BLOOD VOLUME AND OXYGEN CAPACITY IN MAMMALS

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INTRODUCTION

While considerable research reported in the literature deals with changes in total blood volume associated with pathological conditions in man, relatively little information is available with regard to the total blood volume of normal laboratory and wild animals. Changes in total blood volume in various mammals have been reported in regard to ecology, diet, hemorrhage, psychosomatic conditions, and other disturbances.

In studies made at sea level and at altitudes above 11,000 feet, Smith et al. (1924-25) reported a 12 per cent increase with increased altitude in the red blood cells of six men. This was based on a change in the mean total blood volume from 4484 to 5063 cubic centimeters as determined by the carbon monoxide method which is described later. However, with the dye method, also to be described later, the investigators reported an unexplained decrease from 6187 to 6071 cubic centimeters.

On prolonged exposure to a high environmental temperature blood volume tends to increase and to decrease in colder temperatures according to Bazett et al. (1940). Mitchell (1938) reports that temperature regulation in mammals at environmental temperatures from about 15 to 37 degrees centigrade is effected almost entirely by control of the rate of heat loss. Barbour (1921) has shown that a fundamental factor, in which temperature control is necessitated by regulation of heat loss, is the change in the water content of the blood. This movement of water

is reflected by a change in blood volume, and with an increase in blood volume, there may be a marked increase in secretion of sweat. By means of this movement, water, the chief heat transporter of the body, is retained in the tissues in increasing amounts during exposure to cold, but is freed to convey heat to the radiating surfaces of the body during exposure to heat (Mitchell, 1938).

In a study of diuresis in dogs, Evans and Gibson (1937) report that the blood volume decreases "proportionally with the magnitude of the diuresis."

That acute and chronic dehydration produced experimentally in dogs results in a 15 to 30 per cent reduction in plasma volume is reported by Gibson (1941).

There is a 9.3 per cent loss of blood volume in man after a bed rest of three weeks, according to Taylor et al. (1945).

In studies on soldiers of World War I, Robertson and Bock (1919) report that in exudation of wounds the total blood volume was sometimes lowered as much as 2000 cubic centimeters.

"The amount of blood which can be added to the general circulation of cats by contraction of the spleen upon stimulation of the splanchnic nerves is of the order of 2.6-5 p.c. of the total blood volume of the animal," according to Cruickshank (1926).

An increase in erythrocytes in the blood of man is reported to be accompanied by a decrease in the circulating plasma by Brednow (1931).

During emotional excitement in rabbits, there is reported to be a 20.2 per cent increase in the red cell volume and a decrease of 8.5

per cent in plasma volume; in the 11 animals reported on by Nice and Katz (1934), there was a rise of 1.7 per cent of the total blood volume during the emotional excitement.

In cobalt polycythemia, the blood volume rises from 6.38 to 7.89 cubic centimeters per 100 grams of body weight according to Orten et al. (1933). Davis (1940) reports a 17 to 22 per cent greater blood volume in dogs in cobalt polycythemia. In their studies on rats in cobalt polycythemia, Stanley et al. (1946) report that blood volume increased 80 per cent.

"Much of our information with regard to the blood volume and its regulation has come to us through efforts that have been made to develop methods, and often this information is colored by the individual method through which it has been derived," Erlanger (1921). In the following discussion, the various methods are grouped according to their underlying principles.

Bleeding Method

A direct method of blood volume determination was described by Welcker (1858). He took a small measured sample of blood from the animal and diluted it 1:100 in saline solution. The animal was then bled, the blood vessels washed out with water, and the tissues macerated. Water was then added to the collected fluid until its color matched the color of the original blood sample. The total collected fluid divided by 100 gave the total blood volume of the animal. Obviously, this method is not free from serious error. The turbidity of the final solution

prevents exact color matching, the myoglobin of the muscles cannot be distinguished from the hemoglobin, and the loss of hemoglobin as a result of intravascular and extravascular clotting leads to further error. Despite these inaccuracies of the bleeding method, many investigators have used it as may be seen from Table VI..

Indirect Methods

Obsolete Methods

The first method for determining the total blood volume in an animal was described by Valentin (1838). He states that a sample of blood was withdrawn and the percentage of solids was determined. Then a known volume of distilled water was injected intravenously. After a measured time interval, a second sample of blood was withdrawn and the percentage of solids determined. A comparison of the percentage of solids of the two samples was interpreted as an indication of the amount of dilution of the blood caused by the injection of distilled water. From these data the total blood volume was determined. The use of distilled water was objectionable because hemolysis occurred, and a solution of sodium chloride came to be used instead.

Sherrington and Copeman (1893) diluted the blood with 0.75 per cent sodium chloride. They withdrew about one-third of the blood from a rabbit and injected the same amount of sodium chloride solution within 15 seconds. They determined the total blood volume by the degree of dilution on a withdrawn sample which was taken 30 seconds later. The specific gravity was used as an index. Kottman (1906) determined the total blood volume in man by means of the sodium chloride method. He

injected 3.5 cubic centimeters of isotonic salt solution per kilogram of body weight and determined the dilution by means of a hematocrit value obtained before and after injection. Flesh (1909), also working with man, injected a larger quantity of sodium chloride solution, 5.0 cubic centimeters per kilogram of body weight, and calculated the total blood volume from the dilution value which was determined by means of a Flesh colorimeter.

Substances other than sodium chloride have been used in attempts to determine total blood volume. Nelson (1909) reports the results (see Table VI) found in rabbits when an animal is bled first and the amount of blood lost is replaced by an equal amount of serum as suggested by Malassez (1875). Von Bering (1911) used "antitoxin and the dilution was determined biologically." Schurer (1911) injected foreign serum and studied its dilution quantitatively by precipitin reactions. Meek and Gasser (1918-19) used gum acacia as an intravenously injected colloid, and determined gravimetrically at the end of three minutes the amount of gum acacia in a known volume of blood. Robertson and Bock (1919) injected a 6 per cent solution of gum acacia in physiological saline solutions and determined the total blood volume by using the hemoglobin dilution as an index. McQuarrie and Davis (1920) injected a "non-coagulable, highly refractive substance, gum acacia and gelatin solution," and noted the change in the refractive index as the result of the dilution. Lee and Whipple (1921) injected hemoglobin to measure the plasma volume which served as a measure of the total blood volume.

The carbon monoxide method for determination of total blood

volume was introduced by Grehan and Quinquand (1882). Working with dogs, they allowed the animal to breathe a known amount of carbon monoxide from a bag. Then they determined gasometrically the amount of carbon monoxide in a withdrawn sample of blood. This amount was then compared with a standard in which a known amount of carbon monoxide was combined with a known volume of blood. The dilution served as a measure of the total blood volume. Haldane and Smith (1899-1900) adapted this method to the determination of total blood volume in man. Since the publication of their results, many investigators have used the carbon monoxide method to study total blood volume in different species as shown in Table VI. Blood volume for supposedly normal mammals are given in this table following the classification by Simpson (1945).

The disadvantages of the carbon monoxide method have been pointed out by Reeve (1948) who states, "This is one of the oldest and most criticized of the more precise methods." Among the difficulties is the accurate determination of carbon monoxide by gasometric and colorimetric methods, and the amount of carbon monoxide diffusing into the tissues from the blood.

Dye Methods

The dye method for blood volume investigation was first introduced by Keith et al. (1915) of Johns Hopkins University. In looking for a non-toxic dye that remained in the blood long enough for thorough mixing, one that was absorbed but little, and one whose concentration could be determined colorimetrically, these workers had the dye, vital red, suggested to them by Dr. Herbert M. Evans of the Anatomical Department

of the Johns Hopkins Hospital Staff. When Dr. Evans later became Professor of Anatomy at the University of California, Hooper et al. (1920) of the University of California asked Dr. Evans to suggest another dye that might possibly be used for total blood volume determination since the dye, vital red, was not available. He suggested the dye, brilliant vital red, which these investigators used in a study on dogs.

In view of the increasing interest in the dye method among research workers investigating blood volume in animals, Dawson et al. (1920) believed it desirable to study many dye substances which might be of value for future workers.

Sixty-one dyes were divided into three groups for evaluation by studies on dogs. Group I contained 30 dyes resembling the vital red series in that they persist in the blood plasma. Group II contained 14 dyes which leave the blood stream rapidly and appear in the urine. Group III contained 17 dyes which leave the blood stream rapidly, but do not appear to any degree in the urine. The dyes were evaluated for possible errors in colorimetry, mixing time, time of disappearance from the blood, toxicity, and availability.

Studies of Congo red and vital red in dogs and cats to determine which of these two dyes is more suitable for clinical use are reported by Harris (1920). He found that Congo red is better suited for colorimetric studies since the rate of disappearance of vital red is about 12 per cent greater over a 30 minute period. Graff and Clarke (1931), in colorimetric studies of brilliant vital red, demonstrated that hemolysis sometimes occurs in which case the hemoglobin liberated makes color

matching unreliable.

The principle that solutions of different compounds possess characteristic absorption spectra which may be readily detected by spectroscopy was used by Gregersen et al. (1935). They reported that the maximum absorption range for T-1824 (Evans blue) dye was 620 to 630 millimicrons as determined with a spectrophotometer. They state that there is practically no absorption of light by hemoglobin (which has a maximum absorption range of 544 to 576 millimicrons) if hemolysis occurs in the blood sample in the absorption range of the T-1824 dye. However, since the absorption range for the vital red dyes (about 520 millimicrons) is closer to the absorption range for hemoglobin, hemolysis would result in error. The T-1824 dye has subsequently become the accepted dye for blood volume investigations.

The T-1824 dye exhibited no toxic effects on male rats when it was injected intravenously in doses of 20 milligrams per kilogram of body weight (Gibson and Gregersen, 1935). Gibson and Evans (1937) adopted the method utilizing the T-1824 dye as a clinical means of determining total blood volume in man.

In a study of the absorption curve of the T-1824 dye, Gibson and Evalyn (1938) introduced the use of a photoelectric colorimeter for measuring the optical density of the dye with a filter having a maximum transmittancy of light in the range of 620 to 625 millimicrons.

The amount of various dyes absorbed by red blood cells has been studied by different investigators. Gregersen and Schiro (1938) state that T-1824 dye is not absorbed by the red blood cells in dog blood.

Shohl and Hunter (1941) report this is also true for human red blood cells. Lindhard (1926) reports that vital red is absorbed by the red blood cells of an ox, less by the red blood cells of a dog, and no absorption occurs by the red blood cells of man. Rowntree et al. (1929) report that Congo red and vital red do not enter the red blood cells of man.

Ferrebee et al. (1941) report that a trace of T-1824 dye passes into the lymph in dogs during the first 30 to 60 minutes following the time of injection. However, they state that this amount is not "critically significant" in the evaluation of the accuracy of the dye for blood volume determination.

Substances used in blood volume determinations such as sodium chloride, antitoxin, hemoglobin, serum, Ringers solution, and acacia do not give accurate results because of their rapid diffusion from the blood, as pointed out by Erlanger (1921).

Radioactive Methods

The first successful attempt to determine total blood volume by the use of radioactive phosphorus was made by Hahn and Hevesy (1940). They injected a known volume of labelled red blood cells taken from a donor rabbit into the vein of a recipient rabbit. Then they calculated the total blood volume by determining the extent to which the labelled corpuscles were diluted in a sample taken 5 minutes later. The labelled red blood cells were prepared by injecting the donor rabbit subcutaneously with radioactive sodium phosphate over a period of a week. This procedure allowed the red blood cells to incorporate some of the labelled

phosphatide molecules. Hahn et al. (1941) followed the method of Hahn and Hevesy (1940) in the preparation of donor red blood cells to inject into a recipient animal except that they used radioiron in dogs. Anderson (1942) and Hevesy and Zerahn (1942) studied total blood volume and red cell volume, respectively, in rabbits using radioactive phosphorus. Red blood cells were labelled in vitro, washed with physiological saline solution, and injected into the animal. The radioactivity of a sample of labelled, withdrawn blood was compared with a standard made from the injection solution. This served as a measure of the dilution volume in the animal. Govaerts (1942), in studying total blood volume in man and dogs, used the method of Hahn and Hevesy (1940).

A radioactive isotope of iodine (I-131) for determining total blood volume was first used by Fine and Seligman (1943). This work was done to evaluate the total volume of blood in man. In this method the protein fraction of plasma is labelled with I-131. A solution containing potassium iodide plus the carrier-free I-131 and nitric acid was distilled into cold distilled carbon tetrachloride. The carbon tetrachloride solution of iodine was washed with distilled water and added to chilled plasma which was previously extracted with carbon tetrachloride. Sodium carbonate was added and the mixture shaken until the color disappeared. The plasma was separated by centrifugation and then dialyzed for 48 hours against cold running water. The precipitated globulin was centrifuged and the supernatant plasma was ready for use.

