THE RESERVE STORAGE OF RED CELLS AND HEMOGLOBIN AND THEIR PARENT SUBSTANCES DURING GROWTH IN DOGS AS INFLUENCED BY DIET FACTORS.

THESIS

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Introduction

In studies concerning blood regeneration in experimental anemia produced in dogs by blood removal it early became apparent that these animals possessed large reserve stores of material which could be formed into new hemoglobin and red blood cells upon demand. These reserves enabled the dog to manufacture very rapidly red blood cells and hemoglobin in spite of frequent blood withdrawal. Several weeks of extensive blood removal were necessary to exhaust these potential hemoglobin and red cell stores before the desired anemia level could be maintained on the standard basal diet.

We also observed that these reserves seemed to be influenced by diet factors administered during the growth period of these dogs, that is from weaning until maturity. For example, dogs which had received a diet containing approximately 50% of liver apparently had greater storage of potential hemoglobin and red cell precursors than had animals whose diet consisted of salmon bread and milk. The stores of hemoglobin factors of animals which were fed diets consisting of 50% skeletal muscle or pig kidney ranged within these two extremes. It therefore became apparent that a study concerning the influence of these factors was essential as a part of our larger anemia program and this investigation was therefore undertaken.

To supplement the data thus obtained some preliminary experiments concerning the blood picture and weight curves of young puppies during early growth as influenced by a variety of dietary regimes are added. These studies were primarily carried on for the purpose of ascertaining the effect of various food factors upon muscle hemoglobin formation. They have, however, a bearing upon the present investigation.

In order to demonstrate hemoglobin values in the very young particularly during the suckling period and also as possibly aiding the interpretation of some of this data, curves drawn from human studies during early life are also given. The literature abounds with blood studies concerning newborn babies and young children. The blood picture of very young children is very similar to that of young animals; this fact is frequently emphasized. Unfortunately the available data concerning the blood picture of growing dogs are very meager.

These supplements, though appearing perhaps as only distantly related to the present study, do have some bearing upon it and are therefore offered as comparative or confirmative additions.

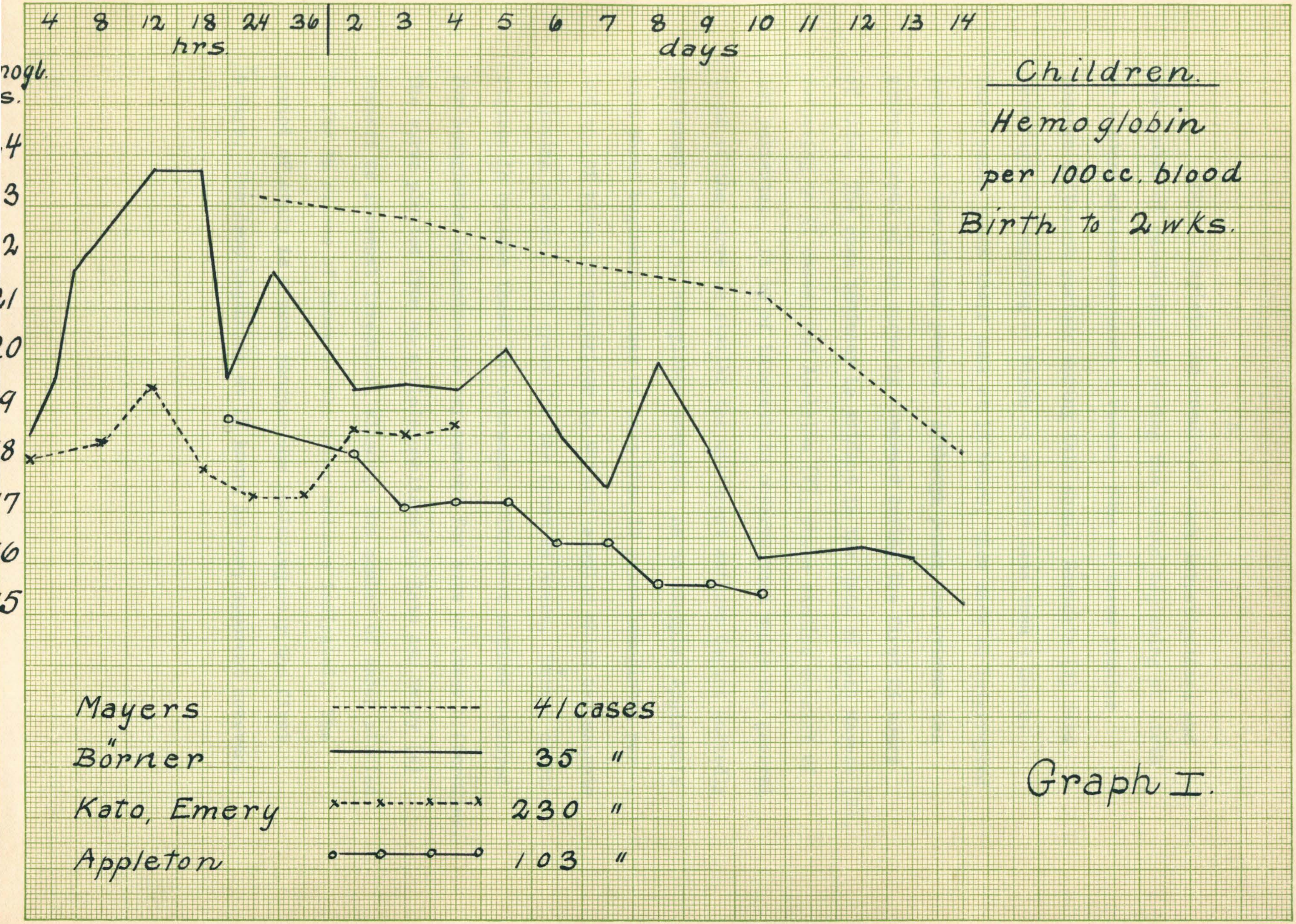
Historical

The blood picture of animals from birth to maturity has been little studied. Isolated observations concerning dogs have appeared in papers describing the blood picture of domesticated animals. Several publications dealing with red cells and hemoglobin data of the usual laboratory animals, rats, mice, rabbits, and guinea pigs have appeared. Many publications concerning humans are available. A scarcity of information concerning diets during these investigations is apparent. The exception to this are studies concerning children.

Most investigators demonstrate in the fetus that red cell and hemoglobin gradually increase until the time of birth. At this time or shortly thereafter the highest level for hemoglobin and red cells is attained when compared to the blood picture during growth or adult age.

Cohnstein and Zuntz (1) cite values for red blood cells of 16.5 million per cubic centimeter of blood for the newborn pup in comparison to 9.7 million for the adult animal. Poggiale, cited by Cohnstein and Zuntz (1), publishes figures of 16.2 million red cells for the newborn dog and 12.6 million for the adult animal. No hemoglobin figures are given. During the next few days of life, particularly during the first twenty-four hours, a sharp drop in the blood picture occurs which continues during the suckling period. Wells and Sutton (2) cite red cell figures ranging from 3.3 to 5 million per cubic centimeter of blood for pups from 3 to 7 days old and from 5.6 to 7.6 million cells as normal values for adult dogs. Burnett and Traum (3) show average figures of 3.9 million red cells for pups up to 20 days old in comparison to average values 6.0 million for adult dogs. Burnett (3) states in general that in the blood of the young of dog, cat, rabbit, sheep, goat and pig the red corpuscles are less in number than those of adult animals.

High hemoglobin values for newborns occur in humans as well as in various animals. Kato and Emery (4) studied 780 children from birth to 2 years of age and cite average values of from 17.3 to 19.7 grams of hemoglobin per 100 cc. of blood during the first 24 hours. The data of various investigators concerning the blood picture at birth with changes as growth progresses have been plotted as curves in Graphs I and II.

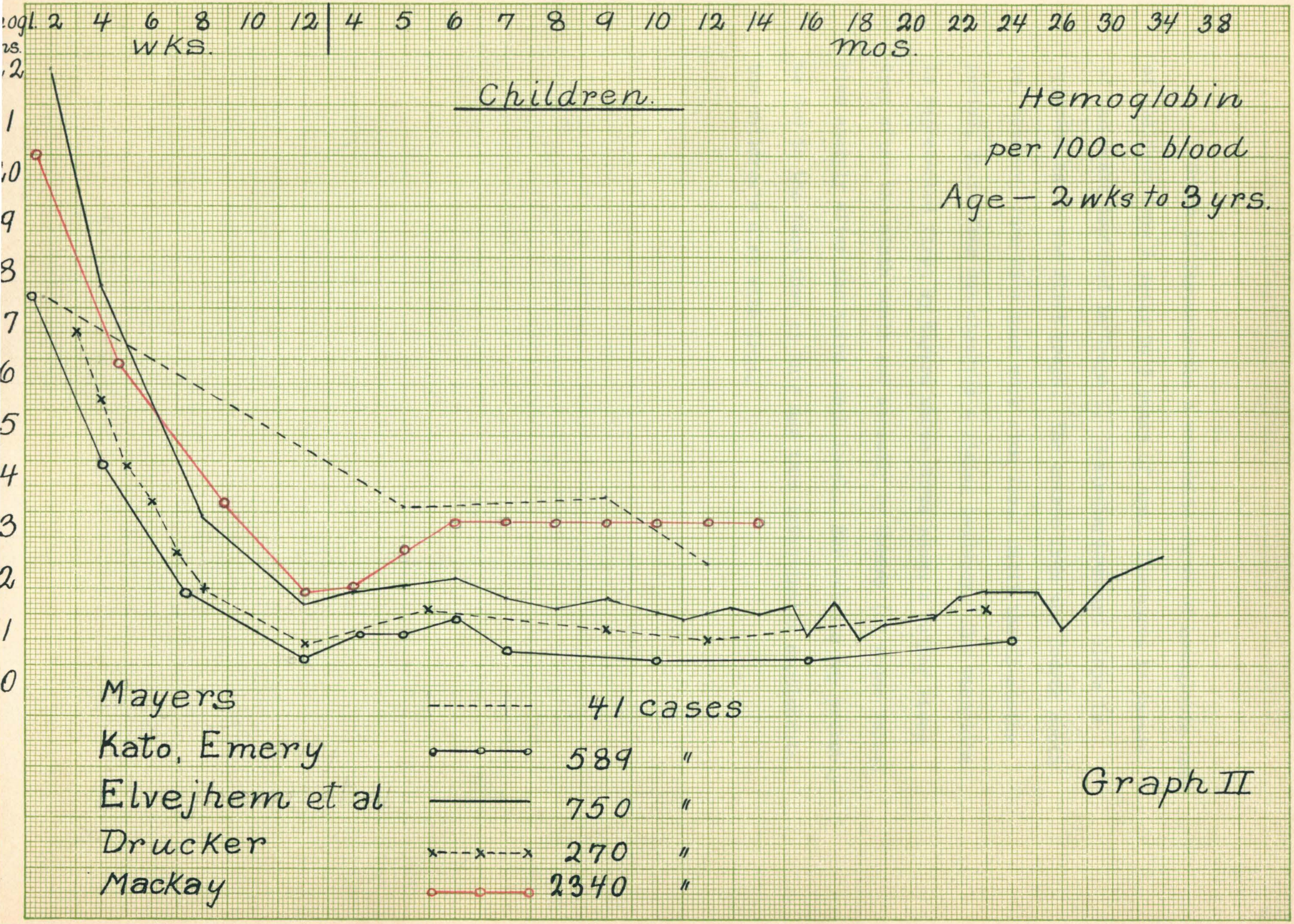


Children.

