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Protein Synthesis in the Animal
Organism

PROTEIN SYNTHESIS IN THE ANIMAL ORGANISM.
CAN NORLEUCINE REPLACE LYSINE FOR THE NUTRITIVE
REQUIREMENTS OF THE WHITE RAT?

BY

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THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF ARTS

IN CHEMISTRY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1920

1920
EC7

UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

June 4 1920

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY
SUPERVISION BY LUCIE EMMA ROOT
ENTITLED PROTEIN SYNTHESIS IN THE ANIMAL ORGANISM. CAN
NORLEUCINE REPLACE LYSINE FOR THE NUTRITIVE REQUIREMENTS
OF THE WHITE RAT?

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR
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TABLE OF CONTENTS.

Page

ACKNOWLEDGEMENT

I	INTRODUCTION	1
	Use of amino acids by the animal organism	1
	Work of Heinriue and Hansen	1
	Work of Abderhalden	1
II	HISTORICAL	2
	Protein before absorpition is broken down to amino acids	2
	Work of Abderhalden	2
	Amino acids in the blood, the building stones of body protein	2
	Van Slyke, Abel, and Abderhalden	2
	Synthesis of amino acids	2
	Willcock and Hopkins, Osborne and Mendel, and Franzen	3
	Lysine isolation and identification by Drechsel and Ellinger	3
	Limiting factor in diet for growth of young rats	3
	Osborne and Mendel	3
	Buckner, Nollau, and Kastle	4
	Hart, Nelson, and Pitz	4
	Norleucine	4
	Isolation and identification by Abderhalden and Weil	4
	Utilization of active and racemic forms by animal organism	5
	Greenwald	5
	Comparison between lysine and norleucine as to structure	5
	Use in the protein molecule	6
III	EXPERIMENTAL	7
	Technic	7
	Food used - how made	8
	Results	8
	Types of growth on	
	Casein diet. Chart I and II	9, 10
	Gliadin diet. Chart III	11
	Gliadin and d-l-norleucine. Charts IV and VII	13, 16
	Gliadin and d-norleucine. Charts V and VII	14, 16
	Gliadin and lysine. Charts V and VI	15
	Relation of food intake to growth	17

IV	SUMMARY	Page 19
	TABLES	20, 21, 22
	BIBLIOGRAPHY	23, 24

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ACKNOWLEDGEMENT

This work was done at the suggestion of and under the direction of Dr. H. B. Lewis of this university. The author wishes to take this opportunity to express her appreciation of the daily help and inspiration of Dr. Lewis.

PROTEIN SYNTHESIS IN THE ANIMAL ORGANISM.

CAN NORLEUCINE REPLACE LYSINE FOR THE NUTRITIVE REQUIREMENTS OF THE WHITE RAT?

I. INTRODUCTION.

During the last ten years, the question of the synthesis of amino acids by the animal organism has been one of increasing interest to biological chemists. It was in the year 1906 that Henrique and Hansen₁ found that cleavage products of protein obtained by acid hydrolysis were not able to maintain an animal in nitrogen equilibrium. When Abderhalden₂ took up this problem in 1909, he obtained a positive nitrogen balance when meat completely hydrolyzed by enzymes was fed, and he decided that animals could live on hydrolysis products of protein. Since that time, the emphasis on the study of the protein minimum has been shifted from a purely quantitative consideration to one both quantitative and qualitative. We now realize that a protein to be adequate for the needs of the organism must not only furnish amino acids in the proper quantity; but it must ^{also} furnish those which are necessary constituents of the body proteins, and which are not synthesized by the organism. The present work deals with the conversion of α amino caproic acid to lysine.

II. HISTORICAL.

We now believe that injected protein before absorption is entirely broken down to amino acids. Abderhaldens was able to maintain dogs on mixtures of amino acids, carbohydrates, and fat during periods of 90 to 100 days. In one case the dog gained 9 kilos in body weight and grew an entire new coat of hair. This study was not limited to animals alone, for Abderhalden₄ also obtained a positive nitrogen balance for a period of 15 days by feeding amino acids per rectum to a boy having an oesophageal stricture. Thus it was proven that man and animals are able to use amino acids in the place of protein.