The technique of Hevesy and Zerahn (1942) for the determination of total red cell volume was modified by Hevesy et al. (1943-44). "A

part of the blood sample containing labelled corpuscles is reintroduced into the circulation. After the lapse of about 5 minutes, a blood sample is secured and the radioactivity of the corpuscles of this sample is compared with the radioactivity of the reintroduced corpuscles of equal weight. The ratio of the radioactivity of the two samples is a measure of the amount of corpuscles present in the circulation."

Nylin (1945) employed the methods of Hahn and Hevesy (1940) and Hevesy and Zerahn (1942) in clinical investigations of total blood volume in man.

A comparative study in dogs was made by Krieger et al (1948), using the methods described by Hevesy and Zerahn (1942) and Fine and Seligman (1943). Nachman et al. (1950) also used radioactive phosphorus to label the red blood cells of man following the methods described by Hevesy and Zerahn (1942) and Anderson (1942). Yalow and Berson (1951) used radioactive potassium ($K-42$) to label red blood cells in vitro. They followed Reeve and Veall (1949), who used the methods of Anderson (1942) and Hevesy and Zerahn (1942) with radioactive phosphorus in man.

METHODS AND MATERIALS USED IN THE PRESENT INVESTIGATION

Two methods of blood volume investigation were followed in the present study. In one method radioactive phosphorus-32 (P-32), which was incorporated in phosphoric acid (H_3PO_4), was used. In the other method, T-1824 (Evans blue) dye was used. A cardiac puncture was made on the animal and a sample of blood withdrawn by means of a needle and syringe coated with heparin. The blood was centrifuged in a No. 2 International centrifuge at 3000 rpm. for 30 minutes. The plasma was removed with care so as not to disturb the red blood cells. The red blood cells were washed 3 times with iced, physiological saline solution and centrifuged after each washing at 3000 rpm. for 5 minutes. A volume of isotonic P-32 solution, which contained approximately 50 microcuries of radioactive phosphorus, was added to the red blood cells. The solution added was equal to the volume of plasma originally present in the withdrawn blood sample. The red blood cells were incubated in this solution in a water bath at 37 degrees centigrade for two hours. About every ten or fifteen minutes the suspension was stirred with a small, glass rod. At the end of the labelling period, the radioactive solution was centrifuged at 3000 rpm. for 5 minutes. The red blood cells were again washed 3 times with physiological saline and centrifuged after each washing at 3000 rpm. for 5 minutes. After each washing, the supernatant was pulled off by a suction pipette so as not to disturb the red blood cells. Iced, physiological saline solution was added to the red blood cells so that the resulting mixture was equal to the original volume of blood

withdrawn from the animal. The mixture was then stirred manually with a small, glass rod and an aliquot was taken for a standard which was made to a definite volume. A known volume of the labelled red blood cell solution was stored in a syringe until it was injected into the jugular vein of the animal. This procedure is essentially the same as that described by Hevesy et al. (1943-44) and modified by Nachman et al. (1950).

A standard and a blank sample for the dye method were prepared during the two hour time interval of labelling the red blood cells. A solution was prepared by diluting a known concentration of T-1824 dye to a definite volume with physiological saline solution. An aliquot of this dye solution was then diluted to a known volume with distilled water. A known volume of the diluted dye solution was then added to a known volume of plasma. The mixture served as the standard solution. The plasma proteins were precipitated by alcoholic phosphotungstic acid solution, a procedure described by Crooke and Morris (1942) for the prevention of turbidity in plasma samples. Precipitation was allowed to occur in the plasma sample for 5 minutes while it was being stirred with a small, glass rod. The suspension was then centrifuged for 5 minutes at 3000 rpm. The optical density of the standard solution was read on a Klett-Summerson photoelectric colorimeter by use of a filter with a maximum transmission of light at 622.5 millimicrons. The filter, as sold by the A. H. Thomas Co., Philadelphia, is their Catalog No. 3788-Q3.

The colorimeter was zeroed by the use of the blank sample which was prepared in the same manner as the standard except that the dye was

omitted. The procedure outlined above follows that of Gregersen (1944). A known volume of the dye in saline solution was stored in a syringe until it was injected into the blood of the animal.

An animal was tied to an animal board in a supine position and etherized. An incision was made over each jugular vein after the area had been clipped to free it from interfering hairs. Alcohol was rubbed on the skin and the jugular vein was then exposed and freed from the connective tissue surrounding it. A probe was placed under the vein to facilitate insertion of the needle. The needle of the syringe containing the P-32 or the T-1824 dye solutions, or both, was inserted into the vein and the injection was made with the point of the needle directed toward the heart. After the solutions were injected, the syringe was flushed two or three times with blood in order to insure the introduction of all of the dye and cell solution into the blood. The needle was quickly removed and the wound pressed with an absorbent cloth. After a definite time interval, a blood sample was withdrawn from the opposite jugular vein into a syringe which had a dried coating of heparin in it. In this withdrawal of blood, the needle on the syringe was pointed toward the head. The withdrawn blood was deposited in a centrifuge tube containing a definite amount of heparin solution. The sample of blood was then centrifuged for 30 minutes at 3000 rpm.

The plasma was separated from the red blood cells and the hematocrit value recorded. A definite volume of the plasma was treated in the same manner as the blank and the standard, and the optical density determined by means of a photoelectric colorimeter. The total plasma

volume and the total blood volume were calculated from the formulae shown on pages 96 and 97 (Appendix II).

The red blood cells of the withdrawn sample were washed 3 times with physiological saline and centrifuged at 3000 rpm. for 5 minutes after each washing. The cells were diluted to a definite volume with distilled water. The radioactivity of this solution and of the standard was then determined. This was accomplished by taking an aliquot of the sample solution and counting the activity on a commercial scaler. In this investigation, two counters were used; one was an Autoscaler manufactured by Tracer Labs., Inc., and the other was a Cyclotron from the Cyclotron Specialties Company. Both scalers were used in conjunction with an immersion-type Geiger Muller tube. Data for corrections of background count from cosmic radiation were made at the beginning of each activity count. From these data the total blood volume was calculated according to the formula shown on page 98 (Appendix III).

Red blood cell counts and hemoglobin concentration were made for each animal. The red blood cells were counted according to the method described by Kolmer and Boerner (1945) and the hemoglobin was determined by the acid hematin method on a Fisher electro-hemometer. The procedures for each method are given in Appendices IV and V.

A detailed description of the procedure for the determination of blood volume by the P-32 and T-1824 dye methods which was followed in this investigation is given in Appendix I.

INVESTIGATIONS OF OPOSSUMS

The opossum, Didelphis virginiana pigra (Bangs), served as a convenient animal for blood volume investigations. Twenty-four specimens were trapped or captured in the wild state from September, 1950, to December, 1951, during which time the blood volume of these animals was investigated.

Blood Volume Determinations with T-1824 Dye and P-32

Blood volume determinations were made on 10 opossums using T-1824 dye and radioactive phosphorus-32. Data concerning these animals are given in Table I. It is noteworthy that the values for the total plasma volume, total red cell volume, and total blood volume is higher in the dye method than in the P-32 method by mean values of 5.8, 4.0, and 9.9 ml., respectively.

The mean value plus or minus three times the standard deviation gives the theoretical limits found in 99.7 per cent of the population according to Simpson and Ree (1939). For the dye method these values are 8.32 to 3.34 ml. and for the P-32 method they are 7.65 to 3.33 ml. of blood per 100 gm. of body weight.

The relationship of the ml. of blood per 100 gm. of body weight of the 10 opossums studied by the T-1824 dye and P-32 methods is shown in Graph I. This shows that there is a decrease in the amount of blood per 100 gm. of body weight with an increase of body weight. The lines of trend were determined for each method by the method of least squares

TABLE I
Opossum, *Didelphis virginiana pigra*

Sex	Wt. (gm.)	Corrected RBC Ht. %	T-1824		ml. of blood per 100 gm. wt.		P-32		TBV
			PV	RCV	T-1824	P-32	PV	RCV	
m	2588	50.9	76.6	79.4	6.0	6.3	89.4	75.4	164.8
f	2640	36.6	72.6	41.9	4.3	4.6	77.8	44.9	122.7
f	1864	28.5	75.8	30.2	5.7	7.1	95.0	37.8	132.8
f	2777	31.0	104.0	46.7	5.4	4.6	87.8	39.5	127.3
f	3085	39.2	101.6	65.5	5.4	5.4	101.4	65.3	166.7
f	3333	35.5	106.4	58.6	5.0	5.0	106.7	58.8	165.5
f	2296	32.1	106.8	50.7	6.8	5.6	86.7	41.2	127.9
f	2909	33.7	117.1	59.5	6.1	5.7	110.0	56.0	166.0
f	3777	35.8	148.8	82.9	6.1	4.8	115.5	64.6	180.1
f	1569	30.5	81.9	35.9	7.5	5.8	62.8	27.6	90.4
Mean	2683	35.3	99.1	55.1	5.83	5.49	93.3	51.1	144.4
Max.	3777	50.9	148.8	82.9	7.5	7.1	115.5	75.4	180.1
Min.	1569	28.8	72.6	30.2	4.3	4.6	62.8	27.6	90.4
S. D.					0.83	0.72			

PV = Plasma volume
RCV = Red cell volume
TBV = Total blood volume
RBC Ht. % = Red blood cell hematocrit percentage
S. D. = Standard deviation

(Simpson and Roe, 1939). The coefficients of correlation determined for the dye and P-32 methods were 36.36 and 35.88 per cent, respectively.

Blood volume was determined by the P-32 method in 4 opossums in addition to the 10 animals listed in Table II. Two of the animals were females of 2105 and 1750 grams of body weight with 6.3 and 6.7 ml. of blood per 100 gm. of body weight, respectively. The other two were males of 2505 and 2050 gm. of body weight with a blood volume of 8.0 and 7.5 ml. per 100 gm. of body weight, respectively. A standard deviation of 1.1 ml. of blood per 100 gm. of body weight was found for the 14 animals whose average weight was 2517 grams. The mean blood volume in these animals was 5.86 ml. of blood per 100 gm. of body weight. Graph II shows the relationship of milliliters of blood per 100 gm. of body weight to an increase in body weight for these animals. The line of trend was determined by the method of least squares as mentioned above. The coefficient of correlation for the 14 animals was 39.19 per cent.

Mixing Time and Rate of Disappearance of Dye and P-32

In addition to the blood volume determinations made on the opossums mentioned above, the mixing time and rate of disappearance of the T-1824 dye and the red blood cells labelled with radioactive phosphorus in the blood of the opossum was studied. In 7 of the animals listed in Table II blood samples were withdrawn at 1, 3, 5, and 10 minutes after injection of the dye and labelled cell solution. On Graph III is shown

the calculated blood volume in milliliters of blood per 100 grams of body weight plotted against time in minutes for the dye method. The points plotted represent the average figure for the 7 animals as calculated for each unit of time. There is a difference of 0.3 ml. of blood per 100 grams of body weight from the 1 to the 10 minute period according to these determinations. This indicates that the dye has become sufficiently well mixed in the blood so that a valid estimation of blood volume may be made on any sample withdrawn 1 to 10 minutes after injection of the T-1824 dye.

Data for the P-32 labelled cells of the 7 opossums used are presented in Graph IV. Here there is a difference of $\approx$ 0.4 ml. of blood per 100 gm. of body weight over the 1 to 10 minute sampling period. This insignificant variation is also an indication that the labelled red blood cells have been sufficiently well mixed by 1 minute so that a valid estimation of blood volume may be made on any sample withdrawn between the 1 and 10 minute period after injection.

Data for blood volume of the opossums used are presented in Table II. These data are shown on Graph V where the lines of trend were determined by the method of least squares. The coefficients of correlation for the T-1824 dye and P-32 methods in the 7 opossums are 34.86 and 44.35 per cent, respectively. The coefficient of variation (in Table II) was determined using the method of Simpson and Roe. From a statistical treatment of the significance of differences between the coefficients of variation for each method, it was found that the "t" value is 2.2. Since this "t" value is only on the borderline of

GRAPH II

M.L. OF BLOOD PER 100 GRAMS OF BODY WEIGHT

12

10

8

6

4

2

0

• = P^{32} METHOD —

RELATIONSHIP OF M.L. OF BLOOD
PER 100 GRAMS OF BODY WT.
TO INCREASE IN BODY WT. IN
OPOSSUMS.

