

**BLOOD VOLUME DETERMINATIONS IN CHICKENS
AND BLOOD LOSS IN DRESSING**

By

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INTRODUCTION

One of the steps through which chickens pass during the dressing operation is the severing of the carotid artery in order to remove as much blood from the carcass as will flow out naturally. A large percentage of the blood of the body is located in the small veins and capillaries, and as soon as the heart stops pumping the blood immediately stops circulating. For these reasons much of the blood remains in the body after death. This blood is present in the carcass of the bird until consumed and manifests itself in the cooked product as black strings which are no more than blood vessels containing coagulated blood. Although this may not change the flavor of the meat, it is objectionable to the consumer, who does not understand what its presence means.

Preliminary investigations on the bleeding of poultry showed that the amount of blood lost during dressing in proportion to body weight varied considerably among individuals of comparable weight groups. This blood loss did not appear to be very closely correlated with the external criteria of satisfactory bleeding. Thus it appeared necessary to determine the amount of blood in normal chickens before proceeding with factors affecting bleeding.

REVIEW OF LITERATURE

As a normal constituent of the body of all animals, blood is present in rather large quantities as compared with other body fluids and has been the subject of considerable research and study. These studies are necessitated by the very character of the action of blood in the body. Even small changes, either in amount or of any constituent of the blood, are immediately reflected in the external behavior or reactions of the body.

Because of inadequate laboratory equipment and techniques for the proper measurement of the amount of blood, early investigators dealt almost entirely with the chemical constituents of the blood. In fact, it was as late as 1628 that Dr. William Harvey was able to demonstrate the mechanics of the circulation of blood in the body.

The methods of determining blood volume fall into two general classifications: (1) the direct method, in which the subject must be sacrificed and the total volume of blood determined by combining the quantity lost by bleeding with the quantity remaining in the body, the latter value having been determined in different ways by the various investigators; (2) the indirect method, in which either dyes or colorless solutions are injected into the blood stream and the dilution of the dye or the blood determined in subsequent samples. Another of the indirect methods used by some investigators was the use of the capacity of the hemoglobin of the blood to displace oxygen with carbon monoxide and the accompanying color change resulting therefrom. The inhalation of a known amount of carbon monoxide and the colorimetric estimation of the amount of oxygen displaced gives a basis for determining the amount

of blood in the body. Each of these methods will be discussed individually, but the indirect method is the only one which is usable for routine clinical diagnoses.

Haller (Welcker, 1854) is reputed to be the first to attempt any determinations of blood volume. His work consisted of weighing the blood lost by criminals on the execution block. He reports only two cases and the weights of the blood obtained were 28 and 29 pounds, respectively. No report was given of the weight of these two criminals, so no percentage figures can be computed. Welcker (1854) used this blood loss technique on small laboratory animals. He also washed out the blood vessels as well as possible and then minced the tissue, washing thoroughly to remove all hemoglobin. He then compared dilutions of a sample of blood from the animal with the combined blood and washings obtained. He divided the dilution of the original sample which most nearly matched the color of the blood washings into the total volume of blood and washings. This gave a value for the amount of blood contained in the body of the animal. This method is subject to several sources of error: (1) the coloring matter of the muscles and tissue interferes with the matching of the color of the two samples; (2) the turbidity added to the solution by the muscles and tissues masks the color and makes matching difficult; (3) the loss of blood pigments due to clotting also leads to further error. These sources of error notwithstanding, his value of 1/13 or 7.7 percent of the body weight being blood was used as standard for several years and many of the workers who came later based their calculations on his values.

Bischkoff (1856 and 1858) applied the technique of Welcker to two condemned criminals and obtained values for man very close to Welcker's values for small animals.

Throughout the next several years many workers tried to improve on Welcker's original technique. Mallassez (1874) attempted to overcome a part of the difficulty by counting red blood cells rather than by colorimetric comparison. He leached the tissue and washed the blood vessels with a solution containing gum arabic, sodium chloride and sodium sulphate. He used this solution in an attempt to prevent hemolysis and also made an attempt to prevent coagulation of the blood.