Hemoglobin per 100cc. blood Birth to 2 wks.

Graph I.

Graph I depicts the result of such investigations and the number of cases studied during the period beginning at birth to 4 hours afterwards until 2 weeks. Some of these studies concern different periods during this span. Börner's (5) data show figures beginning at birth or at ages less than 4 hours. These cases reach their peak at 18 hours; then show an interrupted drop until the 48-hour period. **Although** peaks are evident the general trend of the curve is downward during the first 24 hours of life. The same tendency is evident in the data of both Mayers (6), referring to 41 cases, and Appleton (7), whose studies consist of 103 patients. It is of interest to observe that while the general trend of the curve is similar the different authors depict markedly different levels of hemoglobin content. As an example, Appleton's data shows beginning values of less than 19 grams hemoglobin per 100 cc. of blood in comparison to Mayers' studies which demonstrate beginning values of 23 grams for the first 24 hours. Börner cites figures in between these two extremes. Different methods employed for hemoglobin determination may account for some of these differences. The curve plotted from an article published by Kato and Emery (4) appears as somewhat irregular, although the number of cases studied is far in excess of those of the other authors indicated. The period of study is a much shorter one including only the first 4 days. The drop beginning at 12 hours is very apparent however.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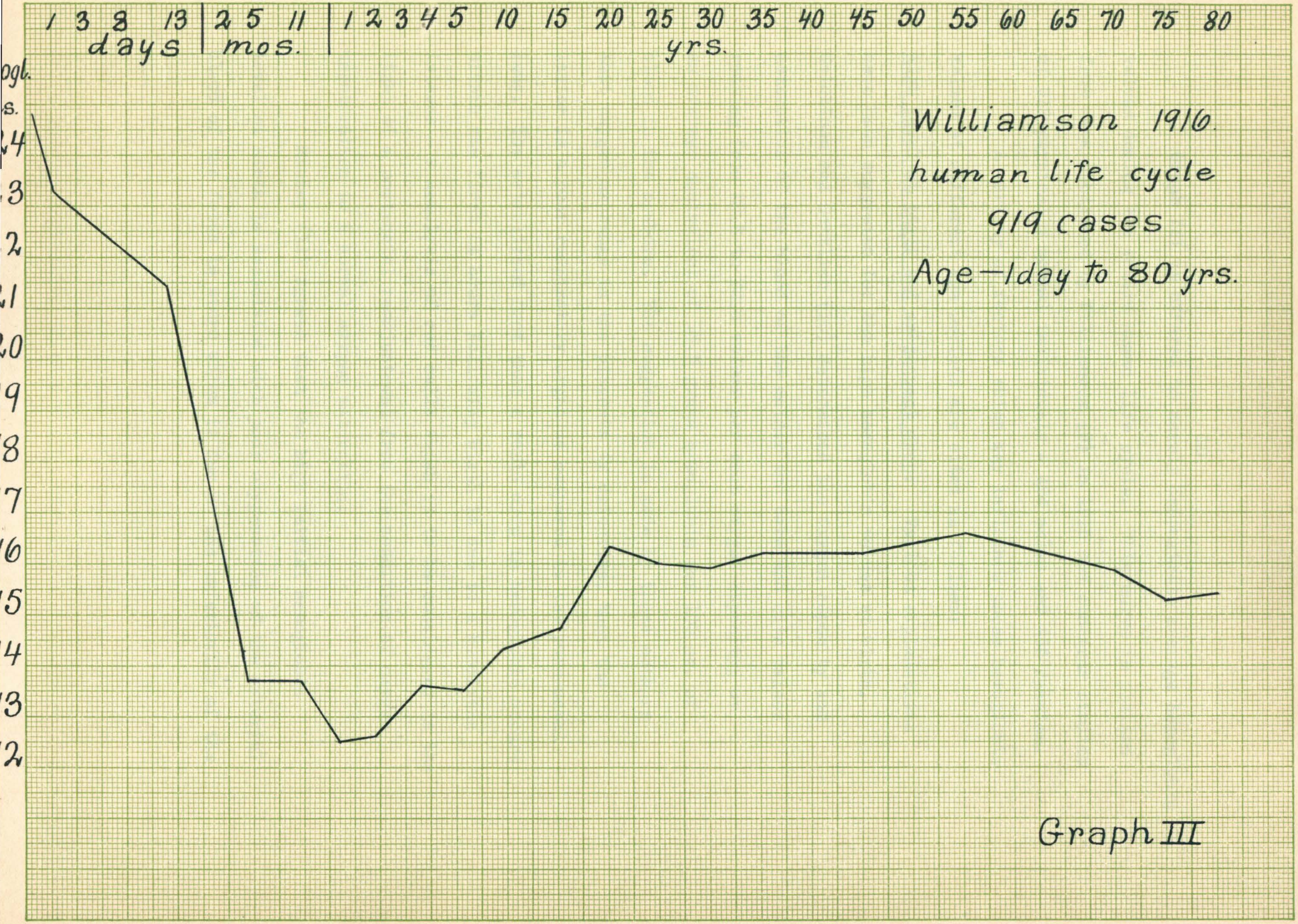
mos.
Children.
Hemoglobin
per 100cc blood
Age — 2 wks to 3 yrs.
Mayers
41 cases
Kato, Emery
589 "
Elvejhem et al
750 "
Drucker
270 "
Mackay
2340 "

Graph II

Graph II demonstrates a composite curve of hemoglobin data of children from 2 weeks to 3 years of age. The curve is devoid of the peaks depicted in Graph I. A similar downward slope is apparent as in the first graph. The course from the fourth month on, however, runs at a much more even level until the 18-months period, thereafter one observes a rising tendency. The exception is represented first by Mayers whose curve shows a rather steep drop after the ninth month and secondly by Mackay (8) whose values rise sharply after the third month level. Her results may probably be due to the fact that at this age this investigator supplements the children's diet with iron.



Williamson 1916.
human life cycle
919 cases
Age—1day to 80 yrs.

Graph III

Graph III is offered to merely demonstrate average hemoglobin values during the entire human life cycle. This curve is plotted from data published by Williamson (9) in 1916 and concerns studies of 919 physically normal individuals - 464 males and 455 females.

A variety of factors which may influence these hemoglobin values at different age periods are cited as follows:

High level at birth and subsequent drop: Various explanations have been suggested for the high hemoglobin values at birth. Goldbloom and Gottlieb (10) determined the oxygen capacity and saturation of the blood of the mother and babe and cite values ranging from 20.8 to 22.8 volume per cent oxygen capacity, or an average equivalent of 16.3 grams hemoglobin per 100 cc. of blood in the newborn. The suggestion is made by these authors that oxygen exchange in the placenta is defective, that the latter is a poor respiratory organ as compared to the post-natal lung. The established fact that a rapid drop in hemoglobin occurs within the first 24 hours following birth is due to a hemolytic icterus - a hemolysis of red cells which is to be considered in the light of a post-natal readjustment from the condition of oxygen unsaturation to a normal oxygen saturation.

Mackay (11) agrees that the high initial hemoglobin level at birth is associated with poor oxygenation of the arterial blood of the fetus in utero which is due to the inferiority of the placenta to the lung as a medium for oxygenation. After birth the red blood cells are reduced by hemolysis. She adds that as cells are already present in excess of body requirements the occuring hemolysis is physiological.

Hampson, cited by Mackay (11), believes a chemical mechanism accounts for the destruction of red cells soon after birth. This investigator finds a much larger difference between the pH of red cells and the pH of the plasma than occurs in the adult. Increased fragility of red cells in the newborn may also account somewhat for increased destruction which usually continues throughout the first month of life. During the same period blood formation is believed to be much diminished.

Greil, cited by Baar and Stransky (12), believes that with loss of the placenta to the fetus the hemolytic function of the reticulo-endothelial apparatus of spleen and liver is much increased. Schwartz, referred to also by Baar and Stransky (12), suggests deficient liver function as the cause of icterus. Any of these factors singly or in combination could easily account for the rapid drop in hemoglobin which occurs.

Growth factor and hemoglobin: Rate of growth and hemoglobin formation are frequently correlated. Mackay (13) states that slow growth may prevent hemoglobin drop and that during the period of slackening growth hemoglobin values may rise.

Wetzel (14) studied human growth in weight both during fetal existence and after birth from the age of six months to 32 years. Calculated on the basis of kilograms per year he found that the rate of growth rapidly increased during fetal life, being equivalent to a rate of 10.1 kilogram per year at birth. After six months there is considerable decrease in the rate, from figures equal to 6.5 kilogram per year at six months of age to a rate equivalent to 1.3 kilogram at the age level of three years. Thereafter a gradual increase again occurs equivalent to a rate of 3.6 kilogram per year at 12 years of age. A gradual drop follows until at 32 years the rate of growth is equivalent to 0.6 kilogram per year.

Mackay's suggestion of correlation between growth and hemoglobin seems plausible. As a result of extensive studies on hemoglobin regeneration in anemic dogs we have been able to demonstrate that the body economy may stimulate production of some factors at the expense of others; a sort of physiological give and take. It has been determined that during a period of rapid accumulation of body weight considerably less hemoglobin is formed than during a period when weight remains stationary. Furthermore experiments give evidence that in rapidly growing puppies hemoglobin formation may not be as active as during adult life when the general growth stimulus is at a minimum.

Stress for one type of growth may retard another type. Certain precursors of body substances may go one way perhaps to form body protein or may be directed into another channel, for example, hemoglobin building. This physiological give and take is apparent in various other ways. It is easily shifted and its direction depends upon the immediate needs of the organism. Grigorowa (15), in studies of Russian children referring to the two functions of the bone-marrow (bone formation and erythropoiesis), suggests that one or the other may take the lead, particularly during the growth period of the organism.

Sex, nationalities and seasonal fluctuations: Such factors as the above and their possible effect on hemoglobin formation have been pointed out. Differences in hemoglobin and red cell values in male or female dogs are very slight. Leichsenring and her coworkers (16) studied possible differences in the blood picture due to sex. Their results in a large series of dogs show a moderate difference only apparent in the hemoglobin figures. Female dogs demonstrated values of 14.4 grams hemoglobin per 100 cc. of blood in comparison to 17.0 grams for the males. Red cells, however, showed no differences. Otto (17) did not find an appreciable difference in the hemoglobin and red cell content of blood in male or female dogs.