BODY WEIGHT IN GRAMS

500 1000 1500 2000 2500 3000 3500 4000

GRAPH III

MIXING TIME IN OPOSSUMS

ML. OF BLOOD PER 100 GRAMS
OF BODY WEIGHT CALCULATED
FROM DATA OBTAINED BY THE
T-1824 DYE METHOD.

VERTICAL LINES
REPRESENT $M \pm 3\sigma$

significance, neither method can be expected to give a more correct blood volume figure than the other.

The mixing time of substances in the blood of other species has been investigated by other workers. The mixing time using T-1824 dye, in man, is 6 minutes according to Gibson (1937) and less than 10 minutes according to Campbell et al. (1948). Robinow (1940) reports that the mixing time for brilliant vital red in man is greater than 1 minute. In the dog, using T-1824 dye, Gilder et al. (1940) report a mixing time of less than 5 minutes; Miller (1947) reports 4 to 6 minutes; and Lawson et al. (1947) report 3 to 5 minutes. Also in dogs, Hahn et al. (1941) report a mixing time with radioiron of less than 10 minutes; Gellhorn et al. (1944), using radioiron, report 4 minutes as being sufficient for mixing. Hahn et al. (1940) report a $2\frac{1}{2}$ minute mixing time in a rabbit for P-32. Cruickshank (1945), in using T-1824 dye, reports that more than 1 minute is necessary for mixing in the cat.

The disappearance rate of the T-1824 dye and P-32 labelled cells was studied by the author in the blood of an opossum which weighed 2777 grams. These data are presented in Graph VI. The per cent dye decreased from 79.8 per cent at 1 hour to 33.6 per cent at 6 hours, 11.2 per cent at 18 hours, 7.2 per cent at 24 hours, and at the end of 48 hours there was no dye present in the blood. At the end of the first hour after injection of the labelled red blood cells, which at one minute had an activity of 2420 counts per minute per ml., 80.8 per cent remained, at 6 hours 55.9 per cent, at 18 hours 36.3 per cent, at 24 hours 29.5 per cent,

GRAPH IV

TABLE II

Opossum, Didelphis virginiana pigra
 Blood Volume in ml. per 100 gm. of Body Weight
 in Relation to Mixing Time

Weight	Methods	M i n u t e s				Average
		1	3	5	10	
2777	P-32	4.27	4.58	4.45	4.87	4.54
	T-1824	5.29	5.43	5.35	5.43	5.37
3085	P-32	5.59	5.45	5.40	5.72	5.54
	T-1824	5.55	5.60	5.42	5.63	5.55
3333	P-32	4.93	4.81	4.96	5.09	4.95
	T-1824	5.02	5.08	4.95	5.33	5.09
2296	P-32	5.10	5.57	5.28	5.58	5.38
	T-1824	6.35	6.85	6.80	6.99	6.75
2909	P-32	5.25	5.70	5.48	5.47	5.47
	T-1824	5.85	6.07	6.09	6.30	6.07
3777	P-32	4.15	4.28	4.76	4.81	4.50
	T-1824	5.93	6.04	6.31	6.20	6.07
1569	P-32	5.29	5.52	5.76	6.15	5.68
	T-1824	7.44	7.52	7.50	7.56	7.50
Mean	P-32	4.94	5.13	5.15	5.38	5.15
	T-1824	5.91	6.08	6.06	6.20	6.05
S. D.	P-32	0.49	0.50	0.42	0.46	0.45
	T-1824	0.74	0.78	0.82	0.77	0.77
V	P-32	0.099	0.097	0.081	0.085	0.087
	T-1824	0.125	0.128	0.135	0.124	0.120
Maximum	P-32	5.93	5.70	5.76	6.15	5.68
	T-1824	7.44	7.52	7.50	7.56	7.50
Minimum	P-32	4.15	4.28	4.45	4.81	4.50
	T-1824	5.02	5.08	4.95	5.33	5.09
M + 3 σ	P-32	6.41	6.63	6.41	6.76	6.50
	T-1824	8.13	8.42	8.52	8.51	8.36
M - 3 σ	P-32	3.47	3.63	3.89	4.00	3.80
	T-1824	3.69	3.74	3.60	3.89	3.74

GRAPH V

GRAPH VI

and at 48 hours 13.8 per cent remained in the blood.

Uptake by Red Blood Cells of P-32

The percentage uptake of radioactive phosphorus-32 by the red blood cells of an opossum was also studied. These data are presented in Graph VII. Twenty milliliters of blood were withdrawn from an opossum by a cardiac puncture into a heparinized syringe. The blood was centrifuged at 3000 rpm. for 30 minutes. The plasma was discarded and the cells were washed with iced, physiological, saline solution and centrifuged 3 times at 3000 rpm. for 5 minutes. These red blood cells were then made to the original volume of blood withdrawn from the animal with physiological saline solution. One milliliter of isotonic radioactive sodium phosphate was diluted with distilled water to make a volume of 250 ml. used as a standard. Then the radioactive sodium phosphate solution was added to an equal volume of the washed red blood cell solution. Six ml. of this mixture was placed in each of 5 centrifuge tubes which were then placed in a water bath of 37 degrees centigrade. Every few minutes the solutions were stirred with small, glass rods. At 5 and 30 minutes and at 1, 2, and 3 hours a tube was removed, the cells washed 3 times with iced physiological saline, and centrifuged for 5 minutes at 3000 rpm. after each washing. The labelled cells were made up to 500 ml. and counted on a Geiger counter as described in Appendix I. A comparison of the activity of the standard with the activity of the sample indicates the amount of P-32 taken up by the red blood cells. If the standard be assumed as 100 per cent there was 11.2 per cent radioactivity

GRAPH VII

PERCENT P^{32} INCORPORATED
IN THE RED BLOOD CELLS
OF AN OPOSSUM

present in the sample at 5 minutes, 28.6 per cent at 30 minutes, 45.3 per cent labelled at 1 hour, 56.4 per cent labelled at 2 hours, and 55.6 per cent labelled at 3 hours.

Other Data in Regard to the Opossum

One opossum which had a red blood cell count of 2,420,000 per mm., and 6.3 gm. of hemoglobin per 100 cc. of blood was evidently abnormal for which reason it was not included in Table III. The animal was a female weighing 1345 gm. and died within a week after capture.

Other data which may have an important bearing on the results reported here in regard to opossums studied are given in Table IV.

TABLE III

Opossum, Didelphis virginiana pigra

Sex	Wt. (gm.)	RBC Count $\times 10^6$	Hemoglobin (gm./100 cc.)	Oxygen Capacity Vol. %	Rectal T. °C.
m	610	3.17	6.8	9.2	32.8
f	1334	3.18	6.2	8.4	34.6
f	2370	6.53	7.3	9.9	33.6
f	2105	5.23	11.9	16.1	35.1
f	1594	6.23	10.8	14.6	34.0
m	3132	6.05	13.9	18.9	35.6
m	2588	6.15	13.2	17.9	35.0
m	2505	5.72	12.2	16.6	35.1
m	2632	6.21	14.5	19.7	35.2
m	2269	6.46	14.0	19.0	35.0
m	2050	6.46	15.4	20.9	36.3
f	1750	3.16	8.9	12.1	34.3
f	2640	6.34	12.2	16.6	36.3
f	1864	4.90	9.9	13.4	36.6
f	2777	4.82	9.9	13.4	33.3
f	3085	5.04	13.2	17.9	34.5
f	3333	5.05	11.3	15.3	35.2
f	2296	5.03	9.0	12.2	35.8
f	2909	4.96	11.7	15.9	36.4
f	3777	4.88	11.8	16.0	36.2
f	1569	5.46	10.0	13.6	36.3
Mean		5.28	11.1	15.0	35.1
S. D.		1.05	2.5	3.4	1.1
Maximum		6.53	15.4	20.9	36.6
Minimum		3.16	6.2	8.4	32.8
$M \pm 3\sigma$		(8.43-2.13)	(18.6-3.6)	(25.2-4.8)	(38.4-31.8)

Scott (1938) reports for D. virginiana

Mean values for 8	5.12		
Mean values for 23		14.9	
Mean values for 16			34.6

BLOOD VOLUME DETERMINATIONS IN OTHER ANIMALS

In addition to the opossums, the blood volume of one raccoon, one dog, 8 rabbits, and 10 swine was investigated, as already mentioned. Data for blood volume in normal animals are given in Table VI.

Raccoon

A male raccoon (Procyon lotor elucus) weighing 1796 gm. had a red blood cell count of 7,770,000 per cmm., and 11.6 gm. of hemoglobin per 100 cc. of blood. This animal had a rectal temperature of 37.6° C.

Dog

The dog, a male, weighed 19,051 gm. It had a red blood cell count of 6,270,000 per cmm., and 12.3 gm. of hemoglobin per 100 cc. of blood. This dog seemed to be part boxer and had been maintained on kennel food (Purina dog chow) for about 2 months before he was used in the experiment. He was not fat, but sleek, had a good hair coat with somewhat of a sheen to it, and was a very docile animal.

Rabbits

Six rabbits used for blood volume determinations had been maintained by Mr. L. R. Arrington on a diet which contained different levels of molybdenum. Two animals were used as controls. The oxygen capacity of each animal was determined by multiplying the hemoglobin concentration in grams per 100 ml. of blood by 1.36 (Bernhart and Steggs, 1943). The data obtained for the rabbits are presented in Table IV.

TABLE IV
Domestic Rabbits

Sex	Wt. (gm.)	RBC $\times 10^6$	Hb $\frac{\text{gm.}}{100 \text{ cc.}}$	Oxygen Capacity (Vol. %)	T-1824 Dye			P-32			Blood (cc/100 gm)		Diet
					PV	RCV	TBV	PV	RCV	TBV	Dye	P-32	
f	2280	5.08	12.0	16.3	78.0	50.6	129.1	64.3	41.4	105.7	4.63	5.66	Normal
m	2269	6.46	14.0	19.0	—	—	—	73.5	42.6	115.7	—	5.10	Normal
m	2140	6.29	13.1	17.8	68.3	33.8	102.1	—	—	—	4.77	—	Low Mo
f	2230	6.43	13.0	17.6	86.8	48.8	135.6	98.9	55.7	154.6	6.08	6.93	Low Mo
m	1947	5.54	10.7	14.5	91.8	53.7	145.5	96.0	54.8	150.8	7.47	7.74	High Mo
f	1825	3.50	7.3	9.9	79.4	21.6	101.0	87.4	24.5	111.9	5.54	6.13	High Mo
m	1255	1.20	3.2	4.3	70.6	8.2	78.4	—	—	—	6.24	—	High Mo
f	913	0.90	2.0	2.7	48.8	2.8	51.6	—	—	—	5.65	—	High Mo

INVESTIGATIONS OF DUROC-JERSEY SWINE

The blood volume of 12 pigs was investigated by Heissler (1886) by bleeding the animal to death and comparing the weight of the collected blood to the net weight of the animal. The pigs he used weighed from 31.95 to 228.0 kilograms. He reported a mean blood value of 4.60 per cent of the body weight.

The blood volume of Hampshire swine using radioactive phosphorus-32 was studied by Hansard et al. (1951). There is a mean value of 6.03 ml. of blood per 100 gm. of body weight for 16 swine investigated. The swine ranged in weight from 3.62 to 167.83 kilograms.

The results of blood volume studies in 10 Duroc-Jersey swine were reported by Burke, Shirley, and Davis (1951). The pigs they used ranged in weight from 10.43 to 96.61 kilograms. The blood volume in 9 of these 10 swine was also investigated by the T-1824 dye method, which has not been previously reported.

Blood Volume Determinations with T-1824 Dye and P-32

The T-1824 dye and P-32 methods were concurrently employed to determine blood volume in these animals. About 10 ml. of blood were removed directly from the heart with a heparinized $\frac{1}{4}$ inch, 22 gauge needle and syringe. Some of the blood was used for a red blood cell count and hemoglobin measurement; the rest of the blood was used as listed in the procedure for the T-1824 dye and P-32 methods. The pig weighing 69,400 gm. was treated the same as the others except that the blood removal and injections

were made through the right and left ear veins, respectively. The T-1824 dye solution was prepared so that it contained either 2 or 4 mgm. per ml. Approximately 10 ml. (the exact amount was recorded) of dye solution was injected directly into the ventricle of the heart and immediately afterwards the P-32 solution was injected. In Table V the data are presented that were obtained for the 10 pigs.

Other Data in Regard to Swine

The oxygen capacity was determined by multiplying the hemoglobin in gm. per 100 ml. of blood by the factor 1.36 mentioned previously.

The mean values for total plasma volume, total red cell volume, and total blood volume as determined with the T-1824 dye method were 65 ml. higher, 3 ml. lower, and 71 ml. higher, respectively, than with the P-32 method.