Other workers tried other methods of improving the technique. For example, Suter and Jaquet (1897) tried flushing the circulatory system, using pulsating pressure and Dreyer and Ray (1910) substituted Lockes solution for the sodium chloride solution. A very complete review of these direct methods as well as the indirect methods with a frank criticism can be found in a review by Erlanger (1921). Inspection of his table listing the results of studies of blood volume of rabbits by the various investigators does not reveal that any of the seemingly better methods yielded any larger volumes.

The determination of blood volume by indirect methods seems to have originated with Valentin (1838). The principles used by him were used later, so a short discussion of his method is in order. A sample of blood is withdrawn and the solids content determined. Then a known amount of water is injected intravenously. After a time lapse which is supposed to suffice for the complete mixing of the injected water with the blood, but before too much water has left the circulation, the

solids content is again determined. It is presumably only a matter of calculation to determine the amount of blood in the body at the time of the injection of the water.

The principles of this method have been used by several workers in an attempt to determine blood volume. Most of them have changed it in some way in an attempt to secure greater accuracy. The use of saline solutions by Sander and Kronecker (1881), who determined hemoglobin dilution, and by Sherrington and Copeman (1893), who used the change in specific gravity of the blood as an index of dilution, are two of these attempts.

The accuracy of the sodium chloride dilution methods is based on two conditions: (1) that the mixing time is sufficiently long to allow complete and uniform mixing of the diluent through the blood stream; (2) that the loss of the diluent from the blood stream between injection and subsequent sampling is negligible. That these two conditions are not fully met is pointed out by Erlanger (1921). He indicates that most of the advocates of this method cite Cohnstein and Zuntz (1888) as having demonstrated the slow disappearance of isotonic saline solution from the circulatory system. However, these two workers, according to Erlanger, used incorrect values for the percentage of the body weight which is blood and thus have unjustly considered their technique accurate. Other workers, Sherrington and Copeman (1893) and Smith and Mendel (1920), have shown that large amounts of the saline solution are lost from the blood stream soon after injection.

Other solutions have been used in an attempt to more nearly satisfy the conditions required for accurate determinations by this method. Loewy (1920) injected isotonic glucose. However, the

objection can be raised that perhaps it is the water which leaves the blood stream and thus the interchange of elements between the blood and the cells of the body introduces error into the calculations. The injection of a compatible serum was suggested by Mallassez (1874) as a means of meeting the condition of low loss from the blood stream during the post-injection period.

Robertson and Bock (1919) tried the injection of Bayliss' solution (6 percent gum arabic in 0.9 percent NaCl) as the diluent and hemoglobin as the index of dilution. These workers had an opportunity to test in the same patient the reading by this method and by the vital red method. The latter method, to be described later, gave a reading 3 percent higher than the former method. Rightly or wrongly, they charge the error against the gum-arabic method.

Quincke (1879) injected blood from normal subjects into the blood stream of subjects suffering from pernicious anemia. He attempted to ascertain the blood volume by determining the degree of dilution by red cell counts. The accuracy with which antitoxin levels in the blood can be determined and the persistence of the antitoxin in the blood led von Behring (1912) to attempt its use in blood-volume determinations. However, differences in levels of the antitoxin in different parts of the blood stream are suggestive of its free passage into the lymph stream. Behring states that the method may be used satisfactorily if all precautions are observed. Several workers followed his suggestions but the method seems to have had very limited usage.

Gréhant and Quinquad (1882) proposed and tested the technique of carbon monoxide inhalation and subsequent determination by gasometric methods of the amount of hemoglobin remaining combined with oxygen.

They used dogs for their blood volume determinations. Haldane and Smith (1899-1900) modified the technique and applied it clinically for determinations on man. In their applications, the patient breathes for five minutes a measured volume of carbon monoxide. A sample of blood is withdrawn and its carbon monoxide content estimated by the carmen titration method of Haldane. The carmen titration method has proved unsatisfactory to many workers, and Oerum (1906) and Zuntz and Plesch (1908) attempted to improve on the carbon monoxide measurement. However, these methods are difficult and hard to master.