In humans variations due to sex are often emphasized. Children up to 15 years of age show practically no difference in hemoglobin, the boys giving values very similar to those of the girls. In adult life it is generally conceded that both hemoglobin and red cell levels are somewhat lower in women than those demonstrated by men.

Nationalities may be an influential factor, however, it would appear as if differences in environmental and dietary conditions would be of much greater importance. In a study of children made by Schiff (18), both in Prague and Budapest, this author found values 30% higher in the Hungarian children than in those living in Prague. In comparing his data obtained in Budapest with that of other Hungarian authors Schiff believes his higher values to be correct. One must not forget, however, that each nation has its own special dietary customs. In view of studies on the influence of diet on hemoglobin production during growth as well as during adult life this particular factor may partially at least explain some of the differences noted.

Seasonal variations are occasionally mentioned as having considerable influence on hemoglobin formation. Most of the studies concerning animals refer to rabbits, very little concerning dogs. The majority of investigators suggest the influence of sunlight in the summer and claim that the peak level of hemoglobin and red cells occurs in late Summer and Fall.

Studies made on the blood of children demonstrate findings somewhat similar. Platt and Freeman (19) investigated hemoglobin values in children ranging from 21 to 44 months of age. This group was classed as a "superior social group" with dietary and environmental conditions above the average. The highest values were obtained early in November and the lowest figures were found in December, January and February. These three months are, of course, the months during which there is the greatest incidence of respiratory infections; this is of great importance to hemoglobin formation. The rise of hemoglobin began in March and increased slightly more in May. Grigorowa (15), in Moscow, studied the hemoglobin values of several thousands of persons of school age, from 17 to 18 years. Late Fall and early Winter demonstrated higher values than Spring and Summer. This author comments that the anemias of childhood have a definite seasonal rhythm with a decrease in the early winter months and a decided increase in Spring and early Summer.

With reference to dietary conditions during the early growth period the data is of the most meager sort. Concerning diets in animals referring particularly to dogs following the suckling period no detailed findings are available. The influence of diet on hemoglobin formation constitutes probably the most important part of the picture. This has been adequately proven by our anemia experiments in dogs.

The literature abounds in publications concerning children. In human studies of very young children the need and effect of iron, particularly during the first years of life, is greatly stressed. The lowered iron stores available to the organism during the latter part of the milk feeding period is frequently blamed for the nutritional anemia of infants. The very large stores of iron at birth are often mentioned as partially explaining the higher values during the early months of life.

As early as 1889 Bunge, cited by Gladstone (20), ascertained that the amount of iron per kilogram of body weight present in the newborn organism was six times greater than that of the normal adult animal. These studies refer to dogs, cats, and rabbits. Similar findings were obtained by Zaleski, cited also by Gladstone (20), who in dogs reported iron values at birth of five times the amount of the adult animal. In the human fetus Hugouneng (20) in 1899 determined that two-thirds of the iron present in the body at full term was laid down in the last three months of intra-uterine life.

With increasing age on a non-supplemented milk diet, the iron stores in the organism are gradually reduced. This applies to both suckling animals as well as humans. Mackay (11) cites four months as the age in children at which surplus stores of iron are exhausted and she then begins to supplement the milk with iron. As soon as a more mixed diet is instituted a moderate rise in hemoglobin becomes evident. Mackay explains her higher values when compared to the data of other investigators as being due to the supplementary change in the milk diet.

In the light of more recent investigations the value of copper as an essential for hemoglobin formation during the suckling period has been stressed. This point first determined in studies concerning the nutritional anemia of rats has also been emphasized in humans. Similar to high iron content in the newborn is the finding of high copper values in their livers.

Ramage, Sheldon, and Sheldon, quoted by Sheldon (21), studied a large series of human livers as to both their iron and copper content. These investigators demonstrated the presence of iron and copper as the only metals in the liver during fetal life. The peak was reached at birth when 0.06% copper (dry weight) and 0.4% iron was found. After birth these figures were seen to drop until stable values were attained at the age of from 12 to 18 months. There may, of course, have been a rise in iron content of the liver during the first few weeks of life since considerable blood destruction occurs and the iron from this hemolytic process may have been stored for the ultimate purpose of taking part in new hemoglobin formation.

The effect of diet other than that of mineral factors on hemoglobin formation in growing animals has been little studied particularly in dogs. Many more data are available for pigs, lambs, and calves.

Subbotin (22), in a paper on the blood picture of various types of animals, cites figures of young growing dogs given a diet for 38 days consisting mainly of starch and fat. These figures indicate decreasing hemoglobin values after the 26th day, from 12 grams per 100 cc. of blood to 9.5 grams. Young puppies shortly after weaning demonstrated 3.5 grams hemoglobin per 100 cc. of blood. Young dogs given meat showed hemoglobin values of 14 grams per 100 cc. of blood.

The influence of various diet factors upon the hemoglobin and potential hemoglobin stores constitute the object of the present investigation. The foregoing data obtained from the literature is offered mainly as evidence which may have a bearing upon hemoglobin formation in the young. Human studies have been cited because the blood picture during growth is similar to that of many animals. Data concerning the blood picture of <u>adult</u> dogs has been purposely omitted as having little bearing on the present work. Many studies referring to blood pictures of normal dogs show such tremendous differences, most of which are no doubt due to environmental and dietary conditions. The subject matter of the accompanying preliminary investigation concerns experiments carried on with young pups from the time of weaning to maturity under a variety of dietary regimes. One purpose of this study was to ascertain the effect of various dietary factors on the formation of muscle hemoglobin (23). For such investigation knowledge as to fluctuations of circulating blood hemoglobin was essential.

Experiments with adult anemic dogs for many years have taught us that the circulating blood hemoglobin is a labile substance subject to fluctuations, rapidly rebuilt and readily controlled by diet factors. The animals were studied from the period of weaning to various ages.

Experimental Procedures

Our experimental plan was to employ several litters of pups bred from our known laboratory stock. These puppies nursed for a period of 6 weeks. At all times during this nursing period they were kept with the mother in a warm, well ventilated and lighted room, bedded with shavings and permitting ample exercise. After weaning the mother was taken away and the pups were put on a bread and milk diet for a few days. During the seventh week the puppies were separated and were placed upon the test rations. One group received liberal amounts of salmon bread and whole milk powder (Klim) mixed into a mash with warm water. The other group received half the ration of the first group plus increasing amounts of either cooked liver or raw beef skeletal muscle until these constituents amounted to 50% of the diet mixture. The liver or muscle meat was put through a meat grinder and intimately mixed with salmon bread mash. Whole milk powder was added to all diets. The salmon bread, while unfavorable for hemoglobin formation in our anemic animals, is adequate for general nutritional maintenance. The present experiments demonstrate that this bread mixture also meets growth requirements. The salmon bread is prepared and baked in our own laboratory. The accompanying formula cites the various ingredients. Pups thrive on this bread ration and show uniform growth curves.

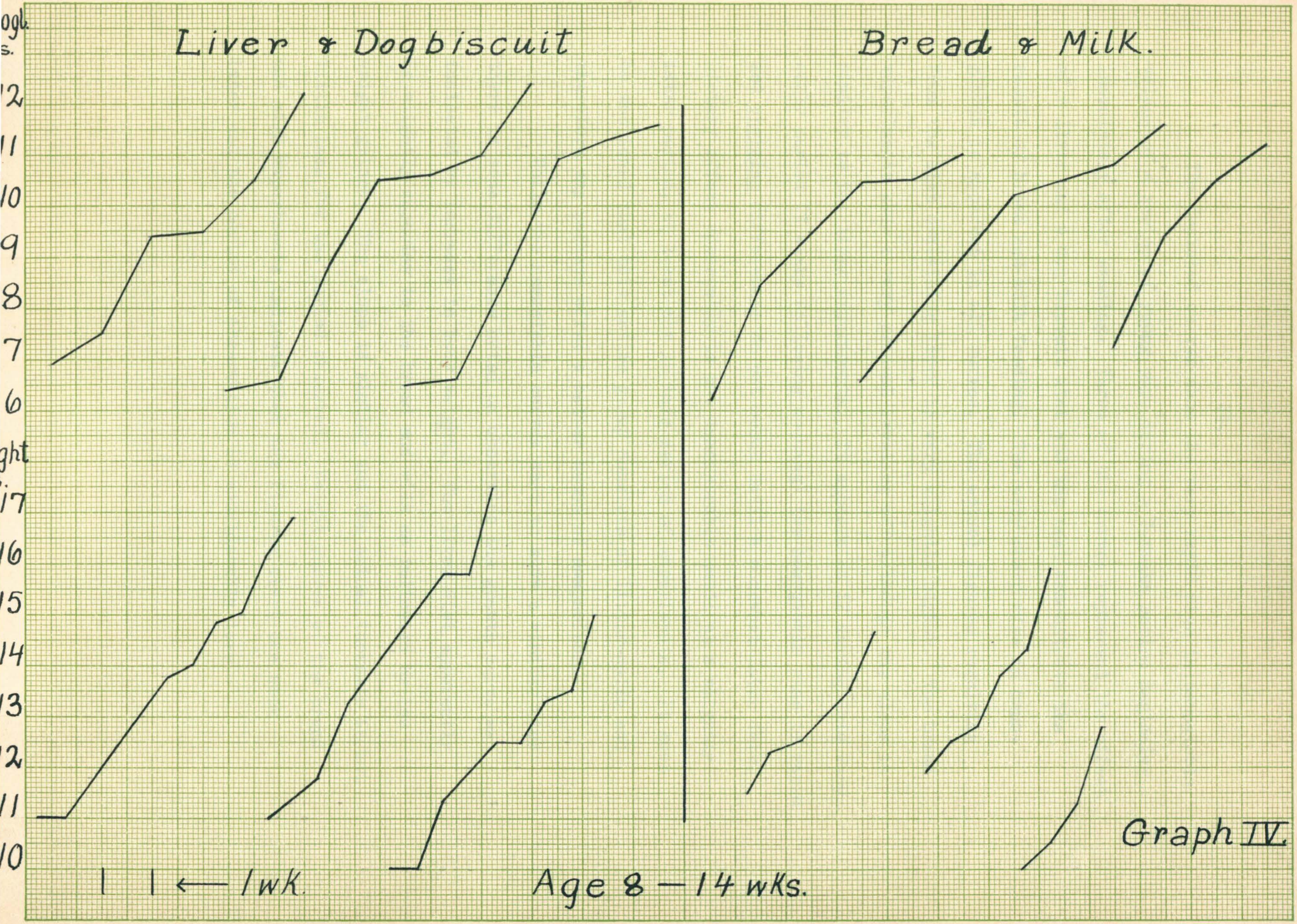
Formula

Salmon Bread

Ingredients	grams	Protein grams	Fat grams	Carbohydrate grams
Wheat flour...........	12,000	1,240	125	8,480
Potato starch.........	6,000			5,400
Bran	2,000	300	86	1,080
Sugar.................	3,000			3,000
Cod liver oil.........	1,000		1,000	
Canned tomatoes.......	2,000	24	4	80
Canned salmon.........	2,500	545	302	
Yeast, compressed.....	455	55	2	96
* Salt mixture........	150			
Water	7,500			
Total.................		2,164	1,419	18,136

Protein, 10.0 per cent.
Fat, 6.5 per cent.
Carbohydrate, 83.4 per cent.
Caloric value 4.8 per gram as fed.
* McCollum and Simmonds' salt mixture with ferric citrate omitted.