By using the expression mean plus or minus three times the standard deviation, the red blood cell count has values of 9.59 to 5.55×10^6 ; for hemoglobin the values are 13.8 to 8.12 gm. per 100 ml. of blood; the values for oxygen capacity are 20.3 to 9.5 volumes per cent; the values for total blood volume range from 15.1 to 2.5 and 14.1 to 3.9 ml. of blood per 100 gm. of body weight for the T-1824 dye and P-32 methods, respectively.

The data showing the relation of milliliters of blood per kilogram of body weight to weight of the swine obtained by the dye and P-32 methods calculated from samples taken 10 minutes after the injection of the dye and labelled cell solutions are presented in Graph VIII. These values indicate that there is a decrease in blood volume per kilogram of

TABLE V.

Duroc-Jersey Pigs

Sex	Wt. (gm.)	RBC $\times 10^6$	Hemoglobin (gm/100 cc)	Oxygen Capacity (Vol. %)	ml. of blood per 100 gm. wt.				P-32		Corrected RBC Ht. %
					T-1824		T-1824		PV	TBV	
					PV	RCV	TBV	P-32	PV	RCV	TBV
m	10430	7.97	12.9	17.5	---	---	---	---	739	362	1101
m	11969	6.89	11.0	14.9	1140	430	1571	10.5	1293	409	1783
m	15775	5.26	10.9	14.8	1377	506	1883	11.9	1210	445	1656
f	15873	7.34	11.2	15.2	1044	439	1483	9.3	844	415	1260
f	20430	8.34	11.8	16.0	1530	777	2308	11.2	1269	647	1916
m	21220	7.99	11.0	14.9	1327	629	2020	9.5	1433	748	2182
f	33566	6.65	11.7	15.9	1689	897	2587	7.7	1842	1101	3244
f	58288	6.51	9.4	12.7	3421	1356	4777	8.1	3346	999	4345
m	69400	6.77	9.6	13.0	2727	1574	4301	6.2	3232	1867	5099
m	96617	7.07	10.8	14.6	3445	1814	5260	5.4	3802	2009	5611
Mean	35656	7.07	11.0	14.9	1966	935	2910	8.16	7.96	1901	938
Max.	96617	8.34	12.9	17.5	3445	1814	5260	11.9	3802	2009	5611
Min.	10430	5.26	9.4	12.7	1044	430	1483	5.4	739	362	1101
S.D.		0.84	0.96	1.8				2.1			

PV = Plasma volume

RCV = Red cell volume

TBV = Total blood volume

RBC Ht. % = Red blood cell hematocrit percentage

S. D. = Standard deviation

GRAPH VIII

body weight with an increase in weight of the swine. This relationship shows a good correlation for each method when viewed statistically. The coefficient of correlation for the T-1824 dye is 87.20 per cent and for the P-32 method is 86.46 per cent. The two best fit lines were determined by the previously mentioned method of least squares.

In line with the findings of Mitchell and Hamilton (1929) and Bull and Carroll (1937) the decrease in blood volume per unit of body weight, which is correlated with an increase in body weight, may be caused by a decrease in water concentration and an increase in fat.

Mixing Time and Rate of Disappearance of Dye and P-32

The rate of disappearance of the P-32 and the T-1824 dye from the blood of a pig was measured. The pig weighing 21,220 grams was used in this experiment. At 30, 45, and 60 minutes the P-32 concentration was essentially the same as at 10 minutes indicating that the blood was thoroughly mixed within 10 minutes and that few of the labelled cells had lost their activity. Values obtained at 1, 3, 5, 18, 24, and 48 hours after injection showed a decrease with time as shown on Graph IX. At 1 hour there was 96.0 per cent of the P-32 remaining in the blood, at 3 hours there was 85.5 per cent, 80.6 per cent at 5 hours, 46.5 per cent at 18 hours, 29.0 per cent at 24 hours, and 13.0 per cent at 48 hours remaining. On Graph IX are also plotted the data in relation to the disappearance of the dye from the blood. At 30, and 45 minutes there was 91.5 and 85.5 per cent T-1824 dye remaining. At 1 hour 80.7 per cent remained,

GRAPH IX

66.2 per cent at 3 hours, 53.0 per cent at 5 hours, 20.4 per cent at 18 hours, 15.6 per cent at 24 hours, and at the end of 48 hours, no measurable amount of dye remained in the blood.

The disappearance rate of P-32 over a period of 5 days was evaluated in a pig weighing 14,969 grams. There was a larger dose of P-32 given to this pig than to the one weighing 21,220 grams since the activity of the radiophosphorus was to be evaluated over a longer period. For the pig weighing 21,220 grams, there was a concentration of P-32 present at 10 minutes of 382 counts per minute per milliliter of blood. For the pig weighing 14,969 grams, the concentration of P-32 at 10 minutes was 961 counts per minute per milliliter of blood. It was found that in the pig weighing 14,969 grams there was 48.5 per cent of P-32 remaining in the blood at the end of 24 hours, 20.9 per cent at the end of 48 hours, 16.3 per cent at 72 hours, 8.7 per cent at the end of 4 days, and 4.0 per cent remained at the end of 5 days. These values are shown on Graph I.

GRAPH X

PERCENT OF P^{32} LABELLED RED
BLOOD CELLS REMAINING IN THE
BLOOD (PIG)

PERCENTAGE

TIME IN DAYS

BLOOD VOLUME VALUES FROM AVAILABLE SOURCES

Blood volume values are presented in Table VI as the milliliters of blood per 100 grams of body weight. The data appearing in parentheses are those reported by investigators as the amount of blood present in per cent of body weight of the animal. In order to make the two values comparable, the author has converted per cent body weight readings to milliliters of blood per 100 grams of body weight. This was done by dividing per cent body weight values by the specific gravity of blood of different animals as reported by Dukes (1937).

From a consideration of the different materials and methods employed in the determination of blood volume in an animal, certain methods have become obsolete as already explained. For this reason an asterisk is placed beside the values in Table VI which seem valid. A "?" indicates that not enough information is available to me concerning methods used to evaluate them positively.

TABLE VI

Blood Volume in ml. per 100 gm. of Body Weight

ORDER Family	No.	Blood Volume	Method	Authority
MARSUPALIA				
Didelphidae				
<u>Didelphis vir-</u> <u>giniana pigra</u>	10	#5.83	Evans blue	Burke
<u>Didelphis vir-</u> <u>giniana pigra</u>	10	#5.49	P-32	Burke
CHIROPTERA				
Vespertilionidae				
<u>Vespertilia</u> <u>noctula</u>	2	(8.3)	Bleeding	Welcker-1858
PRIMATES				
Hominidae				
Man	2	7.2 (7.7)	Bleeding	Bischoff-1856
Man	14	4.5 (4.78)	CO	Haldane-1899-1900
Man (male)	9	5.5	CO	Oerum-1908
Man (female)	3	4.9	CO	Oerum-1908
Man	4	5.2(5.58)	CO	Flesh-1909
Man	2	7.1 (7.5)	CO	Douglas-1910
Man	6	5.95	CO	Salvesen-1919
Man	6	6.8	CO	Smith-1924-25
Man	16	6.42	CO	Chang-1928
Man (infants)	10	7.1	CO	McIntosh-1929
Man(horizontal)	8	7.14	CO	Waterfield-1931
Man (vertical)	8	6.45	CO	Waterfield-1931
Man	12	6.07	CO	Brednow-1931
Man		9.62	CO	Baxett-1940
Man	10	9.7	CO	Asmussen-1941
Man	9	10.13	CO	Hevesy-1943-44
Man	9	8.02	CO	Hopper-1944

TABLE VI (Continued)

Blood Volume in ml. per 100 gm. of Body Weight

ORDER Family	No.	Blood Volume	Method	Authority
Man	12	7.44	CO	Courtice-1949a
Man	4	7.8 (8.3)	NaCl	Kottmann-1906
Man	10	7.4 (7.9)	Tetanus anti- toxin	Fries-1911
Man	18	8.90	Vital red	Keith-1915
Man	5	8.7	Vital red	Bock-1921
Man (infants)	30	14.7	Vital red	Lucas-1921
Man	11	4.6 (4.9)	Vital red	Lindhard-1926a
Man (infants)	35	7.6	Vital red	Darrow-1928
Man	11	8.90	Vital red	Brednow-1931
Man	2	7.12	Vital red	Dieckmann-1934
Man	2	7.4	Vital red	Friedlander-1936
Man (infants)	36	10.1	Br. vital red	Bakwin-1924
Man	6	9.6	Br. vital red	Smith-1924-25
Man	10	9.3	Br. vital red	McIntosh-1929
Man	20	8.7	Br. vital red	Robinow-1940
Man		10.32	Congo red	Bazett-1940
Man (male)	49	8.86	Congo-vital red	Rowntree-1929
Man (female)	25	8.57	Congo-vital red	Rowntree-1929
Man	16	*7.26	Evans blue	Gibson-1937a
Man (male)	49	*7.71	Evans blue	Gibson-1937b
Man (female)	41	*6.61	Evans blue	Gibson-1937b
Man	15	*7.99	Evans blue	Harrington-1940
Man (children)	16	*8.20	Evans blue	Schlutz-1940
Man	10	*9.2	Evans blue	Asmussen-1941
Man (infants)	9	*6.99	Evans blue	Brines-1941
Man (children)	41	*6.99	Evans blue	Brines-1941
Man	11	*7.67	Evans blue	Davis-1942
Man	9	*8.83	Evans blue	Hevesy-1943-44
Man	9	*8.05	Evans blue	Hopper-1944
Man	40	*9.3	Evans blue	Gibson-1946
Man (plasma)	25	*8.07	Evans blue	Nitsche-1947
Man (serum)	25	*7.66	Evans blue	Nitsche-1947
Man	6	*7.8	Evans blue	Mayerson-1948

TABLE VI (Continued)

Blood Volume in ml. per 100 gm. of Body Weight

ORDER Family	No.	Blood Volume	Method	Authority
Man	12	*7.25	Evans blue	Courtice-1949a
Man (male)	25	*8.26	Evans blue	Gregersen-1949
Man (female)	24	*7.78	Evans blue	Gregersen-1949
Man	33	78.13	Sodium thiocyanate	Stewart-1940
Man	2	*7.4	P-32	Govaerts-1942
Man	9	*7.83	P-32	Hevesy-1943-44
Man	6	*7.35	P-32	Nylin-1945
Man	9	*6.98	P-32	Kelly-1948
Man	6	*7.6	P-32	Mayerson-1948
Man	5	*6.73	P-32	Scott-1951
Monkey	2	(8.15)	Sodium chloride specific gravity	Sherrington-1893
LAGOMORPHA				
Leporidae				
Rabbit	5	5.2 (5.55)	Bleeding	Welcker-1858
Rabbit	15	3.9 (4.13)	Bleeding	Ranke-1871
Rabbit	9	7.4 (7.8)	Bleeding	Steinberg-1873
Rabbit	22	4.6 (4.92)	Bleeding	Dreyer-1910-11
Rabbit	52	4.77	Bleeding	Boycott-1911-12
Rabbit (male)	6	6.6 (7.0)	Bleeding	Levine-1941
Rabbit	8	8.0 (8.4)	Sodium-chloride specific gravity	Sherrington-1893
Rabbit	1	7.8 (8.21)	Hemoglobin	Barratt-1909
Rabbit	24	5.3 (5.66)	Serum ppt.	Schurer-1911
Rabbit	2	6.0 (6.3)	Antitoxin	Von Bering-1911
Rabbit	52	4.9 (5.2)	Ringer's	Boycott-1914

TABLE VI (Continued)

Blood Volume in ml. per 100 gm. of Body Weight

ORDER Family	No.	Blood Volume	Method	Authority
Rabbit (male)	7	4.6 (4.85)	CO	Douglas-1906
Rabbit (female)	4	5.0 (5.32)	CO	Douglas-1906
Rabbit	3	6.3 (6.7)	CO	Oerum-1908
Rabbit	22	5.37	CO	Boycott-1909
Rabbit	11	5.3 (5.6)	CO	Nelson-1909
Rabbit	36	5.36	CO	Boycott-1911-12
Rabbit	126	5.1 (5.45)	CO	Dreyer-1913
Rabbit (male)	7	4.95	CO	Salvesen-1919
Rabbit (female)	7	5.0	CO	Salvesen-1919
Rabbit	15	5.77	CO	Courtice-1949b
Rabbit	7	5.44	Acacia	Meek-1918-19
Rabbit	4	6.49	Acacia	McQuarrie-1920
Rabbit	13	8.2 (8.7)	Vital red	Went-1929
Rabbit	11	9.1 (9.6)	Vital red	Nice-1934
Rabbit	24	75.5 (5.82)	Precipitin	Culbertson-1934
Rabbit	60	*7.0	Evans blue	Courtice-1943
Rabbit	15	*5.75	Evans blue	Courtice-1949b
Rabbit	39	*6.98	Evans blue	Aikawa-1950
Rabbit	1	*4.63	Evans blue	Burke
Rabbit	2	*4.7	P-32	Hahn-1940
Rabbit	9	*5.3	P-32	Anderson-1942
Rabbit	2	*5.38	P-32	Burke