Van Slyke's₅ method for amino nitrogen has made possible the quantitative determination of amino acids in the blood. Further experimentation by Abderhalden₆ on large quantities of blood and dialysis in vivo by Abel₇ have resulted in the direct isolation of amino acids from blood. Thus protein is absorbed in the form of amino acids. These in turn are carried by the blood to the tissues where they are used as the building stones for the protein of the body. It is therefore impossible to consider the synthesis of protein without the consideration of these building units, the amino acids.

It has long been recognized that glycocoll can be synthesized by the animal organism. A pig, which was fed sodium benzoate by Abderhalden₈ and his co-workers, excreted more glycocoll as hippuric acid during the period of the experiment than was originally present in all its tissues. But concerning the synthesis of the other amino

acids of the protein molecule we know very little. Results of Will-cock and Hopkins¹⁰,^{and} Osborne and Mendel¹¹ obtained either by feeding mixtures of amino acids from which certain ones had been removed, or by feeding pure proteins in which certain amino acids were lacking or present in low percentage, show that the cyclic amino acids, histidine, and tryptophane and the diamino acid lysine cannot be synthesized by the animal organism. Plants carry out these syntheses, perhaps from ammonia and the products of carbohydrate fermentation, or as Franzen¹² believes, by reactions between HCN and pentoses or hexoses with the addition of ammonia. It was formerly thought that the main difference between these two classes of living organisms lay in the power of synthesis in plants and the lack of this power in animals.

In this study, the special interest was the synthetic power of the animal organism for lysine. This amino acid, which was first isolated from casein by Drechsel¹³ in 1839, was proven by Ellingerr¹⁴ in 1900 to be the α, ϵ , diaminocaproic acid, since on putrefaction it yields cadaverine. Lysine is widely distributed, but the percentage in a number of proteins is quite low.

That lysine is one of the amino acids which cannot be synthesized by the animal organism was first proven by Osborne and Mendel¹⁵ in 1914 in feeding experiments on white rats. Zein, which analysis shows to contain no lysine or tryptophane, plus the missing tryptophane yielded maintenance or very slight growth. Gliadin which contains tryptophane and very small percentages of lysine gave similar results.

In 1914, Buckner, Nollau, and Kastle¹⁶ found that a diet con-

sisting of a grain mixture low in lysine fed to chicks induced very slow growth. As soon as the diet was changed to grain mixtures high in lysine, growth became normal.

In 1915, Osborne and Mendel¹⁷ repeated their former work and found it possible to keep rats for a period of 6 months on a diet low in lysine, i.e., with gliadin as the sole source of protein. The addition of 1.0 or 1.5% lysine to this diet which was formerly adequate only for maintenance permitted normal growth. McCollum, Simonds, and Pitz¹⁸ in 1917 failed to prove that lysine is not the limiting factor in gliadin.

Hart, Nelson, and Pitz¹⁹ in 1918 investigated the synthetic power of the animal organism for lysine from a different view point. These men were of the opinion that it might be possible for the mammary gland to synthesize lysine; since that gland has the power of changing glucose of the blood to lactose. But they found out that, when the rats were kept on diets low in lysine, i.e., on gliadin, the secretions of these glands did not support the growth of the young except through degradation of the tissues of the maternal organism. The work of all these investigators has shown that for growing animals lysine is a necessary factor in the diet.

In 1913, while analyzing the proteins of the brain and nervous tissue, Abderhalden and Weil²⁰ discovered a new amino acid. Later they found this, which they identified as α aminocaproic acid or norleucine, to be a normal constituent of the proteins of the central nervous system. Abderhalden and others²¹ synthesized several dipeptides in which norleucine was one of the amino acids.

Greenwald²² working in the Harrimann Research Laboratory, in New York, found that when the d, l, and racemic forms of norleucine were injected into phloridzinized dogs, sugar was found in the urine. The amount of extra glucose formed was quite comparable to that which would result from this injected material and determinations on the urea nitrogen showed an increase due to the complete deamination of these acids. This work shows conclusively that the racemic form of norleucine is utilized by the animal organism just as efficiently as either the d or the l form alone. These amino acids may go through the path of katabolism as any straight chain amino acid of an even number of carbon atoms.