All experimental pups were weighed once each week and at the same time blood was withdrawn from the jugular vein for hemoglobin and red cell hematocrit determinations. Hemoglobin estimations were made according to the method of Robscheit (24). The extent of the experimental period in these studies varied since it was desirable to determine the muscle hemoglobin content at different age levels. Some of the pups were therefore killed with ether at different intervals of time. The accompanying charts depict hemoglobin data for periods of varying time limits. The results obtained showing the effect of these different dietary regimes on the hemoglobin during this growth period are given in Graphs IV to VIII inclusive.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Liver & Dogbiscuit
Bread & Milk.
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13
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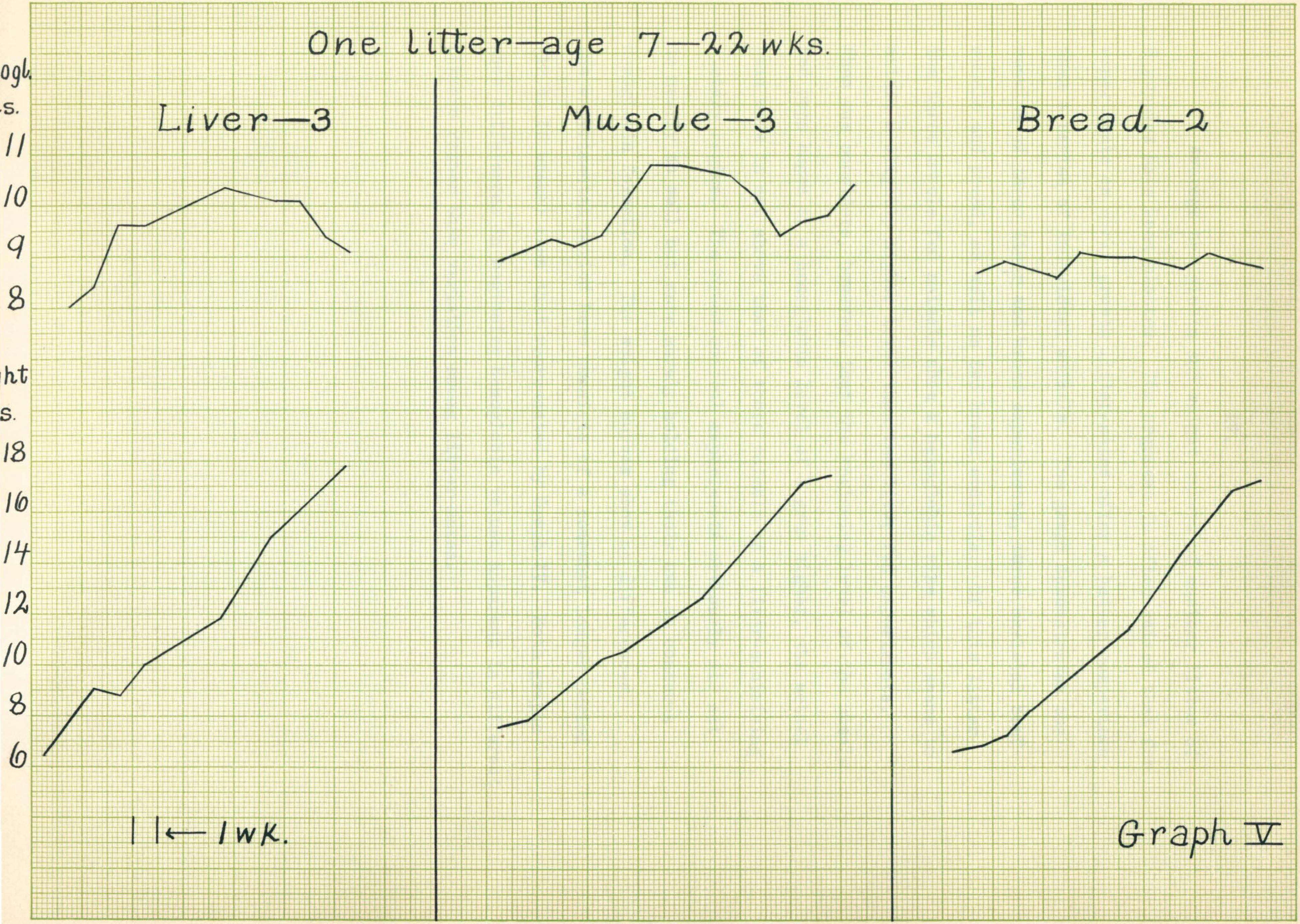
11
10
1 wk.
Age 8 — 14 wks.

Graph IV.

The depicted curves show the very rapid rise in hemoglobin values of a series of young pups during a 7-weeks period beginning immediately after weaning. Three of the curves represent the effect of a liver and a dog biscuit diet and the rest demonstrate the result of bread and milk feeding. The very sharp rise in hemoglobin is apparent in all curves. The actual figures resulting from the diet containing liver is very slightly higher. The bread experiments show a very gradual leveling off although the slope is similar to the liver experiments. It appears as if during this very early period (7 weeks following weaning) the hemoglobin rise is rapid and <u>striking</u> differences do not occur until a somewhat later date. Dietary effect apparently is not demonstrable until at a slightly later period, at least not in this series of experiments. It must be mentioned that this entire group consists of litter-mates and therefore may perhaps offer a better means of comparison.

While the hemoglobin curves seem to show a similar rise there is some difference noted on observing the growth curve as indicated by increase in body weight. The liver diet apparently is slightly superior to the bread and milk as indicated by the weight gained. The slope is similar and the actual increase is but little less for the period observed. Weight curves of the bread and milk experiments indicate that the figures start one week later and the period of observation is considerably less -

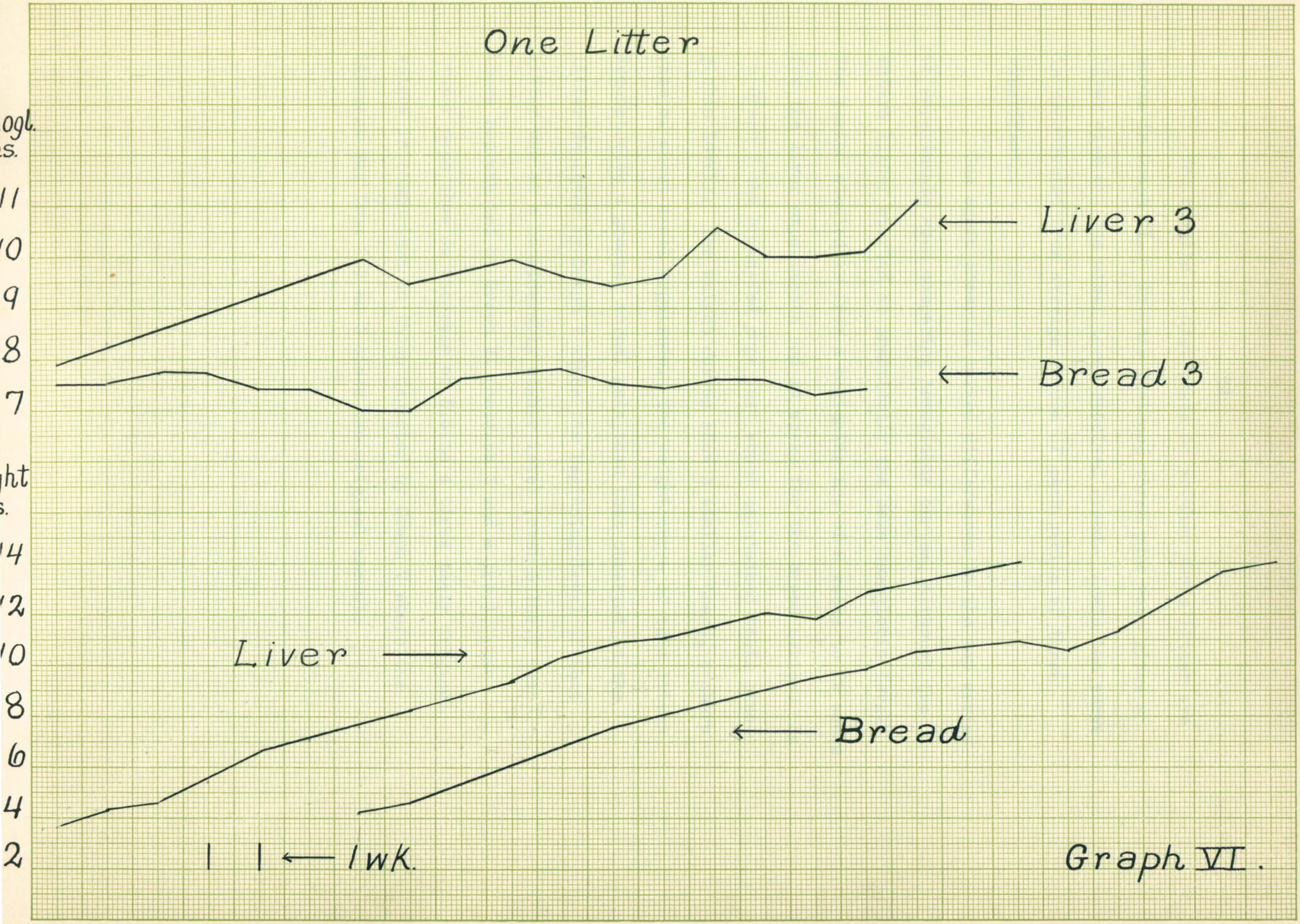
4 to 6 weeks only. The weights of the puppies at the beginning of the experiment were similar to those puppies on the liver and dog biscuit diet.



One litter—age 7—22 wks.

Graph V

Average hemoglobin and weight curves of another litter of 8 puppies have been plotted. The argument is often raised that for experiments on early growth litter-mates offer a better comparison than a series of individual animals. The graph demonstrates the results of three liver and bread experiments, three skeletal beef muscle and bread, as well as two bread controls. Of this litter the three muscle fed animals show a hemoglobin rise which seems to attain a slightly higher level than the liver fed experiments. When one considers, however, that the beginning level of the muscle fed pups began at a hemoglobin level of 1 gram higher, the actual gain made is not above that of those animals on a liver-containing dietary regime. The bread control experiments obviously did not do nearly so well but were merely holding their own as concerns hemoglobin formation. The weight curves show no differences. The experimental period here consists of a somewhat longer period (12 to 15 weeks) than that represented by Graph IV. Both graphs show differences in beginning hemoglobin values apparent in different litters rather than variation among litter-mates.