Van Slyke and Salvesen (1919) developed a simple gasometric method which can be used to determine the quantity of gas quickly and accurately. Salvesen (1919) used this method for the estimation of blood volume in rabbits and in man.

Although the use of the inhalation method in small animals gives fairly consistent results, its use in man has given widely divergent results. One reason for these differences is probably the time allowed by the different workers for the mixing of the gas throughout the blood stream. Another source of inaccuracy was the definition of normal individual subjects given by the various workers. One of the subjects used by Haldane and Smith was very obese, a low percentage of blood volume per unit of body weight being obtained. Plesch reported that some of his subjects were fat. Thus it is hard to draw consistent conclusions from their results.

The use of a vital dye, which is injected into the blood stream, for blood volume determinations was first advanced by Keith, Rowntree, and Geraghty (1915). The dye method, although essentially an injection method, is so much simpler in its application and so widespread in its

usage that a separate discussion of it is included here. In selecting a dye for their determinations, Keith and his coworkers wanted one which was non-toxic. They also had to satisfy the conditions as outlined for the saline solution injections. They decided on the use of vital red for their indicator and found only slight variations between the blood volumes for normal subjects when considered on a body weight basis. Their data tend to refute the findings of Haldane and Smith, and tend to support the findings of earlier workers.

Operating in a base hospital during World War I, Robertson and Bock (1919a), using the method of Keith, determined the plasma volume and hematocrit reading of wounded soldiers admitted to the hospital. They found the method to be very reliable in the determination of blood volume before transfusion and on predicted volume after transfusion.

Hooper, Smith, Belt, and Whipple (1920) in an attempt to improve Keith's method, found that the disappearance of vital red from the blood stream was very slow. They were also unable to demonstrate the presence of the dye in any of the body excretions. Utilizing the fact that the dye disappears slowly from the blood stream, Smith (1920) found that repeated determinations could be made on the same individual at short intervals.

Smith, Arnold, and Whipple (1921), in testing the Welcker, carbon monoxide, and dye methods of blood volume determination, state that some discrepancy may exist through the exclusive use of any one method or substance. They object to the use of the hematocrit for corpuscular volume determination, since the hematocrit readings of blood taken from various parts of the circulatory system will vary. They suggest the use of the dye method for the determination of the plasma volume and either

the Welcker method or the carbon monoxide method for the red cell volume determination. Later workers in this field are explicit in their instructions to use a large blood vein for the sampling, since in these veins the stasis or sludging of blood would be at a minimum and the hematocrit reading more accurate.

In a study to determine whether other dyes were as well suited as vital red for plasma volume determinations, Dawson, Evans and Whipple (1920) tested some 60 different dyes covering the entire color range. Of this large number, they found only three which were usable. These were vital red, already used, and two blue dyes T-1824 and T-1835 (alkaline). These workers indicate in their results that the two blue dyes are preferable for three reasons: (1) they leave the circulation somewhat more slowly than vital red; (2) the blue color can be read much more accurately on the colorimeter of the type they were using; (3) the blue color does not obscure hemolysis, which is a constant source of trouble in this type of analysis. Gibson and Evans (1937), using a spectrophotometer, concur in the use of Evans blue.

As a result of a study of the blood volume of 49 male and 41 female human subjects, Rowntree and Brown (1929) found a low variability when the individuals were grouped according to underweight, standard weight, and overweight. Their results agree with the findings of Haldane and Smith (1899) and Keith et al (1915) in that as the individual approaches obesity the percentages that plasma volume and blood volume are of body weight decreases .

Rowntree and Brown also found a decrease in blood volume as a result of short periods of induced heat. However, Overman and Feldman (1947) found higher values for the blood volume in monkeys in summer

than in winter. The differences in the results of the two studies could well be the acclimitization of the animals in the latter study.

The relationship between age and blood volume in babies up to one year of age has been studied by Lucas and Dearing (1921). They made blood volume determinations regularly on infants from three hours to one year of age. They found the volume to vary widely during the first 15 days, after which the range lessens but the plasma volume increases throughout the entire first year. Bakwin and Rivkin (1924), Marriot (1920), and Darrow, Soule, and Buckman (1928), studying the same problem confirm this observation. Infants seem to have blood volumes too large for their body weight. The change at two weeks is evidently related to some change in erythrocytes, in which the plasma volume increases in relation to red cell volume.