Norleucine closely resembles lysine in structure.

The naturally occurring form of each in the protein of the animal body is the d form. To change norleucine to lysine, would involve only the addition of the ϵ amino group. The possibility of the substitution of an amino group in a position other than the α by the organism of the white rat, has not been previously investigated. Abderhalden²³ evidently considered such a substitution possible, for in his textbook he suggested that norleucine might be the precursor of lysine in the protein of the animal organism.

Furthermore, the α -group is the only amino group of lysine which is joined to other units in the protein molecule. Proteins

treated with HNO_2 in the Van Slyke apparatus at 37°C yield nitrogen which has been found to be equal to one-half the lysine nitrogen. In other words the ϵ amino group of lysine is free in the protein molecule. Taking this fact into consideration, it seemed possible that the norleucine might be linked in the protein molecule in the place of lysine. The only difference between lysine and norleucine in the protein molecule would be the lack of the free ϵ amino group. Thus in the substitution of norleucine for lysine in the nutritive requirements of the white rat there are two possibilities; first, norleucine might be changed to lysine by the addition of an amino group; and second, norleucine might even replace lysine in the protein molecule.

III. EXPERIMENTAL.

Young white rats were used as experimental animals. These were placed on special diets when they had attained the weights of 50 to 60 grams, and were kept on the same diet for periods of 10 to 15 weeks. Weekly weights and food intake were recorded. Some controls were fed milk and dog biscuit, while others were kept on a synthetic diet containing 18% casein. The other diets contained as protein 18% gliadin, or its equivalent in gliadin and lysine or gliadin and norleucine. The exact dietaries were as follows:

casein or gliadin	18.0%	norleucine or lysine	0.5%
protein free milk	28.0%	gliadin	17.5%
corn starch	24.0%	protein free milk	28.0%
butter fat	18.0%	corn starch	22.0%
lard	12.0%	butter fat	18.0%
		lard	14.0%

The casein, gliadin, lysine and norleucine were made in this laboratory. Lysine was prepared from the hydrolysis of casein, while the norleucine was synthetic. Argo Corn Starch and Swifts' Pure Leaf Lard were used each of which were bought in quantity and mixed to give uniformity. Creamery butter was centrifuged two hours at a temperature just above the melting point to precipitate the protein, inorganic salts and water. The supernatant liquid was decanted and kept on ice. The protein free milk was made according to the procedure of Osborne^{and} Mendel²⁴ with one exception. When the temperature of the filtrate from the precipitation of the casein had reached 90° C., dilute NaOH was added until the lactalbumin began to flock out. The mixture was then heated to boiling. The coagulated lactalbumin, was filtered off with suction thru hardened filters. The product, which was evaporated to dryness below 60° C in a cur-

rent of air and ground to a fine white powder, showed on analysis 0.59% nitrogen.

The norleucine used was racemic. In order to insure sufficient quantity of the d-form, to make .5% of the diet, some rats received food containing 1.5% of the racemic compound. During the latter part of the experiment, through the kindness of Dr. C. S. Marvel, we were able to obtain d-norleucine that analyzed 82% of the d form. This was used to the extent of 0.658% of the diet making 0.5% of the d form.

The food was mixed into a paste, kneaded with the hands to insure thorough mixing, and rolled into balls weighing 5 to 10 grams each. When kept on ice, the food hardened sufficiently so that each ball could be weighed before introducing into the food dish. In this form the food was not easily scattered by the animals.

Gliadin was chosen for the basal protein of these diets because it is low in lysine, and contains the other essential amino acids. By the method of Kossel, lysine was isolated directly from the hydrolysis products of gliadin by Osborne. He reported the lysine content as 0.15%. Later by the use of the Van Slyke procedure, the same workers with others increased this figure to 1.3% and by more careful work with the Kossel method the 0.15 was increased to 0.64%.

The results of the experiment are shown in the accompanying tables and charts. Charts I and II for rats 1, 9, and 16, on the casein diet show the normal growth curve which exactly parallels that on milk and dog biscuit as shown by the growth curve of rats 7 and 10. Rats 2, 13, and 17, Chart III show the type of growth ob-

Chart I.

10 grams 10 days

10 grams | 10 days

Chart II.