One Litter
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Liver 3
Bread 3
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s.
14
12
10
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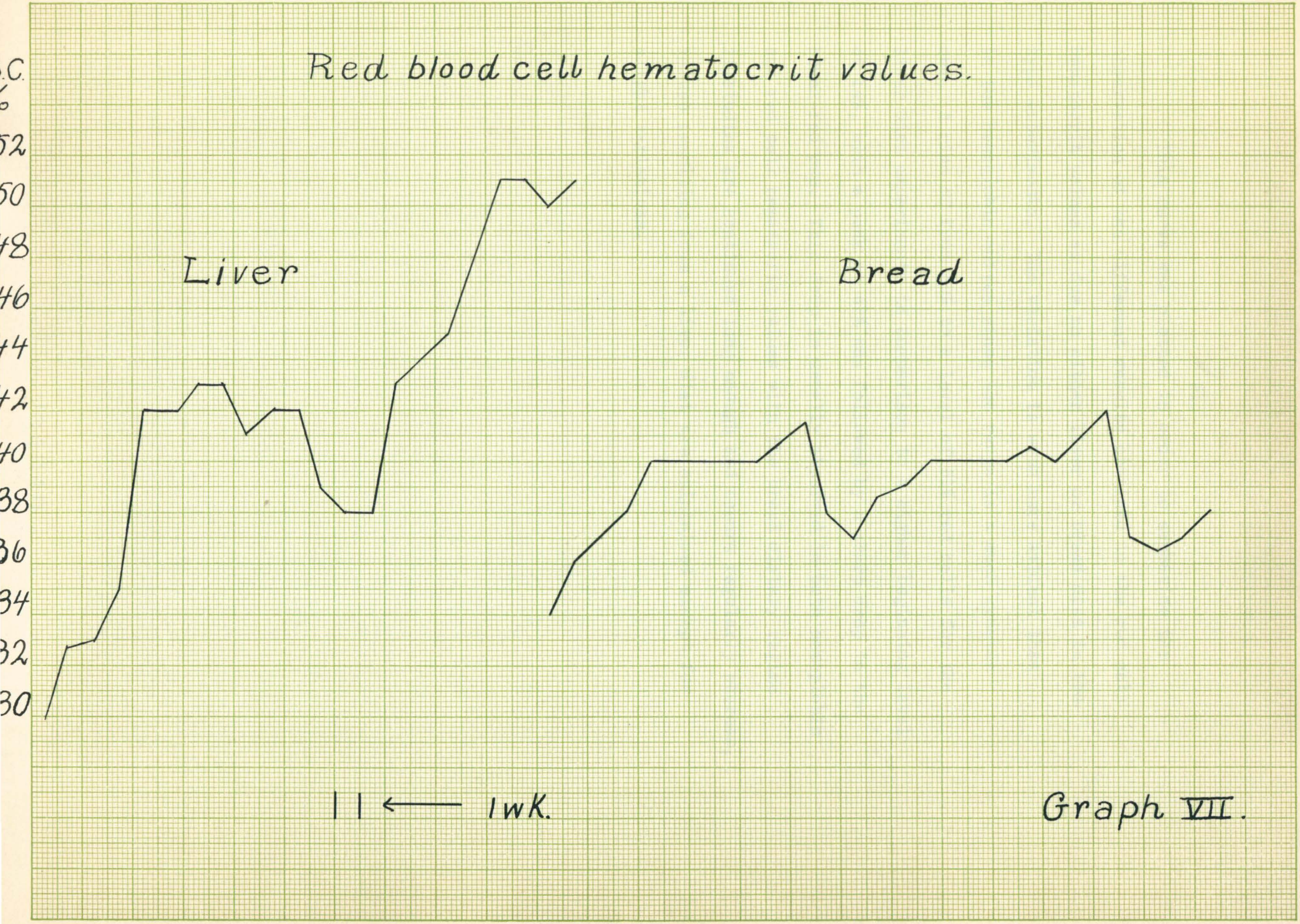
6
4
2
Liver
Bread
1 wk.

Graph VI.

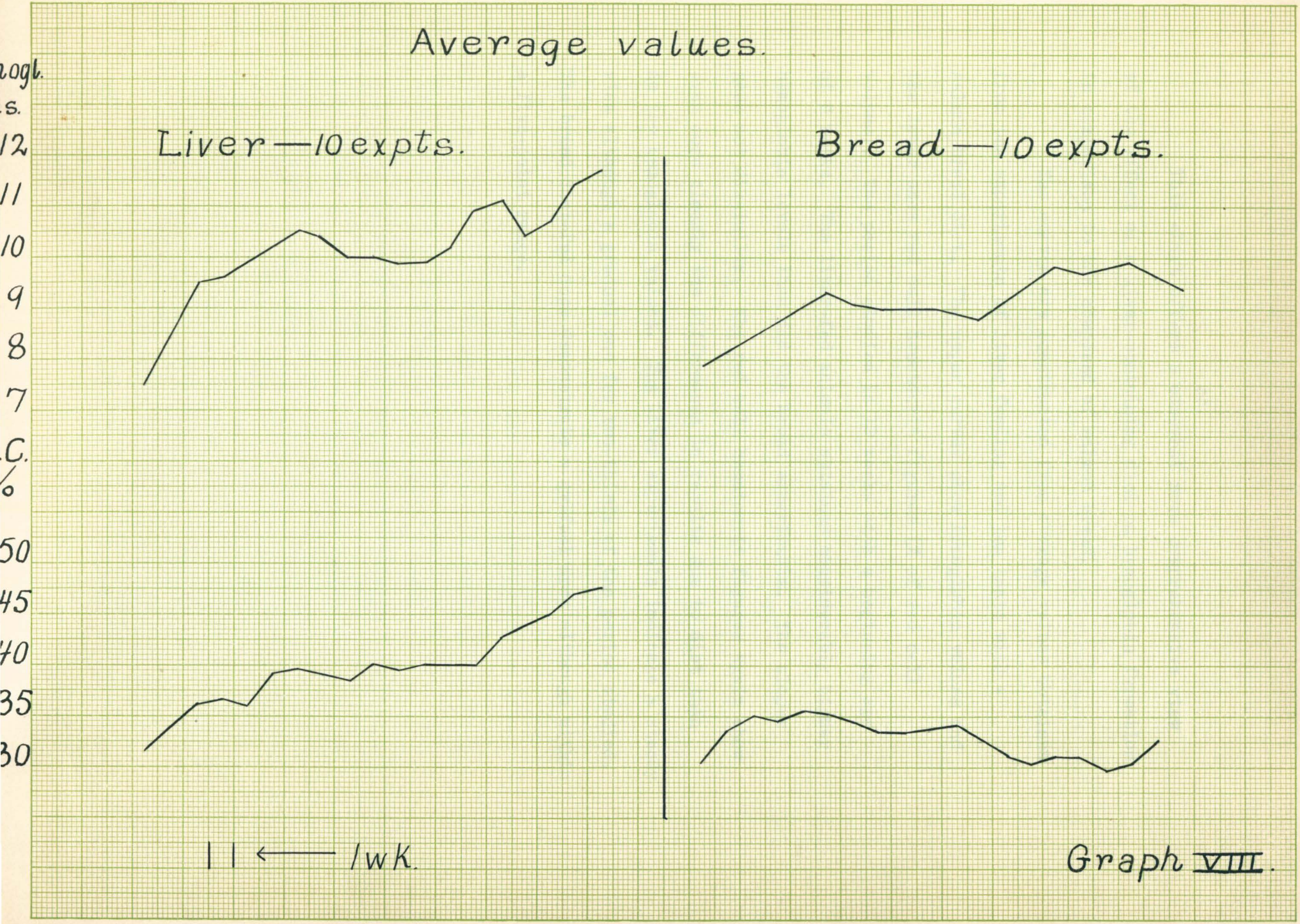
The results of diet tests on another whole litter are shown in Graph VI. In this instance three pups were given a liver diet and the other three received bread. Again the curve is constructed from average values of each series of three experiments.

One again observes marked differences in the two series of experiments when comparing hemoglobin curves, although the entire litter started at practically the same hemoglobin level. The period studied is from a beginning age of 8 weeks to 25 weeks for the liver experiments and 24 weeks for the bread series. As in Graph V the bread-fed pups just about maintained their hemoglobin levels. The weight curves show a similar trend, a sharp rise of about equal magnitude. The weight curve of this litter demonstrates a greater actual gain than that observed in the litter depicted by Graph V since the beginning level of that litter was at a considerably higher level (over 6 lbs.) than those represented by Graph VI (between 3 and 4 lbs.). The latter pups show an increase of 3.5 to 4 times their original weight in comparison to those depicted by Graph V where the increase was from 2.5 to 3 times the original amount.


Red blood cell hematocrit values.

Graph VII.

This curve was plotted in order to emphasize the observation of the different results obtained in the red cell hematocrit values as due to either liver or bread diet in two type experiments. The variation is even more evident than in the hemoglobin curves since the range is a much greater one. A rise of from 30% to a level of 51% red cells for the liver diet experiments in comparison to a higher beginning level of 34%, with a peak at 42% was followed by a sharp drop in the bread-fed pup. This drop is usually observed in both hemoglobin and red cell hematocrit values with the bread dietary regime if studies are continued long enough and occurs regularly whereas, values resulting from a liver diet are maintained except for slight variations.



Average values.

Liver—10 expts.

Bread—10 expts.

Graph VIII.

This chart offers a somewhat more comprehensive view of the effect of a liver-containing diet on hemoglobin formation in comparison to one composed of salmon bread.

The curves are constructed from average values obtained from ten experiments of liver feeding and ten tests with a salmon bread diet. The upper curves show hemoglobin values and the lower ones red cell hematocrit figures (% of red cell volume). The period studied is 19 weeks with a beginning age of 8 weeks. The liver-fed animals attained considerably higher hemoglobin levels than did the bread experiments. The slopes seem not dissimilar. The red cell hematocrit figures again show even more clearly the differences in results due to diet than do the hemoglobin values. The liver experiments indicate a steady rise although a gradual one for two-thirds of the period studied. The bread diet-fed dogs demonstrate a gradual drop although within a five-point range.

Discussion of Preliminary Studies

These preliminary studies concerning the effect of various dietary regimes on the hemoglobin, red cell hematocrit and weight definitely demonstrate the superiority of the liver rich diet in comparison to bread feeding. The bread diet on the whole satisfies growth requirements for the actual differences observed in the weight curves are slight.

During the earlier period of growth for seven weeks following weaning, difference in hemoglobin values are not striking, particularly in the litter plotted on Graph IV. Again in the summarizing Graph VIII the trend of the slope is upward although not quite as steep nor reaching as high a level. Weight increases are about of the same magnitude.

The few experiments concerning skeletal muscle diet vary but little from results obtained with liver feeding. Although the peak seems somewhat higher than the liver experiments the actual gain is no more since the beginning values of the muscle feeding experiments were at a higher level.

