 203
 injections, intraperitoneally, 189
 tolerance, 311
Glutathione, 80, 287
Glycogen, effect of epinephrine on, 131
 in insufficiency, 203
 liver, 197
 mobilization of, 200
 muscle, 199
Glycogenolysis, 197
Goat, 157
Golgi apparatus, 48
Goose-flesh, 127
Grafts, 281, 327
 in Addison's disease, 282, 284
Graves' disease, 207, 220
Growth, in cortical insufficiency, 213
 effect of extracts on, 215
Guanidine, 102
Guinea-pig, 19, 28, 39, 44, 47, 55, 226
 adrenalectomy in, 154
 grafts in, 283
Hagfish, 13
Hamster, 53
Heart, in Addison's disease, 308, 320
 block, 117
 denervated, 92
 effect of epinephrine on, 116
 electrocardiogram of, 166, 308
 injection of, 138
 in experimental insufficiency, 177
 output of, 166, 120
Heat, 100, 168, 364
Hedgehog, 43, 53, 129
Hemangioma, 338
Hemicephaly, 62, 358
Hemolysins, 268
Hemorrhage, 55, 173, 290, 320, 359
 effect of, on epinephrine secretion, 100
Hen, *see* bird
Hermaphroditism, 59, 351
Herpestes mungo, 127
Hibernation, 129
Hirsutism, 348
Hirudo, 12
Histamine, 104, 115, 267, 274, 296
 as antagonist to epinephrine, 268
 for assay of cortical hormone, 249
Histology, 37
History, 1
Homoarterenol, 86
Hormone, cortical, assay of, 248
 chemistry of, 245
 crystallization of, 245
 effect of, on blood sugar, 203
 on glycogen, 200
 on metabolism, 208
 on normal animals, 245, 367
 on reproductive system, 339
 excretion of, 245
 inactivation of, 247
 in infections, 269
 oral administration of, 260
 solubility of, 246
 see extracts
Horse, 43, 53
Hutchison's tumor, 332
Hydrastinin, 103
Hydrocephalus, 62, 358
Hyperfunction, of adrenals, 361
Hyperglycemia, 136, 198
 emotional, 202
Hypernephroma, 336
Hyperplasia, of adrenals, 336, 362
Hypertension, 109, 362
 intermittent, 334
Hyperthyroidism, 207, 220
Hypoglycemia, 136, 198

- Hypophysectomy, cortical hormone
 after, 211
 effects of, 202, 210
Hypophysis, in Addison's disease,
 210, 321
 extracts of, 210
 implants of, 227
 in insufficiency, 173, 209
 relation of, to adrenals, 209
Hypoplasia, of adrenals, 363
Hypotension, 308
 postural, 309
Hystrix cristata, 39, 69
- Ileus, 357
Immunity, 265
Inanition, 265, 290
Infantilism, 218
Infarction, 360
Infections, 264, 265, 364, 367
 cortical hormone in, 269
 epinephrine in, 105
 wound, 266
Influenza, 300, 365
Insufficiency, cortical, chronic, 212
 experimental, 161
Insulin, 101, 173, 201, 202, 229
Interrenal, 11, 31
 accessory, 32, 35
Intestines, in Addison's disease, 320
 for assay of epinephrine, 88
 in cortical insufficiency, 173
 effects of epinephrine on, 123
Involution, of androgenic zone, 60
Iris, denervated, 92
Irradiation, 105, 324
- Jecorin, 296
Juxta-medullary zone, 47
- Kephalin, 296
Kidneys, in Addison's disease, 320
 effect of epinephrine on, 128
 in insufficiency, 170, 173
- Lamprey, 13
Lecithin, 228, 290
- Leech, 12, 74
Lemur, 43
Leucocytes, 55, 167
Ligation, of adrenal vein, 278
Lipase, 296
Lipids, 54, 287, 288
 of androgenic tissue, 62
 anisotropic, 54, 289
 in disease, 265, 290
Lipochromes, 296
Liver, 197
 glycogen of, 174, 199
 in cortical insufficiency, 170, 173
Lobelin, 103
Lymphangioma, 338
Lymphatics, 25
Lymphocytosis, 310
Lymphoid tissue, 175, 321, 358
- Magnesium, 185
Malignancy, *see* tumors
Malnutrition, 55
Mammals, 16, 38
 embryology of, 30
 see individual species
Man, 20, 30, 39, 47, 58
Marchand's bodies, 59
Marmot, 47, 157, 192
Marsupials, 39
 see opossum
Medulla, destruction of, 279
 relation of size of, to cortex, 39
 see epinephrine, chromaphil, etc.
Melanin, 83, 86, 296
Melanophores, 127
Mercury salts, 48, 279
Metabolism, 205, 206, 216
 in Addison's disease, 311
 effect of epinephrine on, 129
Metachirus opossum, 39
Metallic salts, 55, 104
Metamorphosis, of amphibia, 220
Milk, secretion of, 128, 215
Mitochondria, 48
Mole, 47
Molluses, 12, 74
Mongoose, 127