RODENTIA

Muridae

Mice	5	7.3 (7.7)	Bleeding	Welcker-1858
Mice	19	5.4 (5.77)	Bleeding	Dreyer-1910-11
Mice	12	4.9 (5.2)	Bleeding	Furth-1946
Rat (albino)	15	4.5	Bleeding	Jolly-1905
Rat	25	5.8	Bleeding	Chisolm-1911
Rat	87	6.28	Bleeding	Boycott-1911-12
Rat (male)	46	6.35	Bleeding	Oakley-1940
Rat (female)	54	6.29	Bleeding	Oakley-1940
Rat	4	5.59	Bleeding	Gruneberg-1941
Rat (albino)	10	5.25	Bleeding	Barlow-1929

TABLE VI (Continued)

ORDER Family	No.	Blood Volume	Method	Authority
Rat	8	6.4 (6.8)	Vital red	Cartland-1928
Rat	9	7.0 (7.4)	Vital red	Went-1929
Rat (single)	4	9.72	Vital red	Hill-1933
Rat (parabiotic)	7	10.21	Vital red	Hill-1933
Rat	8	6.38	Vital red	Orten-1933
Rat (albino)	20	4.3	Vital red	Griffith-1937
Rat	16	4.5	Vital red	Griffith-1940
Rat	6	6.6 (7.02)	Vital red	Evans-1941
Rat (Wistar)	53	*7.98	Evans blue	Beckwith-1941
Rat (Sherman)	34	*8.9	Evans blue	Metcoff-1944
Rat (Long-Evans)	18	*8.9	Evans blue	Hechtner-1945
Rat (Sprague-Dawley)	9	*8.28	Evans blue	Stanley-1946
Rat	23	*6.17	Evans blue	Benditt-1946
Rat (Wistar 140 gm.)	20	*9.0	Evans blue	Wang-1949
Rat (Wistar 140 gm.)	49	*7.0	Evans blue	Wang-1949
Mice (male)	3	711.27	Evans blue	Wish-1950
Mice (female)	2	711.64	Evans blue	Wish-1950
Mice (male)	6	79.36	Evans blue	Wish-1950
Mice	9	710.3 (10.9)	Evans blue	Furth-1946
Rat	10	6.13	CO	Scott-1924
Rat	125	6.9	Hemoglobin	Lippman-1947
Rat (Slonaker)	12	*4.87	P-32	Berlin-1949
Rat (Slonaker x Curtis-Dunning)	30	*4.52	P-32	Berlin-1949
Mice (male)	3	77.52	P-32	Wish-1950
Mice (female)	2	77.18	P-32	Wish-1950
Mice (male)	6	76.39	P-32	Wish-1950
Rat (Slonaker)	12	*4.75	Fe-59	Berlin-1949
Rat (Sprague-Dawley)	12	*4.87	Fe-59	Sharpe-1950
Rat (Sprague-Dawley)	4	*5.26	Fe-55	Sharpe-1950
Rat (Sprague-Dawley)	6	*4.9	Fe-55 Hemoglobin	Sharpe-1950

TABLE VI (Continued)

ORDER Family	No.	Blood Volume	Method	Authority
Gliridae				
<u>Myoxus glis</u>	3	5.5 (5.8)	Bleeding	Welcker-1858
Caviidae				
Guinea pig	6	(7.8)	Bleeding	Steinberg-1873
Guinea pig	9	(4.10)	Bleeding	Dreyer-1910-11
Guinea pig	18	(6.4)	Vital red	Went-1929
CARNIVORA				
Canidae				
Dog	5	7.0 (7.4)	Bleeding	Welcker-1858
Dog	6	7.4 (7.87)	Bleeding	Spiegelberg-1872
Dog	73	8.0 (8.46)	Bleeding	Collin-1873
Dog	6	7.1	Bleeding	Steinberg-1873
Dog	3	7.7 (8.2)	Bleeding	Flesch-1909
Dog	8	7.1	Bleeding	Harris-1920
Dog	14	8.27	Bleeding	Smith-1921
Dog	4	7.7 (8.2)	Sodium chloride specific gravity	Sherrington-1893
Dog	3	7.7 (8.2)	Hemoglobin-NaCl	Flesch-1909
Dog	18	9.74	Acacia	Meek-1918
Dog	15	9.76	Acacia	McQuarrie-1920
Dog	19	9.2	Hemoglobin	Franke-1920-21
Dog	9	10.13	Hemoglobin	Lee-1921
Dog	9	8.2	CO	Grehant-1882
Dog	4	7.78	CO	Arnold-1921
Dog	14	8.69	CO	Smith-1921
Dog	1	6.88	CO	Govaerts-1942
Dog	16	9.54	CO	Hepper-1944
Dog	6	10.11	CO	Root-1946

TABLE VI (Continued)

Blood Volume in ml. per 100 gm. of Body Weight

ORDER Family	No.	Blood Volume	Method	Authority
Dog	4	8.4 (8.9)	Vital red	Harris-1920
Dog	22	10.13	Vital red	Hooper-1920
Dog (male)	16	7.30	Vital red	Leichsenring-1932
Dog (female)	16	7.06	Vital red	Leichsenring-1932
Dog	106	10.3	Vital red	Crus-1945
Dog	3	12.0	Br.vital red	Smith-1920
Dog	14	10.39	Br.vital red	Smith-1921
Dog	3	8.5-9.0	Br.vital red	Davis-1940
Dog	10	8.22	Br.vital red	Hahn-1942
Dog	6	7.1 (7.6)	Congo red	Harris-1920
Dog	25	11.07	Congo red	Powers-1930
Dog	6	9.3	Dyes	Dawson-1920
Dog	50	*9.27	Evans blue	Gibson-1938
Dog	106	*8.32	Evans blue	Bonnycastle-1942
Dog	29	*7.9	Evans blue	Courtice-1943
Dog	16	*9.52	Evans blue	Hopper-1944
Dog	39	*10.0	Evans blue	Gibson-1946
Dog	6	*10.18	Evans blue	Root-1946
Dog	6	*9.66	Evans blue	Bonnycastle-1947
Dog	10	*10.5	Evans blue	Krieger-1948
Dog	1	*8.92	Evans blue	Burke
Dog	6	*7.77	Radioiron	Hahn-1941
Dog	13	*6.61	Fe-59	Hahn-1942
Dog	1	*6.73	P-32	Govaerts-1942
Dog	10	*9.1 (9.7)	P-32	Krieger-1948
Dog	1	*9.74	P-32	Burke
Dog	10	78.9 (9.4)	I-131	Krieger-1948

Procyonidae

<u>Procyon lotor elucus</u>	1	*8.5	Evans blue	Burke
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TABLE VI (Continued)

Blood Volume in ml. per 100 gm. of Body Weight

ORDER Family	No.	Blood Volume	Method	Authority
Felidae				
Cat	1	6.1 (6.5)	Bleeding	Welcker-1858
Cat	9	7.4 (7.8)	Bleeding	Steinberg-1873
Cat	2	4.5 (4.8)	Bleeding	Harris-1920
Cat	8	5.2 (5.5)	Acacia	Meek-1918
Cat	2	5.9 (6.3)	Vital red	Harris-1920
Cat	6	5.8 (6.2)	Vital red	Went-1929
Cat	4	5.55	Vital red	Friedlander-1936
Cat	3	5.2 (5.5)	Congo red	Harris-1920
PERISSODACTYLA				
Equidae				
Horse	77	8.2 (8.72)	Bleeding	Colin-1873
Horse	38	9.2 (9.75)	Bleeding	Heissler-1886
Horse	2	*7.2	Evans blue	Courtice-1943
ARTIODACTYLA				
Suidae				
Pig	12	4.3 (4.60)	Bleeding	Heissler-1886
Pig (Duroc-Jersey)	10	*7.96	P-32	Burke-1951
Pig (Hampshire)	16	*6.03	P-32	Hansard-1951
Pig (Duroc-Jersey)	9	*8.16	Evans blue	Burke
Bovidae				
Cow	14	7.4 (7.71)	Bleeding	Heissler-1886
Cow	36	4.7	Bleeding	Trowbridge-1915
Cow	10	5.05	Bleeding	Haecker-1920
Cow	14	3.9 (4.13)	Bleeding	Turner-1931

TABLE VI (Continued)

Blood Volume in ml. per 100 gm. of Body Weight

ORDER Family	No.	Blood Volume	Method	Authority
Gow (growing)	54	6.0 (6.25)	Congo-vital red	Turner-1931
Gow (non-lactating)	24	6.1 (6.38)	Congo-vital red	Turner-1931
Gow (lactating)	41	7.7(8.11)	Congo-vital red	Turner-1931
Cow	19	5.97	Br.vital red	Miller-1932
Cow	3	5.4 (5.68)	Ischemolytic serum	Todd-1911-12
Lamb	3	5.5	Bleeding	Barcroft-1937
Lamb (birth)	4	16.9	Congo red	Gotsev-1939
Lamb (1 month)	4	12.9	Congo red	Gotsev-1939
Sheep	42	12.5(13.07)	Bleeding	Colin-1873
Sheep	20	7.6(8.01)	Bleeding	Reissler-1886
Sheep	2	6.3 (6.55)	Antitoxin	Cohnstein-1884
Sheep (term)	3	*6.0	Evans blue	Barcroft-1939
Sheep (6 months)	3	*6.1	Evans blue	Barcroft-1939
Sheep	42	6.47	Br.vital red	Baker-1933
Goat	1	5.8 (6.2)	Bleeding	Welcker-1858
Goat	4	13.3	Chicago blue 6B	Elliott-1934
Goat	30	*7.0	Evans blue	Courtice-1943

DETERMINATION OF OXYGEN CAPACITY OF THE BLOOD

Obviously there is a direct relation between total blood volume and total oxygen capacity of the blood. Various attempts to determine oxygen capacity have been made.

Obsolete Methods

A measurement of the oxygen capacity of a porpoise and of a dog which weighed 15.5 and 13.8 kilograms, respectively, was made by Jolyet and Sellier (1896-1897) who used the carbon monoxide method of determining total blood volume described by Grehant and Quinquand. They measured the saturation of the blood with carbon monoxide by letting the animal breathe a known amount of carbon monoxide and colorimetrically comparing the withdrawn sample of blood with a standard in which a definite amount of blood was saturated with a known amount of carbon monoxide. From this percentage of saturation of the blood, the total oxygen capacity was determinable, since a molecule of carbon monoxide replaces a molecule of oxygen in combination with hemoglobin.

The total blood volume of either animal was not reported, but total blood volume can be determined if total oxygen capacity is known. Jolyet and Sellier report that the total oxygen capacity of the porpoise was 726 cubic centimeters and that the blood had 30.9 cubic centimeters of oxygen combined with 100 cubic centimeters of blood. The total blood volume can be calculated as: $726 \text{ cc.} \times 100/30.9 \text{ cc.} = 2994.2 \text{ cc.}$ of blood. The amount of blood present per 100 grams of body weight is:

$2994.2 \text{ cc.} / 15,500 \text{ gm.} \times 100 \text{ gm.} = 19.3 \text{ cc.} / 100 \text{ gm.}$ body weight. These are the only available data in regard to the total blood volume of a cetacean. Likewise, the total blood volume of the dog can be calculated from their data, since they report that the total oxygen capacity of the dog they studied was 550 cc. and it had an oxygen capacity of 23.6 volumes per cent. The total blood volume would be:

$550 \text{ cc.} \times 100 / 23.6 \text{ cc.} = 2326.5 \text{ cc.}$ of blood. The amount of blood present per 100 gm. of body weight is:

$2326.5 / 13,800 \text{ gm.} \times 100 \text{ gm.} = 16.8 \text{ gm.}$ of body weight. The total blood volume of the dog determined by this method is noticeably higher than the findings of other investigators as reported in Table VI. The total blood volume for the porpoise may likewise be relatively high.