As emphasized these curves merely represent data obtained during an early period of growth and are but complimentary to the next series of experiments.

Experiments

Second Series

The main set of experiments was undertaken to determine the existence and extent of potential hemoglobin reserves and the effect of special diet factors upon the formation of these reserves during the growth period of the dog.

The reserve storage of hemoglobin factors is one of the most important points to consider in the interpretation of any data dealing with blood regeneration in the anemic animal. It is surprising to observe how difficult it may be to exhaust this emergency reserve in the healthy dog. We have made it a rule in our anemia studies to produce this experimental anemia slowly over a 2 to 3 weeks period on the standard bread diet and to maintain this anemia for 2 to 3 months to insure the exhaustion of this reserve. During this period as much as 250 - 330 grams of hemoglobin may be removed by bleeding, representing a tremendous reserve which can scarcely be stored as finished red blood cells. This reserve is probably stored as intermediates which on demand can contribute to the formation of the finished blood hemoglobin. After these reserve stores have once been exhausted the dog will react with a small and constant hemoglobin output to the standard salmon bread diet and will react uniformly to various diet factors as has been published in numerous papers.

Experimental Procedures

For this purpose various litters of puppies were segregated following the weaning period into groups and these groups were raised until the age of 1 year on the different test diets. Diet factors were chosen according to their hemoglobin building potentialities. Our anemia studies rate liver as the optimum diet for hemoglobin and red cell formation and salmon bread as the least favorable for this purpose. The diet factors studied consisted of salmon bread, our regular kennel diet which is composed of table scraps of meat, bread, potatoes, and other vegetable material, and lastly a diet of salmon bread plus the following meat products: kidney, beef skeletal muscle, liver. These organ supplements constituted increasing amounts up to 50% of the diet. A milk powder - "Klim" - was added to all diets. Sufficient quantity of food was given to furnish ample caloric intake. General growth demands were met by all these diets since the pups made satisfactory progress and steadily increased in weight. Detailed studies during this early growth period were not made on this series since this information had already been supplied by the preliminary experiments cited previously (First series). Our main object was to obtain data referring to these reserves of hemoglobin factors indicated by our anemia studies.

As the pups raised under this varied dietary regime attained 1 year of age they were added to our regular anemia colony. Hereafter they were subjected to the same experimental routine as those animals for our anemia studies. This experimental plan is as follows: Practically without exception our dog colony has been healthy during all experimental periods and unless special note is made to the contrary normal conditions obtained throughout the experiments. These experimental animals were born and raised in the kennel and therefore were accustomed to that place of residence, and to the simple procedures used for the removal of blood by venous puncture. The cages are large and comfortable and permit of much exercise. Not more than 12 dogs are kept in any room and every animal is kept at all times in its own cage. Cages are cleaned daily and the dog is transferred to an empty cage and care is taken that there is no access to any food elsewhere in the room during the cleaning period. Water is available at all times. The dog rooms are well ventilated by exhaust fans and a uniform temperature of 65 to 70° F., maintained by thermostatic control. We consider these conditions important for the successful conduct of a long anemia experiment and every care should be taken to maintain uniform temperature conditions day and night. These anemia animals are carefully isolated to prevent infection with distemper.

The dogs are fed once daily in the afternoon and the various food ingredients are so thoroughly mixed that the animals cannot pick out any single food ingredient which may be more to their taste. When bread is used in the mixture about equal parts of water are added to give a hash of suitable texture for thorough mixing. The various blood samples, blood volume determinations etc. are done in the morning and the dogs are so familiar with this procedure that they are not at all disturbed by venous puncture and blood aspiration.

These dogs have a hemoglobin level at one year of age of 120 to 150% (100% is equivalent to 13.8 grams hemoglobin per 100 cc. blood). The desired anemia level is induced by three, four or more bleedings spaced at one or two-day intervals. Care must be used else too rapid bleeding and blood concentration may cause fatal shock. This anemia level of 40 to 50% hemoglobin is then maintained as near a constant as possible by hemorrhages graded as to frequency and amounts by the readings of small samples taken at intervals each week for hemoglobin and hematocrit readings. Fluctuations of plasma volume must be taken into consideration in estimating the required bleeding in various experiments.

Hemoglobin pigment in grams is determined in every aspirated bleeding sample. Following every hemorrhage after an interval of 48 hours there are determined the hemoglobin and red cell hematocrit. Blood plasma volume and red cell counts are done each week independent of these estimations of hemoglobin and red cell hematocrits. No blood volume determinations are made less than 48 hours following any hemorrhage.

Blood plasma volume is measured by a method described in detail in various publications (25). This method utilizes the dilution of an inert red dye (brillian vital red) by the circulating plasma volume.

Hemoglobin is measured in the form of acid hematin colorimetrically against a known standard as described by Robscheit (24). This standard at 100% contains 13.8 grams of hemoglobin. In order to facilitate interpretation of the accompanying tables all figures pertaining to hemoglobin values are given in grams.

Red cell hematocrit values are obtained from a sample of blood taken into a 1.6% solution of sodium oxalate (isotonic to dog's blood) in graduated, calibrated 15 cc. centrifuge tubes. These tubes are centrifuged at 2200 revolutions per minute for 30 minutes which experience has shown gives uniform and maximal packing of cells.

Total circulating hemoglobin represents the amount of hemoglobin contained in the total circulating blood of the animal. This figure is obtained by multiplying the number of grams of hemoglobin pigment contained in 100 cc. of blood by the total blood volume divided by 100.

The bread mixture used in this experiment consists of salmon bread which is a rather unfavorable diet for hemoglobin and red cell formation. The various ingredients have previously been described in "Experimental Procedures" pertaining to the preliminary groups of experiments cited.

Table 1.

Hemoglobin Reserve and Diet.

Salmon Bread

Dog No.	Wt. at beg.	Non-Anemic					Anemic					Hb. Reserve	
		Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Days for Remov.	Hb. Remov.
		Total	Plas.	R.B.C.			Total	Plas.	R.B.C.				
	Kgm.	cc.	cc.	cc.	gm.	gm.	cc.	cc.	cc.	gm.	gm.		gm.
27-231	12.1	1137	497	629	21.8	248	864	700	157	5.6	49	50	168
27-236	16.2	1161	550	602	19.2	223	1055	835	209	4.7	50	51	167
27-238	14.8	1180	610	560	19.2	227	1073	850	217	6.4	68	55	216
30-114	14.8	1440	720	714	17.8	257	1138	894	239	6.1	69	49	196
30-116	14.6	1370	730	633	17.1	234	1080	812	263	6.1	66	49	229
30-120	16.2	1223	536	683	22.0	269	1079	800	273	5.8	63	49	210
32-5	14.5	1435	690	738	19.3	277	1043	780	254	6.5	69	54	198
32-6	14.4	1370	640	718	19.2	263	1149	806	326	8.4	97	56	286
33-13	16.2	1295	629	660	19.1	247	1214	941	266	6.2	75	57	241
Ave.	14.9				19.3	249				6.3	67	52	212

$$\text{Total circulating Hb.} = \frac{\text{blood vol.}}{100} \times 13.8 \text{ gm.}$$

This table contains a detailed blood picture of that group of dogs raised on the salmon bread diet to adult age and then made anemic making possible the estimation of the reserve storage of hemoglobin factors as produced by this diet. This group can serve as a control for the subsequent tables to follow (Tables 2 - 7). This table is discussed in great detail in order to aid in the interpretation of those to follow.

One observes first the blood picture of these animals during their normal or non-anemic state and the total amount of circulating hemoglobin during this phase. The same picture is again drawn following the removal of the reserve stores mentioned. The columns concerning the anemic state indicate the last blood volume determination following the removal of hemoglobin in grams cited in the last column of the chart. Besides the blood volume data, the total circulating hemoglobin in grams is estimated. This gives us a composite picture of the blood before and after the exhaustion of the reserves of hemoglobin factors. This study concerns a group of nine dogs raised from the weaning period to adult age on the salmon bread diet. The animals represent individual dogs from different litters although several of them are litter-mates. The weight indicated denote the weight in kilograms at the beginning of the experiment, that is when the accompanying blood volume was determined.

The blood volume is indicated first as <u>total circulating blood volume</u> and the next two columns give figures for its two main constituents, the plasma and red blood cell volumes cited in cubic centimeters. The column "hemoglobin per 100 cc. of blood" is self-explanatory. The total circulating hemoglobin computation is explained under experimental procedures.

The weights of the dogs vary from 12.1 kilograms to 16.2 kilograms with average values of 14.9 kilograms. With the exception of this one lower animal members of the group were of similar size. Blood volume figures always show individual variations. The average hemoglobin per 100 cc. is 19.3 grams per 100 cc. of blood. Total circulating hemoglobin figures are relatively within a narrow range, giving an average of 249 grams. During the normal non-anemic state one observes a group of dogs having an average weight of 14.9 kilograms with a total circulating hemoglobin volume of 249 grams. The animals, after having been rendered anemic, demonstrate the following points:

Total blood volumes have been considerably decreased with the exception of dog 33-13 whose anemic value is relatively little changed from the original amount. The amount of decrease in total blood volume varies greatly in the different animals. The decreases range from 9 to 27%. Dogs 30-114, 30-116, and 32-5 show loss in blood volume of 21%, 21% and 27% respectively. The rest show a lesser loss - 9 - 16%.

Occasionally one finds a dog which in spite of an unfavorable diet necessitates removal of considerably more hemoglobin than others, for example, dog 32-6 in this series. These individual variations are met with in all studies concerning living animals. This figure of 212 grams of hemoglobin represents withdrawal of hemoglobin of 85% of the non-anemic total circulating hemoglobin. The anemic level still shows an average total circulating hemoglobin of 67 grams; adding the amount removed or 212 grams we obtain a figure of 279 grams total to compare with the original non-anemic value of 249 grams, indicating a very slight reserve storage.

Occasionally one finds a dog which in spite of an unfavorable diet necessitates removal of considerably more hemoglobin than others, for example, dog 32-6 in this series. These individual variations are met with in all studies concerning living animals. This figure of 212 grams of hemoglobin represents withdrawal of hemoglobin of 85% of the non-anemic total circulating hemoglobin. The anemic level still shows an average total circulating hemoglobin of 67 grams; adding the amount removed or 212 grams we obtain a figure of 279 grams total to compare with the original non-anemic value of 249 grams, indicating a very slight reserve storage.

Table 2.

Hemoglobin Reserve and Diet.