- Monkey, 21, 28, 39
 adrenalectomy in, 156
 Monotremes, 39
 Morphine, 103, 267
 for assay of cortical hormone, 249
 effect of, on epinephrine discharge, 101
 Mouse, 39, 47, 226
 adrenalectomy in, 151
 androgenic tissue in, 63
 Muirhead treatment, 325
Mus musculus, see mouse
Mus norvegicus, see rat
 Muscle, effect of epinephrine on, 129
 glycogen of, 200
 see asthenia
Mustela foina, 39
Mycosis fungoides, 320
 Myoma, 338

Nephthys scolopendroides, 12
 Nerves, of adrenal, 25, 26
 Nervous system, 177
 Neurin, 103, 296
 Neurofibromatosis, 333
 Nicotine, 101, 102, 267
 Nitroid crisis, 365
 Non-protein-nitrogen, of blood, 180, 192, 310

 Obesity, 348, 357
 Opossum, 28, 43, 69, 157, 192
 Oposonins, 269
 Oral therapy, 260
Ornithorhynchus, 39
 Osmotic pressure, 187
Osteitis fibrosa, 222
 Osteomalacia, 357
 Ovaries, 227
Ovis aries, 21, 54
 Oxidation, 80

 Palmitic acid, 289
Paludina vivipara, 12
 Pancreas, 173, 176, 229
 Pancreatectomy, 204

 Paraganglion, 26
 aorticum, 26
 epinephrine content of, 74
 suprarenale, 26
 Paraganglioma, 333
 Parathyroids, 222
 Parenteral therapy, 260
 Pathology, of Addison's disease, 313
 of the adrenals, 54
 of cortical insufficiency, 161
 Pepper type, 332
 Peptone shock, 104
 Perfusion, for epinephrine assay, 89
 Peritonitis, 54, 266, 270
 Pernicious anemia, 54, 323
 Petromyzon, 12
 pH, of blood, 187
 Phaeochrome tissue, 11
 Phaeochromocytoma, 333
 Phenol, 55, 279
 Phenolsulphonophthalein, 180
Phocaena communis, 39, 40
 Phosphates, in blood, 187
 Phrenosin, 289
Physeter macrocephalus, 21
 Physostigmin, 103, 115
 Picrotoxin, 102
 Pig, 21, 69
 Pigeons, adrenalectomy in, 150
 Pigmentation, in Addison's disease, 303, 313
 in cortical insufficiency, 172
 origin of, 86
 Pilocarpin, 101, 103, 115
 Pilomotor muscles, 126
 Piquè, 97, 197
 Pisemsky's method, 90
 Pithing, 48
 Pituitary, basophilism, 217, 223, 353
 see hypophysis
 Pituitrin, 219
 Plasma volume, 183
 Pneumonia, 269, 279
 Porcupine, 39, 69
 Porpoise, 39, 40
 Potassium, blood, 185, 192

- Precocity, 343
 Precursors, of epinephrine, 82
 Pregnancy, in Addison's disease, 312
 adrenalectomy in, 147
 effect of, on adrenals, 43, 226
 epinephrine in, 225
 Progeria, 218, 357
Propteris annectens, 15
 Prostate, 126
 Protein, blood, 183, 310
 Protocatechuic acid, 75
Pteropus medius, 39
 Pulmonary artery, 120
 ventilation, *see* respiration
 Pulse, in Addison's disease, 308
 effect of epinephrine on, 116, 121
 in experimental insufficiency, 166
 Pupil, of eye, 124
Purpura lapillus, 12

 Quinine, 103

 Rabbit, 28, 35, 47, 69, 226
 adrenalectomy in, 152
 grafts in, 283
 innervation of adrenals in, 25
 Radium, 209
Raja, *see* elasmobranchs
 Rat, 20, 21, 41, 49, 226
 adrenalectomy in, 21, 28, 151, 252
 assay method, 251
 grafts in, 283
 Regeneration, after injury, 265
 of medulla, 284
 Reproduction, in Addison's disease, 312
 after adrenalectomy, 227
 in experimental insufficiency, 170, 215, 224
 Reptilia, 16
 Respiration, in Addison's disease, 312
 effect of epinephrine on, 122
 in cortical insufficiency, 164
 Reticular zone, 40, 44
 Reversal effect, of ergotoxin, 115
 Rhinoceros, 39, 47