The values for oxygen capacity of man, oxen, a rabbit, a horse, and a sheep were reported by Haldane and Smith (1899-1900). These values are given in Table VIII. An apparatus was described by Barcroft and Haldane (1902) in which they state oxygen capacity of the blood can be determined manometrically with as little as 1.0 cc. of blood. They report a difference in oxygen capacity of only 0.04 and 0.05 volume per cent for the ox and the cat, respectively, with their method in comparison with the carbon monoxide method.

Present Day Methods

Van Slyke Method

A manometric apparatus for the determination of oxygen capacity was described by Van Slyke (1918). Van Slyke and Stadie (1921) reported

a 0.92 volumes per cent higher mean value for oxygen capacity studied in 5 men with the Van Slyke apparatus than with the carbon monoxide method. The Van Slyke method of determining oxygen capacity has been used almost universally since 1921.

Roughton-Scholander Syringe Method

A micro-method whereby only 39.3 cmm. of blood are needed for an oxygen capacity determination was described by Roughton and Scholander (1943). The determination is carried out in a 1.0 cubic centimeter syringe with a fused capillary attached to the syringe. They report an average discrepancy for 3 minutes of only 0.05 volumes per cent with their method in comparison with the Van Slyke method. Grant (1947) modified the syringe method so that aeration of the blood sample could be completed within the syringe. In a study of 6 dogs, he reports the same mean values by means of the micro-method as obtained by use of the Van Slyke apparatus.

With relation to the size of the blood sample needed for a determination, Roughton and Scholander's syringe method is very adaptable to study oxygen capacity in small animals. The procedure involves the delivery of 39.3 cubic millimeters of blood into the syringe by a pipette (Fig. 1) where the blood is mixed with physiological saline solution. Fig. 2 shows the transfer of blood under saline solution from the pipette to the capillary; in A, the pipette containing the blood is fitted into the bottom of the cup of the capillary; in B, the blood has been withdrawn into the syringe by withdrawal of the plunger; in C, the saline solution is drawn into the syringe on top of the blood. Aeration of the blood sample is accomplished by turning the syringe on its horizontal axis for

Fig. I

Fig. 2

5 minutes with an occasional turn at right angles. Once a minute during the aeration, the air supply is replenished. Then a solution containing potassium ferricyanide, potassium bicarbonate, and saponin is introduced into the syringe. The ferricyanide converts the hemoglobin to methemoglobin or reduced hemoglobin, and in doing so, releases the gases oxygen, nitrogen, and carbon monoxide. The bicarbonate supplies the carbon dioxide in which the gases from the blood are taken up, and the saponin lyses the red blood cells. A buffer solution containing sodium acetate and glacial acetic acid is added to the syringe which acts to keep the pH constant when the alkalies are later added. Urea is added to the syringe because of its protein dissolving ability. After the acetate buffer and urea solution is added, the mixture in the syringe is shaken (Fig. 3) to evolve the gases from the blood. Then sodium hydroxide is added to absorb the carbon dioxide as shown in Fig. 4 (A, before absorption, B, after absorption). Alkaline pyrogallol is then added to absorb the oxygen. Temperature equilibration is carried out at room temperature in a beaker of water, before and after the pyrogallol is added, as shown in Fig. 5. The difference in readings of the divisions on the capillary of the syringe before and after the introduction of the pyrogallol represents the oxygen capacity of the blood.

A detailed procedure followed by the author for the oxygen capacity investigation is outlined in Appendices VI, VII, VIII, and IX.

Oxygen Capacity Determinations of the Present Investigation with the Syringe Method

The author determined the oxygen capacity per unit of blood of 43

Fig. 3

Fig. 4

C

Fig. 5

mammals by the Roughton-Scholander syringe method. The mammals investigated were: 20 albino rats (Rattus norvegicus), 3 cotton mice (Peromyscus gossypinus), 3 Florida wood rats (Neotoma floridana), 3 domesticated guinea pigs (Cavia sp.), 7 golden hamsters (Cricetus auratus), and 7 bats (Myotis austroriparius). The data obtained in this study are presented in Table VII.

The albino rats were laboratory raised. Their body weights ranged from 7 to 302 grams. The rat weighing 7 gm. had an oxygen capacity of 5.3 volumes per cent. This rat was 5 days old at the time of the oxygen capacity determination. In rats with greater body weight, the oxygen capacity of the blood was greater as shown in the table. The mean oxygen capacity of 8 rats with a mean weight of 249 gm. was 18.1 volumes per cent.

Some data are available in regard to the number of red blood cells and the amount of hemoglobin per unit volume in rats of different ages. The red blood cell count of 162 rats from 1 to 8 days of age was 2,000,000 per cmm. according to Reich and Dunning (1943). Gardner (1947) reports that "the average number of red blood cells in the adult rat, regardless of sex or strain, is 8,520,000 per cmm." She also reports that the hemoglobin content of the blood of young rats averages 11.8 gm. per 100 cc. while the hemoglobin content of the blood of an adult rat averages 14.4 gm. per 100 cc. From these data it may be inferred that the rat with the least number of red blood cells present in the body has less hemoglobin to combine with oxygen, and consequently, the oxygen capacity per unit of blood for that animal is less than for an adult rat which has approximately

TABLE VII

Oxygen Capacity of Animals Determined in this Investigation

Animal	Sex	Wt. (gm.)	Oxygen Capacity (Volumes per cent)
<u>Rattus norvegicus-albino</u>	f	7.0	5.3
" " "	f	13.5	5.8
" " "	m	17.0	9.0
" " "	m	50.0	13.2
" " "	f	60.0	10.5
" " "	m	64.0	11.7
" " "	m	67.0	10.4
" " "	m	69.0	14.4
" " "	f	69.0	15.0
" " "	m	72.0	14.0
" " "	m	72.0	14.4
" " "	f	153.0	13.0
" " "	m	203.0	17.1
" " "	f	204.0	16.2
" " "	f	230.0	17.9
" " "	m	256.0	18.4
" " "	m	256.0	19.1
" " "	f	260.0	18.3
" " "	m	281.0	19.2
" " "	m	302.0	18.8
8 Adults Mean		249.	18.1
<u>Peromyscus gossypinus</u>	m	36.0	17.9
" "	m	28.0	20.6
" "	m	42.0	19.3
Mean		39.0	19.3
<u>Neotoma floridana</u>	m	172.0	18.6
" "	f	220.0	19.0
" "	f	237.0	15.0
Mean		209.0	17.5

TABLE VII (Continued)

Oxygen Capacity of Animals Determined in this Investigation

Animal	Sex	Wt. (gm.)	Oxygen Capacity (Volumes per cent)
<u>Cavia sp.</u>	m	380.0	9.7
" "	m	381.0	12.4
" "	f	427.0	13.2
	Mean	396.0	11.7
<u>Gricetus auratus</u>	m	62.0	16.8
" "	f	102.0	16.7
" "	m	121.0	17.8
" "	f	132.0	12.1
" "	f	139.0	13.0
" "	f	157.0	13.9
" "	f	127.0	18.9
	Mean	120.0	15.6
<u>Myotis austroriparius</u>	m	5.0	20.3
" "	m	5.1	21.2
" "	f	5.2	20.5
" "	m	5.3	20.9
" "	m	5.3	24.8
" "	m	5.5	16.4
" "	m	5.6	23.1
	Mean	5.2	21.0

4 times the number of red blood cells present. It may be seen in Table VII that the oxygen capacity in volumes per cent increases as the weight of the rat increases.

The 3 cotton mice and 3 wood rats, in which the oxygen capacity of the blood was determined, were adults and had been in captivity for at least 2 years.

The oxygen capacity of the blood of 7 bats was determined on February 22, 1952, the day after they were captured.

Blood for oxygen capacity determinations was collected from the tail of the large albino rats. The tail was clipped, and drops of blood were collected in a paraffin block. In the case of the other animals, except the bats, blood was taken from the heart with a heparinized syringe and 3/4 in. no. 24 needle. With the bats, blood was sucked directly into the pipette from the incised heart.

Oxygen Capacity Values from Available Sources

The manometric method of Van Slyke (1918) has persisted in use today because of its simplicity of operation and accuracy in repeated determinations. However, other methods such as the syringe, hemoglobin, spectroscope, and copper sulfate-specific gravity methods give comparable results with the Van Slyke method as shown in Table VIII.

It may be seen from Table VIII that the carbon monoxide and the Bancroft manometric methods give comparable results in cats (Bancroft, 1902). Roughton and Scholander (1943) obtained practically the same

results in man with the syringe and Van Slyke methods. In opossums, almost the same values were obtained by the author, using the hemoglobin as the values reported by Scott (1938) who used the Van Slyke method. No comparative evaluations for the spectroscope and copper sulfate-specific gravity methods are available.

In Table VIII an asterisk is placed beside the values which appear valid. For the reason stated above, the spectroscope and copper sulfate-specific gravity methods are indicated by "?".

TABLE VIII
Oxygen Capacity in Volumes Per Cent

ORDER Family	No.	Oxygen Capacity	Method	Authority
MARSUPIALIA				
Didelphidae				
<u>Didelphis virginiana</u> <u>pigra</u>	23	*14.9	Van Slyke	Scott-1938
<u>Didelphis virginiana</u> <u>pigra</u>	21	*15.0	Hemoglobin	Burke
CHIROPTERA				
Vespertilionidae				
<u>Myotis austroriparius</u>	7	*21.0	Syringe	Burke
PRIMATES				
Hominidae				
Man	14	18.5	CO	Haldane-1899-1900
Man	5	18.66	CO	Van Slyke-1921
Man	5	*19.58	Van Slyke	Van Slyke-1921
Man	2	*20.7	Van Slyke	Bock-1924
Man	16	*19.5	Van Slyke	Chang-1928
Man	33	*18.2	Van Slyke	Stewart-1940
Man	3	*22.20	Van Slyke	Roughton-1943
Man	6	*21.3	Hemoglobin	Drastich-1928
Man	7	*20.0	Spectroscope	Hall-1936
Man	20	*21.62	CuSO ₄ -specific gravity	Phillips-1943
Man	3	*22.27	Syringe	Roughton-1943
Man (male)	25	*17.5	Syringe	Gregersen-1949a
Man (female) (Chinese)	24	*14.6	Syringe	Gregersen-1949b

TABLE VIII (Continued)
Oxygen Capacity in Volumes Per Cent

ORDER Family	No.	Oxygen Capacity	Method	Authority
LAGOMORPHA				
Leporidae				
Rabbit	1	14.58	CO	Haldane-1899-1900
Rabbit	22	13.6	CO	Boycott-1909
Rabbit (male)	4	15.95	CO	Salvensen-1919
Rabbit (female)	2	9.97	CO	Salvensen-1919
Rabbit	3	*15.4	Hemoglobin	Drastich-1928
Rabbit	2	*17.6	Hemoglobin	Burke
Rabbit	4	715.6	Spectroscope	Hall-1936
RODENTIA				
Sciuridae				
Woodchuck	11	20.7	CO	Rasmussen-1915
Castoridae				
Beaver	6	*17.7	Van Slyke	Irving-1939
Cricetidae				
<u>Cricetus auratus</u>	7	*15.6	Syringe	Burke
Muskrat	3	*25.0	Van Slyke	Irving-1939
<u>Peromyscus gossypinus</u>	3	*19.3	Syringe	Burke
<u>Neotoma floridana</u>	3	*17.5	Syringe	Burke
Muridae				
Mice	6	*19.1	Hemoglobin	Drastich-1928
<u>Rattus norvegicus</u> (albino, > 200 gm.)	8	*18.1	Syringe	Burke

TABLE VIII (Continued)

Oxygen Capacity in Volumes Per Cent

ORDER	Family	No.	Oxygen Capacity	Method	Authority
	<u>Rattus norvegicus</u> (albino, < 200 gm.)	12	11.3	Syringe	Burke
	Caviidae				
	<u>Cavia</u> sp.	3	*11.7	Syringe	Burke
	Chinchillidae				
	<u>Viscacha</u> (<u>Lagostomus</u>)	1	*15.8	Spectroscope	Hall-1936
CARNIVORA					
	Canidae				
	<u>Vulpes fulva</u>	1	*21.0	Van Slyke	Irving-1939
	Dog	1	23.6	CO	Jolyet-1896-97
	Dog	2	*15.2	Hemoglobin	Drastich-1928
	Dog	1	*16.7	Hemoglobin	Burke
	Dog	6	*15.77	Van Slyke	Grant-1947
	Dog	6	*15.80	Syringe	Grant-1947
	Procyonidae				
	<u>Procyon lotor elucus</u>	1	*15.7	Hemoglobin	Burke
	Felidae				
	Cat	1	12.25	CO	Barcroft-1902
	Cat	1	12.20	Barcroft	Barcroft-1902
	Cat	2	*15.0	Hemoglobin	Drastich-1928