Mixed Diet

Dog No.	Wt. at beg.	Non-Anemic					Anemic					Hb. Reserve	
		Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Days for Remov.	Hb. Remov.
		Total	Plas.	R.B.C.			Total	Plas.	R.B.C.				
	Kgm.	cc.	cc.	cc.	gm.	gm.	cc.	cc.	cc.	gm.	gm.		gm.
24-59	16.9	1593	654	924	17.4	277	1282	946	314	6.5	83	54	227
25-23	15.8	1232	578	648	16.7	206	1001	760	236	6.8	68	43	165
26-18	14.6	1325	776	535	15.5	205	1200	939	255	6.1	73	51	261
26-102	12.9	1155	612	532	17.8	205	1012	807	201	5.8	59	54	234
26-164	12.8	1148	562	574	18.8	216	978	758	215	6.6	65	48	187
29-65	12.0	1280	520	750	23.0	295	928	740	184	6.5	60	35	209
29-66	13.4	935	391	540	22.4	209	1067	836	221	6.2	66	48	230
29-67	15.7	1603	745	841	20.1	323	1154	929	220	5.7	65	54	232
29-68	14.4	1503	640	848	21.2	319	1131	926	200	5.4	61	54	247
Ave.	14.4				19.2	251				6.2	67	49	226

Similar studies as described in detail in discussing Table 1 are depicted by Table 2. This group of animals were raised on our usual kennel diet, which consists of a mixture of table scraps.

One observes much greater individual changes in these animals than those of the preceding group. It should be mentioned that "such a mixed diet" varies from day to day more in the amounts of the various ingredients rather than perhaps the individual food factors per se, and the rigid dietary control in this group does not exist as is the case in the experiments cited in all the other tables.

The average weight of the animals closely approximates that of the salmon bread group with similar individual variations. Similar decreases in total blood volume figures are apparent with the exception of that of dog 29-66 whose anemic blood volume is somewhat greater than his original non-anemic one. The greatly increased plasma volume offers the explanation. We observe a plasma volume when the dog was anemic of more than double that during his non-anemic state. Generally blood volume changes are similar to those in Table 1, although original blood volume figures vary widely. Dog 29-65 to 68 inclusive are litter-mates.

Similar marked individual variations apply to the hemoglobin figures as evident in both hemoglobin per 100 cc. and consequently in the total circulating hemoglobin. <u>Average</u> figures in comparison with those of Table 1 agree very closely. We observe a total circulating hemoglobin figure of 251 grams in comparison to 249 as cited in Table 1. In fact the average values of these two tables are in close agreement. The anemic level of total circulating hemoglobin is the same as that of the bread-fed group. The actual hemoglobin removed is but slightly more than that indicated by Table 1, a removal of 90% of the original circulating hemoglobin present in comparison to 85%.

With the average removal of 226 grams and a circulating hemoglobin of 67 grams when anemic a total figure of 293 grams is obtained which in comparison to the normal circulating hemoglobin of 251 grams represents very slight storage - only 17%. Therefore animals raised to adult age on a mixed diet such as represented by our hospital table scraps store only slight reserves of hemoglobin factors.

Table 3.

Hemoglobin Reserve and Diet.

Kidney

Dog No.	Wt. at beg.	Non-Anemic					Anemic					Hb. Reserve	
		Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Days for Remov.	Hb. Remov.
		Total	Plas.	R.B.C.			Total	Plas.	R.B.C.				
	Kgm.	cc.	cc.	cc.	gm.	gm.	cc.	cc.	cc.	gm.	gm.		gm.
27-239	11.4	1306	541	754	21.4	280	962	724	234	5.2	50	56	281
27-240	12.3	1412	500	898	23.3	329	1082	845	225	6.1	66	50	238
27-241	12.3	1148	482	654	21.7	249	960	714	238	7.2	69	57	235
Ave.	12.0				22.1	286				6.2	62	54	251

This group raised on a diet of salmon bread and kidney - approximately 50% of each - consists of three experiments. These three dogs were somewhat smaller than those cited by Tables 1 and 2. An average weight of 12 kilograms compares with 14.4 and 14.9 kilograms in the preceding groups. Similar blood volume fluctuations are apparent in this group as were observed in the other two.

The average hemoglobin per 100 cc. is over 22 grams in contrast to 19.3 in the bread and 19.2 in the mixed diet series. The circulating hemoglobin also shows the highest value of the entire series. However, one must stress the fact that we have but three experiments to report and a larger series might modify the figures both of hemoglobin per 100 cc. of blood as well as that of the total circulating hemoglobin. The latter value of 62 grams for the anemic state is very slightly below that of the preceding groups. The hemoglobin removal amounts to 251 grams. While this figure actually appears of greater magnitude than the value indicated by the mixed diet series it actually represents 90% of the original amount of circulating hemoglobin or the same percentage value as that indicated in Table 2.

If one computes the sum of the hemoglobin removed and the circulating hemoglobin when anemic, a result of 251 + 62 grams or 313 grams hemoglobin is obtained. This figure compared to the value of the non-anemic circulating hemoglobin of 286 grams shows a sum of 110% or an excess or reserve of but 10%. We believe that a larger series of experiments would raise this value somewhat.

Table 4.

Hemoglobin Reserve and Diet.

Skeletal Muscle

Dog No.	Wt. at beg.	Non-Anemic: Blood Volume Total	Non-Anemic: Blood Volume Plas.	Non-Anemic: Blood Volume R.B.C.	Non-Anemic: Hb. per 100cc. blood	Non-Anemic: Total circulating Hb.	Anemic: Blood Volume Total	Anemic: Blood Volume Plas.	Anemic: Blood Volume R.B.C.	Anemic: Hb. per 100cc. blood	Anemic: Total circulating Hb.	Hb. Reserve: Days for Remov.	Hb. Reserve: Hb. Remov
	Kgm.	cc.	cc.	cc.	gm.	gm.	cc.	cc.	cc.	gm.	gm.		gm.
30-117	15.3	1457	688	762	19.3	283	1281	986	290	6.5	83	55	330
30-118	13.4	1081	531	546	18.5	200	1054	797	251	7.0	74	55	247
30-121	14.7	1516	744	766	18.5	281	1165	886	274	6.8	79	57	305
32-1	13.2	1248	600	642	20.1	252	964	725	234	6.9	66	55	217
32-3	12.8	1196	610	580	18.6	223	994	762	224	6.6	66	55	228
Ave.	13.9				19.0	248				6.8	74	55	265

This series demonstrates the effect on stores of hemoglobin factors in five experiments of a diet composed of salmon bread and beef skeletal muscle. The weights of these dogs varied from 12.8 to 15.3 kilograms with an average of 13.9 kilograms weight levels in between the bread and kidney groups.

The blood volume changes are similar to others cited, a considerable decrease in total blood volume with increased plasma volumes. Dog 30-118 is the exception, the anemic blood volume is practically of the same amount as the non-anemic volume.

The circulating hemoglobin during the anemic state is somewhat greater than that of the preceding groups. The removal of 265 grams of hemoglobin represents a little more than the total original circulating hemoglobin volume or 107%. A summation of the amount of hemoglobin removed 265 grams plus that amount circulating within the anemic dog gives a total of 339 grams or 137% of the original value. This represents an excess of 37%, quite a considerable reserve storage of hemoglobin factors.

Table 5.

Hemoglobin Reserve and Diet.

Liver

Dog No.	Wt. at beg.	Non - Anemic					Anemic					Hb. Reserve	
		Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Days for Remov.	Hb. Remov.
		Total	Plas.	R.B.C.			Total	Plas.	R.B.C.				
	Kgm.	cc.	cc.	cc.	gm.	gm.	cc.	cc.	cc.	gm.	gm.		gm.
25-97	15.3	1616	735	864	16.8	272	1295	927	356	6.8	88	57	308
27-233	11.5	1026	510	500	19.0	195	968	760	188	6.5	63	54	233
27-234	14.0	1361	724	625	17.4	237	1073	836	229	6.6	71	56	307
27-235	14.4	1325	512	800	24.6	326	1048	836	206	6.1	64	56	297
30-115	13.0	1187	624	558	16.3	194	1060	809	241	6.1	65	55	349
30-119	13.3	868	484	381	15.5	135	891	696	187	5.3	47	58	233
32-2	15.1	1515	765	736	18.2	276	1179	988	182	5.0	59	56	276
32-4	14.1	1210	530	682	20.7	251	1075	800	267	6.9	74	55	273
Ave.	13.8				18.6	236				6.2	66	56	285

This group of animals, fed a diet rich in liver (50%), demonstrates the very favorable effect that this diet has on hemoglobin formation. The eight experiments cited show average weights similar to the skeletal muscle group. In some cases, dogs 27-233, 30-115, 32-4, the blood volume changes between the non-anemic and anemic periods are only moderate. The anemic blood volume of dog 30-119 is even slightly above his non-anemic level. Hemoglobin per 100 cc. of blood and the total normal circulating hemoglobin is somewhat less than that of the preceding series. The amount of hemoglobin removed in order to render these dogs anemic is greater, however, demonstrating more extensive reserve storage. The hemoglobin removal figure of 285 grams represents 120% of the amount of hemoglobin originally circulating within the animal. The anemic level still demonstrates an amount of circulating hemoglobin equal to 66 grams. Adding to this the amount of hemoglobin removed or 285 grams, one obtains a total of 351 grams or 149%. We observe therefore, a reserve storage of hemoglobin factors of 49%, quite a considerable amount.

Table 6.

Hemoglobin Reserve and Diet.

Miscellaneous

Dog No.	Wt. at beg.	Non-Anemic					Anemic					Hb. Reserve	
		Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Blood Volume			Hb. per 100cc. blood	Total circulating Hb.	Days for Remov.	Hb. Remov.
		Total	Plas.	R.B.C.			Total	Plas.	R.B.C.				
	Kgm.	cc.	cc.	cc.	gm.	gm.	cc.	cc.	cc.	gm.	gm.		gm.
Apricots													
33-11	11.8	881	392	484	20.7	182	698	468	226	9.0	63	52	194
Iron													
33-12	12.7	820	395	418	20.4	168	702	496	203	7.6	53	52	179
Blood													
33-14	11.5	916	428	484	19.9	182	878	704	170	5.5	48	54	176

This table cites three miscellaneous experiments which are merely offered as isolated experiments rather than as comparative data.

The first experiment consists of a diet in which the salmon bread is supplemented with cooked apricots equivalent in amount to 100 grams of the dried fruit. The amount of hemoglobin removed in this instance to maintain a uniform anemia level is 194 grams or 107% of the original circulating hemoglobin when the dog was not anemic. The circulating hemoglobin during the anemic period is 63 grams. The sum of 194 added to this figure or 257 would indicate considerable reserve storage of hemoglobin factors. We do not feel that one is justified in drawing any conclusions from this single experiment.

The next test represents an addition of iron in amount equivalent to 40 milligram as Fe to the salmon bread diet. Iron is favorable for hemoglobin formation in the type of experimental anemia we are studying - the simple hemorrhage anemia (26). Again one observes that the amount of hemoglobin removed is slightly greater than the amount originally circulating, a value of 107% as in the apricot experiments cited. The total circulating hemoglobin in the anemic animal is somewhat lower than the apricot experiment, 53 grams in comparison to 63 grams. The actual reserve hemoglobin factors would give results of practically the same magnitude as the apricot experiment 38% in comparison to 41% (apricot). This data must be augmented by further experiments before considering the value of the results obtained.