The blood volume of rabbits, goats, dogs, and horses is reported by Courtice (1943). His results show a high correlation between body weight and blood volume for the different species. His values for blood and plasma volumes for dogs agree closely with the results of earlier studies except that his values for a group of greyhounds show a much larger per unit volume than other animals. This, he explains, is due to the more heavy musculature of the body in relation to total body weight.

With the recent advances in the use of radioactive isotopes, their use in the field of clinical investigations has been studied. Gibson, Peacock, Seligman, and Sack (1946), comparing the radioactive iron and the dye methods for the determination of blood volume, found the former gave consistently lower results. Meneely, Wells, and Hahn (1947) found considerable individual variations in blood volumes when using the

radioactive iron method and concluded that much additional work was necessary on the technique before much faith can be put in the results secured.

The comparison of blood volumes obtained by use of radioactive phosphorous and by the use of T-1824 (Evan's Blue) dye is reported by Mayerson et al (1948). They found only slight variation between individuals of the same weight as well as between radioactive and dye methods of blood volume determinations. Their use of a procedure first outlined by Hahn and Hevesy (1940) and simplified by Nieset et al (1948) increases greatly the possibilities of this technique for future usage.

At the present time the dye method of determining blood volume seems to be the best for routine clinical investigations. Many observations of great value were made by the use of the dye method of Keith in its original form. However, these subsequent studies revealed that it was by no means fool-proof. Efforts to erradicate sources of error and inaccuracies led to many modifications. Many of these modifications made the dye method a rather elaborate and time-consuming procedure, requiring highly specialized and expensive equipment. Gibson (1936) described a modification which simplifies the procedure considerably, and Gibson and Evans (1937) discussed its theoretical basis. They also gave the results of volume determinations on several human subjects, indicating only slight variations between individuals. They employed a spectrophotometer for dilution determinations and extrapolated the results of samplings subsequent to dye injection to zero time. By this procedure they obtain a value for the blood volume at zero time or at the time of the injection of the dye.

Gregersen (1944) further simplified the Keith technique by making one sampling of the blood exactly 10 minutes after the injection of the dye into the blood stream. He also injected a standardized amount of dye from an ampule. In this way, calculation of the plasma volume was eliminated and was determined by reference to a table of densities predetermined from various dilutions of the dye in plasma.

Nitshe and Cohen (1947) also used only a 10-minute period for mixing before sampling the dyed blood. However, they did not use ampules as Gregersen did; instead, they calculated a value called K. This value was a constant for a particular spectrophotometer and for a particular stock solution of dye. It was calculated in order that samples of plasma containing dye might be compared spectrophotometrically with undyed samples.

This is by no means a review of every piece of work on blood volume in the literature. Many of the studies have dealt with blood volume determinations under pathological conditions and are not relevant to the present study. Only those works which present techniques or modifications of present techniques have been reviewed.

The results of the study by Gibson and Evans (1937a) using their method on humans gave bases for the following conclusions: "(1) the total blood volume of normal males is greater than that of females, the difference being due to the greater red cell volume of the males; (2) blood volume declines with increasing age after middle age; (3) the blood volume per unit of weight is high in muscular and thin individuals and low in obese persons".

From this study as well as others reviewed, values of 70 to 100 cc. of blood and 40 to 60 cc. of plasma per kilogram of body weight are representative for normal human subjects.

There has been some work done on laboratory animals such as dogs, goats, monkeys, and horses. The values for the blood volume of these animals fall in the same ranges as those for man. The smaller laboratory animals, such as rats and mice, have been the subjects of a very limited amount of research on blood volume. This is no doubt due to their small size. Lippman (1947) summarizes the results of several studies of blood volume determinations on rats and the values fall in the same range for these small animals as those given above.

There are very few reports in the literature of studies on the blood volume of fowls. Why this should be is hard to deduce, except that the use of the fowl as a laboratory animal in the testing of pathological conditions affecting humans is necessarily limited.