TABLE VIII (Continued)
Oxygen Capacity in Volumes Per Cent

ORDER Family	No.	Oxygen Capacity	Method	Authority
Mustelidae				
<u>Mustela vison</u>	22	*23.9	Van Slyke	Scott-1939
<u>Martes americana</u>	2	*24.3	Van Slyke	Scott-1939
Otariidae				
Steller's Sea Lion	1	*19.8	Van Slyke	Florkin-1931
Phocidae				
<u>Phoca vitulina</u>	11	*29.3	Van Slyke	Irving-1935
CETACEA				
Phocaenidae				
Porpoise	1	30.9	CO	Jolyet-1896-97
Porpoise	1	742.5	Van Slyke	Sudzuki-1924
Porpoise	1	*22.2	Van Slyke	Green-1933
Porpoise	6	*19.0	Hemoglobin	Drastich-1928
Delphinidae				
<u>Tursiops tursio</u>	1	33.4	CO	Joliet-1902
<u>Tursiops truncatus</u>	1	*19.1	Hemoglobin	Eichelberger-1939
Physeteridae				
<u>Physeter macrocephalus</u>	1	29.09	CO	Joliet-1902
Balaenopteridae				
Blue and Fin Whales	17	*14.1	Van Slyke	Laurie-1933

TABLE VIII (Continued)
Oxygen Capacity in Volumes Per Cent

ORDER Family	No.	Oxygen Capacity	Method	Authority
PERISSODACTYLA				
Equidae				
Horse	1	18.39	CO	Haldane-1899-1900
Horse	3	*14.2	Hemoglobin	Drasrich-1928
ARTIODACTYLA				
Suidae				
Pig	10	*14.9	Hemoglobin	Burke
Camelidae				
<u>Llama huanachus glama</u>	4	723.5	Spectroscope	Hall-1936
<u>Llama vicugna</u>	1	717.5	Spectroscope	Hall-1936
Bovidae				
Ox	6	18.12	CO	Haldane-1899-1900
Ox	1	19.70	CO	Barcroft-1902
Ox	5	17.5	CO	Abeloo-1928
Ox	1	19.74	Barcroft	Barcroft-1902
Ox	1	*23.53	Van Slyke	Van Slyke-1918
Ox	5	*18.6	Van Slyke	Abeloo-1928
Cow	2	*14.7	Hemoglobin	Drastich-1928
Sheep	1	17.30	CO	Haldane-1899-1900
Sheep	3	16.2	CO	Abeloo-1928
Sheep	1	*15.8	Hemoglobin	Drastich-1928
Sheep	3	*17.5	Van Slyke	Abeloo-1928
Sheep	114	*14.98	Van Slyke	Baker-1933

TABLE VIII (Continued)

Oxygen Capacity in Volumes Per Cent

ORDER Family	No.	Oxygen Capacity	Method	Authority
Sheep	5	715.9	Spectroscope	Hall-1936
Lamb (birth)	4	*20.5	Van Slyke	Gotsev-1939
Lamb (1 month)	4	*17.2	Van Slyke	Gotsev-1939
Goat (male)	1	*14.8	Hemoglobin	Drastich-1928
Goat (female)	3	*12.1	Hemoglobin	Drastich-1928
Goat (2 days)	4	*21.4	Van Slyke	Elliott-1934

TABLE IX

Oxygen Capacity in Various Mammals as Determined
by Acceptable Methods

Species	Oxygen Capacity in Volumes Per Cent	Carnivora	Rodentia	Artiodactyla	Chiroptera	Primates	Cetacea	Lagomorpha	Marsupialia	Perissodactyla
Harbor seal	29.0	x								
Muskrat	25.0		x							
Weasel	24.0	x								
Marten	24.0	x								
Guamoo	23.5			x						
Stellar's sea lion	21.0	x								
Southeastern little brown bat	21.0				x					
Fox	21.0	x								
Man	20.0					x				
Porpoises	19.0						x			
Dolphins	19.0						x			
White mice	19.0		x							
Cotton mice	19.0		x							
Cow	18.5			x						
Laboratory rats	18.0		x							
Vicuna	17.5			x						
Florida wood rat	17.5		x							
Rabbits	16.0							x		
Goats	16.0			x						
Hamsters	15.5		x							
Dogs	15.5	x								
Raccoon	15.5	x								
Viscacha	15.5		x							
Cats	15.0	x								
Pigs	15.0			x						
Sheep	15.0			x						
Opossums	15.0								x	
Blue and Fin whales	14.0						x			
Horses	14.0									x
Guinea pigs	11.5		x							

SUMMARY AND CONCLUSIONS

This study was undertaken to determine blood volume and oxygen capacity in mammals by the use of various techniques.

Small, and supposedly young, individuals of a species have a greater blood volume per unit of body weight than adults as demonstrated with swine and opossums. In swine the blood volume values decrease from an average of 10.5 ml. per 100 gm. at 1000 to 2000 gm. of body weight to 6.0 ml. at 96,000 gm. Opossums exhibit the same relation of a decrease in blood volume per unit weight to an increase in body weight as swine although the relationship is not as pronounced. No differences of blood volume with regard to sex are apparent.

Values determined by acceptable methods compiled from Table VI of blood volume for opossums are 5.5, 6.0 for sheep, 6.5 for rabbits, 7.0 for laboratory rats, 7.2 for horses, 7.5 for man and swine, and 9.0 for dogs, in milliliters of blood per 100 gm. of body weight. It is evident from these values that blood volume in adults in relation to size, weight, or taxonomic groups is comparable.

Thorough mixing of the T-1824 dye and P-32 labelled red blood cells was effected in one minute in the blood of opossums and by 10 minutes in the blood of swine.

The dye and P-32 in opossums and swine decreased with time until at 48 hours there was no measurable amount of dye present, and 13.8 and 13.0 per cent of P-32 remained, respectively.

In 21 opossums mean values for red blood cell count, hemoglobin, and rectal temperatures are 5,280,000 per cmm., 11.1 gm. per 100 cc., and 35.1° C., respectively. In 10 swine the mean value for red blood cell count is 7,070,000, and is 11.0 grams of hemoglobin per 100 cc. of blood.

Oxygen capacity per unit of blood is less for young animals than adults as demonstrated in this investigation with laboratory rats. The values range from 5.3 for a 5 day old animal to 19.2 volumes per cent for an adult.

It is difficult to make comparative evaluations for the different mammals listed in Table IX, which was compiled from values in Table VIII, inasmuch as data for age, weight, and sex are not reported in most cases.

In considering the data in Table IX from a taxonomic viewpoint, it is evident that the differences in values are not on an ordinal basis. Nor are these differences due to size as shown by values given for the muskrat, weasel, and marten which possess an oxygen capacity that is very different from that of the opossum, although all of these animals are of about the same size. Also large animals, as illustrated by horses and harbor seals, possess great differences in oxygen capacity values. The oxygen capacity of animals in the same family, such as dogs and foxes, is very different. It is also noted that there is a great difference in the oxygen capacity of diving mammals. For instance, the

oxygen capacity of blue and fin whales is very different from that of harbor seals and Stellar's sea lions.

From the information at hand, it appears that values for oxygen capacity vary with the species.

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APPENDIX

The procedures used by the author in this investigation are described in the appendix.

APPENDIX I

PROCEDURE FOR BLOOD VOLUME DETERMINATION

The following procedure was employed in this investigation for the determination of total blood volume by the use of T-1824 (Evans blue) dye and radioactive phosphorus-32.

1. Approximately 8.0 ml. of blood was withdrawn by a cardiac puncture using a heparinized syringe which was well-greased and a 2 in., no. 20 hypodermic needle. The blood was placed in a 15.0 ml. hematocrit tube which contained a solution of heparin. I used a heparin solution which was prepared according to Nachman et al. (1950). One hundred milligrams of dried heparin was dissolved in 14.5 ml. of physiological saline solution. Nachman et al. state that 0.05 ml. of this solution prevents the coagulation of 5.0 ml. of blood.
2. The withdrawn blood was centrifuged at 3000 rpm. for 30 minutes and the hematocrit recorded.
3. The plasma was removed from the red blood cells by a pipette and the plasma proteins were precipitated. The precipitating reagent described by Crooke and Morris (1942) was found to be very satisfactory for this purpose. The reagent was prepared by dissolving 15.0 gm. of phosphotungstic acid in 100.0 ml. of 98% alcohol, shaking manually, and centrifuging at 3000 rpm. for 5 minutes in order to separate the small amount of alcohol-insoluble matter. The amount of insoluble matter varied in different

samples of phosphotungstic acid. The solution remained stable for about 14 days when stored in a dark place in a tightly-stoppered bottle. One milliliter of concentrated hydrochloric acid with a density of 1.18 was mixed with 6.0 ml. of the alcoholic phosphotungstic acid and, if necessary, the solution was filtered. The solution, which is light sensitive, must be kept clear and must be freshly prepared each 48 hours. A blue color develops after this time rendering the solution worthless.

4. Two milliliters of plasma were treated with 14.0 ml. of the precipitating reagent, stirred manually with a small, glass rod for 5 minutes, and centrifuged at 3000 rpm. for 5 minutes. The optical density was read on a Klett-Summerson photoelectric colorimeter using a filter with a maximum transmission of light having a wavelength of 622.5 millimicrons. The maximum absorption of light of the T-1824 dye is 620 to 625 millimicrons. This reading is referred to as the blank reading.
5. Fifty milligrams of T-1824 dye were completely dissolved in 100.0 ml. of physiological saline solution; this mixture served as the primary dye solution.
6. Ten milliliters of this primary dye solution were diluted to 100.0 ml. which served as the secondary dye solution.
7. Two-tenths of a milliliter of this secondary dye solution was then added to 1.8 ml. of plasma. This solution of dye in plasma was deproteinated with the precipitating reagent as described in step (4). The optical density of this standard solution was read on

the colorimeter.

8. The red blood cells from step (3) were washed with iced, physiological saline solution 3 times and centrifuged at 3000 rpm. for 5 minutes. The supernatant was pulled off with a suction pipette after each washing.
9. Isotonic P-32 solution, containing approximately 50 microcuries of activity, was added to the red blood cells until the original volume of blood withdrawn in step (1) was reached.
10. This suspension was incubated for two hours in a water bath at 37° C. The solution was stirred every 10 to 15 minutes with a small, glass rod.
11. The suspension was then centrifuged at 3000 rpm. for 5 minutes and the supernatant was removed by suction. The labelled cells were washed 3 times with iced, physiological saline at 3000 rpm. for 5 minutes, and the supernatant was discarded after each washing.
12. Physiological saline solution was added to the washed, labelled red blood cells until the original volume of blood withdrawn in step (1) was reached. This suspension was stirred with a small, glass rod. A 1.0 ml. aliquot of this suspension was diluted to 200.0 ml. with distilled water. A 10.0 ml. aliquot of this solution was diluted to 100.0 ml. with distilled water. This diluted mixture served as the standard solution.
13. Five milliliters of the primary dye solution and 5.0 ml. of the labelled red blood cell suspension from step (12) were injected into the jugular vein of the animal using a 3/4 in. no. 24 needle.

Before removing the syringe from the vein, it was rinsed with blood two or three times.

14. Approximately 5.0 ml. of blood was withdrawn at intervals of 5, 10, and 20 minutes from the opposite jugular vein from that into which the injections were made.
15. The withdrawn blood was placed in 15.0 ml. centrifuge tubes to which heparin had been added as in step (1). The blood was centrifuged at 3000 rpm. for 30 minutes.
16. Plasma was separated from the red blood cells by the use of a small pipette. The plasma proteins of a 2.0 ml. plasma sample were precipitated as in step (4).
17. The optical density of the plasma samples was read on the photoelectric colorimeter. The total plasma volume and the total blood volume were calculated from the formula as shown on pp. 96-97.
18. The red blood cells from step (16) were washed according to the procedure of step (11).
19. The washed red blood cells were hemolyzed and diluted with distilled water to 100.0 ml.
20. The activity of the standard and sample red blood cell solutions were measured on 20.0 ml. aliquots using an immersion-type Geiger-Müller tube.
21. The total blood volume and the total red cell volume were calculated from the formula as shown on page 98.