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{ 10
grams }

{ 10 days }

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tained when the casein is replaced by an equivalent amount of gliadin. Slight growth ensues. These results are the same as those obtained by Osborne and Mendel in the work to which we have previously referred to. Rat 3, Chart IV, was fed a diet in which 0.5% d- l-norleucine replaced 0.5% of gliadin. The curve of growth for this rat closely resembles that for the rats kept on gliadin alone. The animals which received the diet of 1.5% norleucine plus gliadin, and rats 12/14, Charts IV and V, did not make any better growth than those receiving the smaller percentage of this amino acid. Rat 11, Chart IV, shows the effect of the 0.5% norleucine diet on an older rat after he had grown normally on the casein diet for three weeks. The slight loss of weight by this animal towards the end of the experiment was due to some pulmonary infection, the only case of ill health in the colony. In order to be sure that this failure to grow was not due to the inability of the organism to resolve the racemic norleucine, we fed rat number 8 for six weeks on food containing 0.68% of norleucine analyzing 82% of the d form. The growth as shown by the curve in Chart V is not at all different from that resulting from the feeding of the racemic mixture.

To prove that the failure of these rats to grow was due to the deficiency in lysine, 0.5% and 1% lysine was added to the gliadin diets and norleucine was replaced by lysine. Charts V and VI for rats 4, 5, and 6 show the effect. Immediately, even the first day after the addition of the missing amino acid, growth began and remained normal as long as lysine was fed. As soon as lysine was again replaced by norleucine, there was an immediate cessation of growth, a slight drop and then a very slow rise. This effect on

Chart IV.

{ 10 grams }
10 days

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Chart V

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Chart VI

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10 grams 120 days

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growth is exactly the same as that produced when a rat is changed from a casein diet to one of gliadin and norleucine as Chart IV for rat 11 shows. Rat 8, which was on the d-norleucine diet showed marked increase in growth when norleucine was replaced by lysine (Chart V). That the growth in this case was not quite normal on 0.5% lysine may be explained by the fact that a less pure sample of lysine was used.

Thus in every case of gliadin feeding without lysine, there resulted a stunting of growth. The appearance of the rats was quite different from that of normally growing ones. The stunted rats seemed to increase in length but not in body weight. This gave their faces an old look and made their limbs feel thin and fragile. Their hair which normally grows rather coarse and thick with maturity remained downy and fine. The characteristics of a norleucine fed rat were in no way different from those of a gliadin fed one. As soon as lysine was fed and growth began, these other characteristics disappeared, the hair became coarse, the frame filled out, and the sex organs developed.

The total food intake for each rat is tabulated in the table, with the total gain in weight during the periods on the different diets. It may be noted in the column under Food per 100 gram rat, that the gliadin rats ate more than the casein rats. This seeming discrepancy may be explained in two ways. First, rats 2 and 13 were kept on this food for 13 weeks, time enough for them to become accustomed to the diet. Their body weight increased very little in this time and they gradually ate more and more of the food. Second, a casein rat of the same age would be actively growing, and although

the amount of food eaten would increase, yet not at the same rate at which the body weight increased. As a matter of fact, the amount of food eaten by the gliadin animals was quite comparable to that eaten by the casein fed ones in proportion to their body weight.

In the last column in the same table, the gain per 100 grams food, it is shown that 100 grams of casein food induced a gain of 17-20 grams, while the same amount of gliadin food or norleucine-gliadin yielded a gain of 3-4 grams only. 100 grams lysine-gliadin food made possible a gain of 15 to 22 grams. The gain per 100 grams food is lower, the shorter the period of time, because any gain takes place after the animal has become accustomed to the food, i.e., after 4-6 weeks on the diet. On the other hand, the figures showing the gain by rat 4 on casein are abnormally high; because they represent a time when his body weight was increasing most rapidly. These figures show that gliadin and lysine make a diet comparable to that containing casein as its protein. It is easily seen that over equal periods of time a mixture of gliadin and norleucine was no more efficient for growth than was gliadin alone.

IV. SUMMARY.

1. Gliadin comprising 18⁰/o of a synthetic diet has been shown to be inadequate for normal growth of the white rat. On this type of diet maintenance or slow growth only results. This is in harmony with the work of previous investigators, notably Osborne and Mendel.