Welcker, in the course of his studies, 1856-1893, on the blood volume of a great number of species of animals studied some of the fowl species, including chickens. He reported in 1903 that the value for blood weight as a percent of body weight in the *Gallus Bankiva Domesticus* was 3.68. This is the result of a study of only one individual. His values of blood volume of geese are 5.71 and 6.75 percent of the body weight. He studied the blood volumes of several of the wild birds also, reporting values varying from 3.43 percent to 9.35 percent for small and large birds, respectively. He found values of 11.3, 13.7 and 20.8 percent for the blood volume in relation to body weight for chicks on the day of hatching. These high values for very young chicks are in close agreement with the findings of Lucas and Dearing (1921), who also found relatively high values for human infants.

Weighing the blood lost by 131 chickens, Zaitschek (1908) found that the chickens lost 3.8 percent of the body weight when the jugular vein was severed. The close agreement of this value and that of Welcker leads to the conclusion that Welcker, in his study, weighed only blood lost. Zaitschek found small variations in blood loss between the individuals used in his study. The average blood loss was 41.6 grams per individual, with a standard deviation of only 7.94 grams.

Magnan (1912) studied the blood volume of several species of wild fowl and, using the original Welcker technique, determined the weight of the blood lost when the carotid artery was cut. To this he added the weight of the blood obtained by flushing the circulatory system with a 0.7 percent saline solution. He obtained values for the weight of the total volume of blood ranging from 39 to 86 grams per kilogram of body weight. The larger values were not for heavier birds but rather for birds whose natural habitat was near the sea and which were also good divers.

PROCEDURE

The blood volume was determined for a group of New Hampshire chickens ranging in age from six weeks to adult stock. The young chickens were all battery raised. The hens were maintained in batteries and were all in laying condition. The adult male birds were from the laying pens on the University poultry farm.

All blood volume determinations were made by the dye method, using T-1824 (Evans Blue) dye. The procedure, which is described in detail here, was that of Nitshe and Cohen, loc cit. The constant or K for the solution and spectrophotometer was determined as follows:

1. A stock solution was prepared by dissolving 800 mg. of Evan's Blue dye in 200 ml. of 0.9 percent saline solution. This stock solution was also used for subsequent blood volume determinations. The saline solution was used to prevent hemolysis.
2. One ml. of the dye solution was delivered into a 50 ml. volumetric flask and made up to volume with distilled water.
3. One-half ml. of the dye solution was delivered into a 50 ml. volumetric flask and made up to volume with distilled water.
4. From each of these flasks 0.6 ml. was delivered into separate absorption cells and 5.4 ml. of plasma from the species of animal being tested was added. This gave concentrations of 0.008 and 0.004 mg. dye per ml. solution respectively.
5. The optical densities of the dye-containing samples were determined with a spectrophotometer a wave length of 610 millimicrons.

The control or undyed sample was made up of 0.6 ml. of 0.9 percent saline and 5.4 ml. of the same plasma.

6. The formula, $K = \frac{D}{C}$, where D is density and C the concentration, was used in the calculation of the K value.

For the actual determination of the blood volume, a sample of blood was withdrawn from a cubital (brachial) vein. This constituted the undyed sample to be used as zero optical density or standard. Approximately 0.3 mg. of dye per kilogram of body weight was then injected into the brachial vein, using a very carefully calibrated syringe. This was allowed to circulate in the blood stream until sufficient time had elapsed for complete dispersal throughout the circulatory system of the body. This time has been determined to be 10 minutes for humans. Since the pulse rate is higher and the body much smaller in chickens than in humans and since the disappearance rate of the dye from the blood stream is so slow, the 10-minute period was thought to be a sufficient time and was used throughout this study. As soon as this mixing time had elapsed, a sample of blood was withdrawn from the opposite cubital (brachial) vein for the blood volume determination.