The last experiment of the group concerns a diet supplement of cooked or coagulated whole dog blood in amount equal to 200 grams. The non-anemic circulating hemoglobin level is similar to that of the dog given apricots; the anemic circulating hemoglobin level is considerably below that of the fruit experiment, 48 grams in the former in comparison to 63 grams of the latter. The amount of hemoglobin removed (176 grams) of the animal which received the blood diet represents 97% of his original circulating hemoglobin. Adding 176 grams or the amount of hemoglobin removed to the circulating hemoglobin during the anemic state or 48 grams, a total of 214 grams is obtained indicating a modest reserve of hemoglobin factors of 18%.

Table 7.

Hemoglobin Reserves.

Comparison of Diets Tested

Diet	No. Expts.	Total circulating Hemoglobin		Hb. Removed	Total Hemoglobin		Reserve %
		Non-anemic	Anemic		Grams	% of Original	
Bread	9	249	67	212	279	112	12
Kennel food	9	251	67	226	293	117	17
Kidney	3	286	62	251	313	110	10
Muscle	5	248	74	265	339	137	37
Liver	8	236	66	285	351	149	49

Table 7 permits a general comparison of the value of the various diets described as to their effect on the storage of potential hemoglobin precursors.

The headings of the chart are self-explanatory. The number of experiments in each group vary, this depends upon the size of the various litters studied. The salmon bread, kennel food and liver series offer the best means of comparison since the number of experiments were nearly the same. The miscellaneous group for obvious reasons has not been included in Table 7. One observes that the column indicated as "total hemoglobin" is the sum of the amount of hemoglobin removed to exhaust reserve storage plus the amount of circulating hemoglobin when the animal is anemic. This figure gives a sum of varying amounts over 100%, where 100% represents the amount of circulating hemoglobin in the non-anemic dog. The portion over 100% indicates that excess or reserve stores of hemoglobin factors.

The bread diet results in storage of the least reserves and liver indicates the greatest accumulation of stores. The actual figure for the kidney diet group is, of course, the lowest; however we feel confident as a result of much experimentation with this food factor in our regular anemia studies that the value of a kidney diet in a large series probably would be greater than indicated by the present series. The results of kennel food and skeletal muscle fall between the bread and the liver extremes.

Table 8.

Hemoglobin Reserves.

Calculated per Kilogram Body Weight

Diet	Circulating Hb. per Kg. Body Wt.		% of Original Circulating Hemoglobin		Total % of Original
	Non-Anemic	Anemic	Removed	Remaining	
Bread (9)	17.0	3.7	85	27	112
Kennel food (9)	18.0	4.9	90	27	117
Kidney (3)	24.0	5.2	88	22	110
Muscle (5)	18.0	6.8	107	30	137
Liver (8)	17.0	4.8	120	28	149

Because of not inconsiderable differences in weight of the dogs studied this chart may offer additional information. The data used for computation is that cited in Table 7, but instead of total circulating hemoglobin we have computed these figures as circulating hemoglobin per kilogram of body weight of the dog for both the non-anemic and anemic states. The next two columns cite the amount of hemoglobin withdrawn and that still present in the anemic animal as percentage values of the original amount of circulating hemoglobin. One again observes that the kidney diet group shows the greatest amount of circulating hemoglobin in the non-anemic state. The other group demonstrates values agreeing very closely in amount. This table indicates the reserves of potential hemoglobin factors as does Table 7 in the last column.

Discussion - Second Series

Considerable variations in blood data in the different animals are apparent. We believe these differences to be individual rather than that litters vary as a whole. Litter-mates do not necessarily show similar findings even though raised on the same diets (see Table 2, dogs 29-65 to 68 inclusive). Frequently a litter contains one particularly active hemoglobin regenerating animal (Dog 32-6, Table 1) even though the diet may be an unfavorable one for hemoglobin formation. This demonstrates that an insufficient number of experiments in a series may lead to some confusion in the interpretation of results.

One observes that following the removal of large quantities of blood during the time interval cited in Tables 1 to 6 inclusive the total blood volume has not regained its original amount which is in contrast to the usual results obtained after an interval of 48 hours following a single hemorrhage even though this be an extensive one. The body makes a great effort to restore this loss of fluid at once possibly from tissue fluids and other stores as evidenced by a considerably increased plasma volume in the anemic animal.

It is surprising how rapidly these dogs adapt themselves physiologically to the anemic state, i.e. to the greatly lowered blood levels. They behave as perfectly normal animals and are very active. No one observing them would realize that these dogs are carrying on their normal physiologic functions with only from 22 to 34% of the amount of their original normal circulating hemoglobin.

The salmon bread diet group of dogs show relatively slight storage of hemoglobin factors; an estimated figure of 12%. This fact is borne out by our studies on blood regeneration in our anemic dogs which have demonstrated that salmon bread is a very poor diet for hemoglobin formation (27). It has been determined that these standardized anemic dogs form but from 2 to 5 grams of hemoglobin per week above their maintenance requirements. The latter term signifies the repair necessary to rebuild that amount of red cells and hemoglobin destroyed during general wear and tear of circulation.

The experiments concerning kennel diet show greater individual variations, particularly as concerns the non-anemic total circulating hemoglobin. The diet itself probably offers a partial explanation because it obviously may vary from day to day; more perhaps in the proportionate amount of its ingredients rather than in different constituents. These tests with this mixed diet were made in order to determine the effect of what represents probably the customary dietary regime to which the usual dog is subjected.

The group which were given a kidney and bread diet is really not sufficiently large to permit conclusions. It is of interest that these three experiments all demonstrated high beginning hemoglobin values. It happens that these dogs were litter-mates although the remaining two dogs of this litter were not particularly active hemoglobin regenerating animals. The non-anemic circulating hemoglobin value is also higher than that of any group of the entire series. We believe these three dogs well represent the individual variations one encounters in animals. A great many observations made on anemic dogs show kidney to be an excellent diet for red cell and hemoglobin formation (29), being equal to 85-90% of that resulting from liver feeding. The actual stores of hemoglobin factors resulting in the tested group of three show a low value, a reserve of but 10%. More extensive experimentation is essential to ascertain whether this value will remain at the present level.

Skeletal muscle feeding shows a very favorable influence on the laying down of stores of hemoglobin factors. The series of five experiments demonstrate a storage of 37% which is a sizeable amount, considerably greater than that apparent in both the mixed diet and kidney fed series.

It is of interest to observe that the non-anemic circulating hemoglobin approximates in value so closely that of the bread and mixed diet group. Skeletal beef muscle does not give nearly the uniform results in stimulating hemoglobin formation in the anemic dog that is evident when testing other meat products or organs (30).

Liver is the optimum food for hemoglobin and red cell formation (31). The response to this diet is uniform, anemic dogs in many and frequently repeated tests manufacture over 100 grams of hemoglobin above their maintenance requirements, as a result of a two weeks period of liver feeding. In the particular application of this dietary to the present test - its influence on storage of hemoglobin precursors - we again find it the diet par excellence, one which produces the greatest reserve stores. The group given the liver diet offers an excellent means of comparison with particularly the salmon bread tests. The number of experiments cited in both of these groups are somewhat greater than most of the other series and we are here dealing with the maximum and minimum responses. One observes that the non-anemic circulating hemoglobin value in the liver experiments is the least of any of the series excepting those single experiments cited in Table 6, but the hemoglobin removed is greater in amount than any of the other groups. The hemoglobin withdrawn equals 120% of the original amount present. Taking into consideration the volume of hemoglobin removed and the amount of circulating hemoglobin remaining in the anemic animal it is evident that considerable reserve stores have been accumulated, an excess equal to 49%.

Concerning the miscellaneous group cited, composed of single experiments with a variety of diet factors, it is obvious that one cannot estimate true values as to reserve storage of hemoglobin factors from isolated experiments. They do indicate a trend and confirm data obtained with these dietary supplements in our anemia studies. Apricots are favorable for hemoglobin regeneration (28), most of the effect obtained with this fruit is due to its mineral content, of which iron is one of the component parts.

Iron is the most potent mineral for hemoglobin formation. In a large series of experiments we have ascertained that 40 mg. of Fe added to the bread ration for a two-weeks period permits of a hemoglobin formation of approximately 50-60 grams (27), this is in comparison to 100 grams as a result of a two-weeks feeding period of a liver containing diet.

In similar tests blood has comparatively little effect on hemoglobin building.

The various diets compared as to their potency in laying down stores of hemoglobin factors show definite gradations. The kidney group in further tests would in all probability show up more favorably when considering the results of repeated tests made on anemic dogs, the present results therefore are left for modification by further experiments. This same gradation of potency is again observed when one compares the actual amount of hemoglobin removed in the different diet groups, the bread series necessitating the least withdrawal of hemoglobin in order to exhaust these stored reserves of hemoglobin factors and liver the greatest.

Summary

A study of the stored reserve of potential hemoglobin factors in growing dogs as influenced by a variety of dietary regimes has been made.

Because of accepted similarity in the hemoglobin curve during early growth in humans with that of some animals several graphs depicting such data are added. In order to aid in interpretation of these papers a variety of factors which may influence hemoglobin formation in the new born and growing organism are discussed.

A series of preliminary experiments dealing with the effect of different dietary factors on hemoglobin formation during various ages of growing dogs are cited. The results of this investigation demonstrate that at the age of from 8 - 14 weeks the difference in effect on hemoglobin curves of liver fed pups and those given a diet of bread and milk are not striking. The average hemoglobin values of the bread and milk fed group are only slightly lower than those of the group on the liver diet. The hemoglobin curves of both series show a sharp rise. A weight curve shows a similar rise demonstrating that the salmon bread diet meets the necessary requirements of growth. At later age periods differences between bread and liver fed groups are pronounced.

A diet rich in either liver or skeletal muscle is very favorable for hemoglobin formation during the growth period. The salmon bread diet in contrast is not. Growing dogs show some rise in the hemoglobin curve, but of only moderate extent. This level is maintained with minor fluctuations for some weeks after which a slight drop occurs.

The difference in potency for hemoglobin building in the liver diet as compared with the bread ration is even more apparent in curves depicting red cell hematocrit values.

The main and second series of experiments concerns the effect of different diets on storage of hemoglobin factors during the growth period of dogs.

The results of these experiments show that a salmon bread diet results in only slight storage of hemoglobin factors, a value of a 12% excess.

The group given a mixed diet composed of table scraps demonstrate a storage of 17%.

The kidney fed series represent too small a number to permit conclusions. This type of diet is a very favorable one for hemoglobin regeneration in anemic animals, therefore further experimentation to determine the influence of this diet on reserve stores of hemoglobin factors is essential.

Skeletal muscle is potent in laying down stores of hemoglobin factors, an excess of 37% is observed.

Liver demonstrates the greatest potency. A reserve of 49% is attained as a result of the feeding of a diet rich in liver.

A salmon bread diet is the least effective in accumulating reserve of hemoglobin factors and liver represents the optimum diet for this purpose.

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