 Rickets, 357
 Ritter's disease, 359

 Saliva, 127
 Salt, therapy, 193, 261
 see metallic, sodium, *etc.*
 Santonin, 102
 Saponin, 290
 Sarcoma, 331, 333, 338
 Scarlet fever, 54
 Schridde's disease, 359
 Scurvy, 265, 294
Scyllium, 29
 Secretions, *see* specific items
 Semilunar ganglion, 321
 Sensory stimulation, 98
Sepia officinalis, 86
 Sex, effect of, on survival after adrenalectomy, 148
 on weight of adrenals, 225
 Sheep, 21, 54
 Shock, anaphylactic, 104
 epinephrine in, 135, 273
 extracts in, 276
 histamine, 274
 peptone, 104
 relation of adrenals to, 271
 surgical, 270
 Siderophile zone, 44
 Simmond's disease, 210, 217
 Size, variations in, 23
 Skate, 13, 14, 29, 149
 Sodium chloride, in Addison's disease, 193
 in cortical insufficiency, 184, 310
 in diagnosis, 323
 therapy, 326
 Solar plexus, 321
 Spartein, 103
 Sphingomyelin, 289
Spina bifida, 62, 358
 Splanchnic nerves, 96
 Spleen, 126, 173
 Spongy zone, 44
 Squirrel, 28, 157
 Stannius corpuscles, 15

- Starvation, 54, 105
Status lymphaticus, 175, 321, 358
Stearic acid, 289
Stomach, in Addison's disease, 320
 effect of epinephrine on, 123
 in experimental insufficiency, 173
Stone-marten, 39
Streptococcus, 279
Strophanthus, 101
Strychnine, 101, 267
Sugar, *see* glucose
Sulphate, blood, 287
Sulphur, 287, 288
Suprarenal, 2
Suprarenin, 75
Surgery, epinephrine in, 134
 in hypertension, 109
 on human adrenal, 280
 procedures used, 279
Sus scrofa, *see* pig
Sweating, 127
Sympathoblast, 330
Sympathoblastoma, 331
Sympathogone, 330
Sympathogonioma, 331
Sympathomimetic action, 71
Syphilis, in Addison's disease, 319,
 326
 effect of, on androgenic tissue, 61

Tamandua, *see* ant-eater
Teleosts, 14
Temperature, 130, 217
Testudina, 16
Tetanus toxin, 105, 267, 279
Tetrahydronaphthylamine, 103
Theelin, 228
Theobromine, 102
Therapeutic use, of cortical hormone,
 326, 367
 of epinephrine, 134
Thrombosis, 320, 360
Thymus, 173, 175, 321, 358
Thyroid, extracts of, 219
 relation of, to cortex, 219
 to epinephrine, 221
 to metabolism, 205
Thyroidectomy, 206, 219
 action of epinephrine after, 130
Thyrotropic hormone, 217, 218
Thyroxin, 221
Toad, skin glands of, 74
Toxemias, 365
Toxicology, of epinephrine, 133
Toxins, 264
Tragulus javanicus, 47
Trauma, 320
Trichosurus, *see* opossum
Trypanosomiasis, 269
Tuberculosis, 315, 320
 miliary, 316
Tumors, of androgenic tissue, 339
 as cause of Addison's disease, 319
 classification of, 329
 of cortex, 335
 Grawitz, 336
 of medulla, 330
 metastatic, 329
 renal, 336
Tunica dartos, 126
Typhoid, mouse, 270
 vaccine, 267, 269
Tyrosinase, 83

Urea, 180, 192
Ureter, 126
Uric acid, 182
Urine, effect of epinephrine on, 128
 in cortical insufficiency, 184
Urodela, 16
Uterus, for assay of epinephrine, 90
 effect of, epinephrine on, 125
 lecithin, 228
 in cortical insufficiency, 227

Vaccination, 365
Vagina, 126, 228
Vas deferens, 126
Vasomotor nerves, 169, 277
Veins, of adrenal, 25
 effect of epinephrine on, 121
Venom, cobra, 267
Veratic acid, 75
Vesperugo, 53

- Virilism, 58, 347
Vitamin, A, 292
 B₁, 292
 B₂, 295
 C, *see* ascorbic acid
 G, 295
Vomiting, 163, 307
Vulpian's reaction, 71

Water, balance, 182
 content, of adrenals, 287
 of blood, in Addison's disease, 192
 in insufficiency, 182
 of muscles, 192

Weber-Fechner law, 118
Weight, of adrenals, 19
Whale, 21
Wound infections, 266

X-rays, 105
X-zone, 63
Xanthophyll, 296

Zona arcuata, 43
Zones, of adrenal, 40
Zuckerkindl's bodies, 26
 pressor effects of, 28
 tumors of, 334