APPENDIX II

CALCULATIONS FOR THE T-1824 METHOD

Calculation of Total Plasma Volume According to Gregersen (1944)

The concentration of dye in the plasma sample obtained after the injection of the primary dye solution, designated here as (C_2), is to the concentration of dye in the primary solution injected (C_1), as the volume of the primary dye solution injected (V_1) is to the total plasma volume (V_2). Therefore

$$V_2 = \frac{C_1 V_1}{C_2} .$$

Since the concentration of dye is directly proportional to the optical density, the equation may be written with the optical density values D_1 and D_2 may be substituted for the concentration C_1 and C_2 . Therefore

$$V_2 = \frac{D_1 V_1}{D_2} .$$

From steps (5), (6), and (7) of the procedure on page 93, it may be seen that an aliquot of the primary dye solution was diluted 100 times. The optical density of this diluted dye solution was read on the colorimeter as the standard. Therefore

$$V_2 = \frac{(D_1 \times 100) V_1}{D_2} .$$

Hematocrit values

$$\text{Hematocrit red cell value} = \frac{\text{red cell volume of hematocrit}}{\text{whole blood volume of hematocrit}}$$

$$\text{Hematocrit plasma value} = (1 - \text{hematocrit red cell value})$$

Chapin and Ross (1942) report that the amount of plasma trapped between the red blood cells of centrifuged blood samples is 8.5% of the total plasma. Mayerson et al. (1948) corroborated the findings of Chapin and Ross. For this reason, all of the hematocrit red cell values used in the present investigation were corrected by multiplying by the factor 0.915. It follows that the

Corrected hematocrit plasma value = $(1 - \text{corrected hematocrit red cell value})$.
From these data, and from the previously determined total plasma volume, the total blood volume may be determined.

Total Blood Volume

$$\text{Total blood volume} = \frac{\text{Total plasma volume}}{\text{Corrected hematocrit plasma value}} .$$

APPENDIX III
CALCULATIONS FOR THE P-32 METHOD

Total Blood Volume

The counts per minute of the blood sample taken for analysis, designated here as (S), is to the counts per minute of the injected solution (I), which may be calculated from data given in step (12) of the procedure on page 94, as the volume of the blood sample (V_1) is to the total blood volume (V_2). Therefore

$$V_2 = \frac{I V_1}{S} .$$

APPENDIX IV

PROCEDURE FOR RED BLOOD CELL COUNT

Procedure used in the present investigation for counting red blood cells according to Kolmer and Boerner (1945):

1. Draw blood to the 0.50 mark in the Thoma pipette.
2. Draw in Hayme's diluting fluid to the 101 mark while rotating the pipette.
3. Place a coverglass over the ruled area of a Levy-Hauser counting chamber.
4. Wipe off the first drop of diluted blood and allow the next drop to flow under the coverslip without filling the trough. Allow 3 minutes for the blood cells to settle.
5. Use the "bright light" counting chamber and count the number of red blood cells in 8 small squares in a horizontal row and in 10 small squares in a vertical row to get a total of 80 small squares counted. Count only the red blood cells on the top and left lines and those inside the squares.
6. Count the red blood cells on the lower part of the counting chamber as was done in step (5) and average the two counts.
7. Add 4 ciphers to the average found in step (6) which gives the number of red blood cells per cubic millimeter of blood.

APPENDIX V
HEMOGLOBIN DETERMINATION

Procedure used in this investigation for the determination of hemoglobin on the Fisher Electro-hemometer:

1. Fill pipette to the mark "20" line with blood.
2. Add 0.1 N hydrochloric acid to the upper pipette "filled" mark.
The standard dilution is 20 cubic millimeters of blood in 5.0 ml. of 0.1 N hydrochloric acid.
3. Hemolyse for at least 30 minutes.
4. Adjust the hemometer to read zero.
5. Adjust the reading to zero with the standard cell in place.
6. Read the per cent hemoglobin or g/100 cc. of hemoglobin with the sample in place.

APPENDIX VI

APPARATUS USED IN THE SYRINGE METHOD

A procedure for the determination of the oxygen capacity of the blood as described by Roughton and Scholander (1943) and modified by Grant (1947):

The instrument used in the syringe method described by Roughton and Scholander (1943) consists of "a 1 cc. pyrex tuberculin syringe, with arresting clip on the plunger to prevent it from slipping and with a standard precision bore 0.5 mm. pyrex capillary fused to its nozzle. The top of the capillary is expanded to a cylindrical cup of about 2.5 mm. bore and 1.5 cm. length." The distance between the two marks on the cup is approximately 5 mm. The blood pipette is made from thin-walled glass tubing (1.0 to 1.5 mm. bore) and it is ground smooth at the tip so as to fit snugly into the bottom of the glass cup. This pipette is calibrated so as to deliver 39.3 cubic mm. which is equal to 100 divisions of the capillary, and also 43.3 cc. A detachable rubber cup of about 1.0 cc. capacity is fitted to the top of the glass cup.

Five 5 cc. and one 10 cc. syringe are necessary for storing and delivering reagents.

APPENDIX VII

REAGENTS USED IN THE SYRINGE METHOD

1. Distilled water.
2. 0.9 per cent sodium chloride.
3. Caprylic alcohol.
4. Ferricyanide solution:
 - 12.5 gm. of potassium ferricyanide
 - 3.0 gm. of potassium bicarbonate
 - 0.5 gm. of saponin

These substances are dissolved in water and made up to 50.0 cc. The solution should not be used for more than 3 days; otherwise appreciable loss of carbon dioxide may occur.

5. Acetate buffer:

70.0 gm. of sodium acetate, $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$, are dissolved in 100 cc. of distilled water and 15 cc. of glacial acetic acid are added.

6. 45 per cent urea.
7. 10 per cent sodium hydroxide.
8. Pyrogallol solution:

15.0 gm. of powdered pyrogallol are added to 100 cc. of 20 per cent sodium hydroxide in a rubber-stoppered bottle and covered with a layer of oil 2 cm. thick on top. The pyrogallol is dissolved under the oil by stirring with a glass rod.

Reagents 3, 4, 5, 6, and 8 are conveniently stored in 5 cc. syringes, and reagent 7 in a 10 cc. syringe. The syringes have fine-tipped glass nozzles attached and the rear part of the plungers are well greased.

9. Heparin.

APPENDIX VIII

PROCEDURE WITH THE SYRINGE METHOD

1. Blood was sucked directly into the 39.3 cubic millimeter pipette which had been previously flushed with anti-coagulant solution (heparin) and dried in a current of air. Blood was taken directly from a vessel or the blood was collected in a paraffin block which contained cup-shaped cavities.
2. The 1 cc. syringe was flushed 3 times with separate portions of saline, emptied, and the cup filled to the lower mark with saline.
3. The tip of the pipette containing the blood was passed carefully into the cup which contained saline and pressed firmly against the bottom.
4. Blood was sucked into the syringe by a smooth withdrawal of the plunger. Once the pipette was emptied, it was quickly removed and the saline in the cup was drawn in after the blood.
5. The blood-saline mixture was lowered far enough into the syringe to permit entry of an air volume of approximately 1.0 cubic centimeters.
6. The syringe was rotated on its long axis with an occasional rotation at right angles to spread a small layer of blood on and over the inside of the syringe and thus ensure uniform exposure of blood to the air. Once a minute during this 5 minute procedure the air was renewed by alternately running the blood up to the capillary and lowering to the 1.0 cc. mark.

7. Two capillary divisions of caprylic alcohol was added which prevented the formation of transverse blood films which made the expulsion of air without loss of blood difficult.
8. Air was expelled and the blood-saline mixture was pushed up to the bottom of the cup. The cup was filled to the upper mark with ferricyanide solution, and the solution was then lowered to the bottom of the cup.
9. The cup was filled to the upper mark with acetate buffer and the latter was drawn down to the bottom of the cup.
10. The cup was then immediately filled to the top with 45 per cent urea, and then closed firmly with the finger.
11. The closed apparatus was vigorously shaken in the horizontal position and the plunger was gradually drawn out as the carbon dioxide and other gases were evolved, with the pressure in the syringe kept roughly at existing pressure. A volume of about 0.75 cc. of gas was evolved by shaking for about 2 minutes.
12. The syringe plunger was manipulated so as to keep the gas meniscus in the capillary as the finger was gradually released. A small amount of urea was allowed to run down into the capillary and left there until the walls were perfectly clean.
13. Three-fourths of the urea solution in the glass cup was removed, and the rubber cup was fitted in place and filled with 10 per cent sodium hydroxide without trapping air bubbles.
14. A little sodium hydroxide was drawn into the syringe. This absorbed some carbon dioxide, causing a partial vacuum which quickly

sucked in more sodium hydroxide until only a small bubble consisting of oxygen, nitrogen, and carbon monoxide was left at the top of the syringe. The absorption took a few seconds and just before it was completed the residual bubble was screwed slowly and carefully up into the capillary by manipulation of the plunger.

15. The rubber cup was removed and the glass cup emptied.
16. The capillary was placed for one-half minute in a beaker of water at room temperature.
17. It was then removed, dried by light wiping, and care was taken that the capillary was not handled, and the volume of the bubble read, and recorded as V_1 divisions.
18. The glass cup was then filled with pyrogallol solution and the oxygen of the bubble was absorbed by pulling the gas bubble down to the bottom of the capillary and back again a few times until the reading was constant. Finally the bubble was moved very slowly up into the top part of the capillary and after a further temperature equilibration its volume was read again and recorded as V_2 divisions.

To obtain a blank value for the reagents as well as for physically dissolved oxygen of the blood, an analysis is performed exactly as described in the procedure, but instead of delivering the usual 39.3 cubic millimeters of blood, 43.3 cubic millimeters of 0.9 per cent sodium chloride is substituted. Sendroy et al. (1934) have shown that 1.1 cc. of

aerated saline solution contains the same volume of physically dissolved oxygen as 1.0 cc. of blood. The procedure is continued as if blood were used instead of saline solution.

To wash the instrument, the plunger is pulled out under a stream of running water and the blood mixture poured out. The syringe is filled and emptied several times with water before the plunger is restored. The plunger should never be forced inwards if there is a resistance due to precipitation. Occasionally, the whole syringe should be rinsed with dichromate solution. The time necessary to run a sample is about 12 minutes.

In the measurement of the bubbles it is essential to move them very slowly and evenly so as to assure good drainage. These delicate adjustments are readily made by rotating the plunger gently with a screwing motion as it is being drawn in or out. Slipping back of the plunger is prevented by a proper adjustment of the arresting clip. Careful control of the movement of the bubble is secured by using the little finger as a brake against the plunger.

APPENDIX IX

CALCULATIONS OF OXYGEN CAPACITY WITH THE SYRINGE METHOD

$$\text{Volume} = \pi r^2 h$$

$$= 3.1416 \times (0.25)^2 \times 100$$

$$= 19.6350 \text{ mm. total volume of capillary.}$$

$$\frac{19.6350}{50} = 0.39270 \text{ mm. per division of capillary.}$$

$$1 \text{ division of capillary} = 2.0 \text{ mm.}$$

The capillary is marked off in 50 divisions.

$$50 \times 2 = 100 \text{ mm.} = \text{length of capillary.}$$

$$\text{Diameter of capillary} = 0.5 \text{ mm.}$$

$$\text{Oxygen content} = (V_1 - V_2 - c) \times f$$

V_1 = length of gas bubble before pyrogallol is added.

V_2 = length of gas bubble after pyrogallol is added.

c = correction of oxygen content of reagents as determined by the blank.

f = correction factor for temperature, aqueous vapor pressure, and the barometric pressure. This factor may be read from a table such as that given by Peters and Van Slyke (1932) volume 2:129, Table 15.

BIOGRAPHY

Jack Denning Burke was born April 24, 1919, in Clarksburg, West Virginia. He attended elementary schools in Charleston, West Virginia, and was graduated from Woodrow Wilson High School in Beckley, West Virginia, in 1937. He attended Blue Ridge School for Boys in Hendersonville, North Carolina, from 1937 to 1938, and West Virginia Wesleyan College in Buckhannon, West Virginia, from 1939 to 1940.

He was employed by E. I. du Pont de Nemours Company in Belle, West Virginia, from 1940 to 1945 at which time he entered the U. S. Naval Reserve. He served on active duty until April, 1946.

He entered the University of Tennessee at Knoxville in June, 1946, and received the Bachelor of Arts degree in 1948. The Master of Science degree was awarded in 1949 by West Virginia University in Morgantown, West Virginia. He entered the University of Florida in June, 1949, in order to continue his graduate studies.

He was appointed a graduate assistant in biology in July, 1950, and held that appointment until September, 1951. At that time he was appointed a teaching assistant in biology and filled that position until June, 1952.

He is a member of the Phi Sigma Biological Society, American Association for the Advancement of Science, Association of Southeastern Biologists, and the Florida Academy of Sciences. He is a 32nd degree Mason, a Shriner, and a member of the Lambda Chi Alpha social fraternity.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 9, 1952

Dean, College of Arts and Sciences

Dean, The Graduate School

SUPERVISORY COMMITTEE:

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