2. Lysine in amounts equivalent to approximately 3⁰/o of the total protein of the above diet without further alteration renders the diet adequate for normal growth.

3. Neither racemic norleucine nor the d form is able to supply the deficiency of the gliadin diet as does lysine. When norleucine is replaced by lysine in the experimental dietaries normal growth ensues.

4. No evidence was obtained which would indicate that norleucine is a precursor of lysine or that the organism of the white rat can substitute norleucine for lysine in the synthesis of its proteins.

No.	Diet	Duration days	Initial wt. gms.	Final wt. gms.	Gain gms.	Total Food gms.	Food per 100 gms. rat gms.	Gain per 100 gms. food gms.
I	Casein	98	97.3	250	152.7	877.2	32.2	17.8
IX	Casein	84	63.1	190	126.9	616.6	37.4	20.5
XVI	Casein	56	62	206	144	453	40	31.9
II	Gliadin	91	100.4	116.8	16.4	456.5	34.2	3.7
XIII	Gliadin	91	68.6	88.2	19.6	451.2	50.0	4.3
III	Gliadin + .5% d-l- norleucine	91	101.3	131.6	30.3	496	31.4	4.0
XI	Gliadin + .5% d-l- norleucine	91	150.4	149.1	-1.3	609.9	31.5	-.21
XII	Gliadin + 1.5% d-l- norleucine	98	130.3	140.	9.7	558.4	29.9	1.7
V Period	(a) Gliadin	70	48.8	58.5	9.7	271.4	43	3.5
	(b) Gliadin + .5% lysine	42	58.5	112.7	54.2	243.3	39.8	22.2
	(c) Gliadin + 1.5% d-l- nor- leucine	35	112.7	115.	2.3	173.4	25.5	1.5
VIII Period	(a) Gliadin	42	75.7	76.7	1.	183.9	36.4	.54
	(b) Gliadin + 1.5% d-l- nor- leucine	34	76.7	87.7	11.	349.3	32.8	3.1
	(c) Gliadin + .5% d- norleucine	42	87.7	90.7	3.	171.3	31.0	1.7
	(d) Gliadin + .5% lysine	42	90.7	120	19.3	207.6	31.	9.3

No.	Diet	Dura- tion days	Initial wt. gms.	Final wt. gms.	Gain gms.	Total Food gms.	Food per 100 gms. rat gms.	Gain per 100 gms. food gms.
VI								
Period	(a) Gliadin	28	63.5	62.5	-1.	133.6	43.3	-0.75
	(b) Gliadin + .5% d-l- nor- leucine	56	62.5	73.6	11.1	230.1	38.0	4.8
	(c) Gliadin + 1% lysine	42	73.6	123.5	49.9	248.3	35.0	20.0
	(d) Gliadin + .5% d-l- nor- leucine	35	123.5	126.0	2.5	204.8	27.8	1.22
IV								
Period	(a) Casein	28	58.7	105.9	47.2	140.1	35.1	33.7
	(b) Gliadin + 1.5% d-l- nor- leucine	63	105.9	118.1	12.2	397.3	34.4	3
	(c) Gliadin + .5% lysine	35	118.1	166.8	48.7	249.1	28.8	15.5
	(d) Gliadin + 1.5% d-l- nor- leucine	35	166.8	159.7	-7.1	234.2	24.2	-3.0
XIV								
Period	(a) Gliadin	84	62	62	0	281.1	34.8	0
	(b) Gliadin + .5% lysine	35	62	97	35	142.6	35.2	24.4
XV								
Period	(a) Gliadin + 1.5% d-l- nor- leucine	56	57	57	0	199.5	44	0
	(b) Gliadin + .5% d-nor- leucine	42	57	65	8	110.2	30	7.2

No.	Diet	Dura- tion days	Initial wt. gms.	Final wt. gms.	Gain gms.	Total Food gms.	Food per 100 rat gms.	Gain per 100 food gms.
XVII								
Period	(a) Gliadin	56	57	59.3	2	224.5	50	0.89
	(b) Gliadin	63	59.3	62.4	3.1	184.8	34.4	1.6
	+ 1.5% d-l- nor- leucine							

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