Most of the methods of blood volume determinations require the withdrawal of five to ten ml. of blood for the hematocrit and plasma volume determinations. Because of the small size of the chicken and because of the necessity of having at least seven ml. of plasma in the absorption cell, it was decided to modify the technique slightly. This modification consisted of the withdrawal of only three ml. of blood. One ml. was used in the hematocrit and two ml. was diluted with eight ml. of 0.9 percent saline. The undyed sample was processed in the same

manner in order that both samples would have the same optical density except for the amount of dye contained in the dyed sample.

Using this sample of dyed blood, the percentage of red and white blood cells were determined. This was accomplished by using Wintrobe hematocrit tubes. Both the hematocrit and the dilute sample of dyed blood were centrifuged for 30 minutes at approximately 3000 rpm. The percentages of red and white cells were added together and recorded as corpuscular volume.

Nitshe and Cohen stated that the light absorption by the dyed as compared with the undyed sample was determined by use of the spectrophotometer with a 620 millimicron filter. The spectrophotometer available for use in this study had only a 610 millimicron filter available. However, examination of the light absorption curve for Evan's Blue as presented by Gibson and Evans (1937) showed the effect of this difference of 10 millimicrons in wavelength to be less than 0.01 in optical density.

After both dyed and undyed samples have been centrifuged and the optical density determined, the plasma volume (PV) can be determined by using the following formula: $\frac{MK}{D} = PV$, in which M = No. of Mg. of dye injected, K = Constant previously described, and D = Density reading of the spectrophotometer.

This PV will be approximately 4 times too large because of the dilution with water. The exact dilution (Plasma Volume Correction Factor, PVCF) can be determined by the formula:

$$PVCF = \frac{2 - (\%CV \times 2)}{8 + (2 - (\%CV \times 2))}, \text{ in which } \%CV = \% \text{ of corpuscular volume as determined from the hematocrit.}$$

The value thus obtained is multiplied by the PV of the previous formula in order to determine the true plasma volume (TPV).

$$TPV = PV \times PVCF$$

The total blood volume is calculated from the formula:

$$TBV = \frac{TPV}{P} \times 100$$

P - corrected volume of plasma per 100 ml.

calculated as follows:

$$P = 100 - \left\{ \left(\frac{\text{Volume of cells}}{\text{Volume of plasma and cells}} \times 100 \right) \right\}$$

Thus the total blood volume is reported in ml. per animal, in which form it may be related to body weight, surface area, or any other measure desired.

To test the validity of this technique in vitro, samples were prepared by filling beakers with varying amounts of blood and the volume determined in accordance with the above outlined procedure. Eight-tenths milliliter of the stock solution of Evan's Blue was added to each beaker and mixed thoroughly. The samples were withdrawn with the same syringe which was used for injections. The values reported below are not only results of duplicate samplings from the same in vitro sample but also from different samples containing the same number of ml. of blood. Some of the values were also determined at the conclusion of the study in order to determine whether the dye had changed in concentration during the period of study. Table 1 shows the results of these trials.

Table 1. Blood volume determinations by the dye method on measured samples of blood

No. of samples	Actual volume cc	Average calculated volume cc
7	50	49.1 \pm 2.7
4	60	59.4 \pm 3.6
3	70	70.4 \pm 1.2
6	100	99.5 \pm 1.7

It will be noted from this table that the blood volume as determined by the spectrophotometer was very close to the measured blood volume. There is some variation present in these readings, a part of which may be due to the quantity of blood clinging to the side of the graduate when pouring into the beaker. The coefficient of correlation between measured and calculated blood volume was $r = 0.990$. This high correlation seemed ample justification for the technique as described.

RESULTS AND DISCUSSION

The results of this study are presented in two parts, first, the determination of the blood volume of New Hampshire chickens, and second, the results of the application of these blood-volume data to the measuring of the completeness of bleeding of chickens killed by different methods.

Part I. Blood Volume of New Hampshire Chickens

Before proceeding with the determination of blood volume of a large number of chickens, it was decided to test by in vivo methods the accuracy of the method. This was accomplished by determining the blood volume twice on the same chicken. It was felt that, if the values thus obtained were approximately the same, the procedure would be valid for further use. Twenty birds were used (8 males and 12 females) varying in weight from 900 to 3600 grams. It was fortunate that these weights were selected, as later work showed this to be the weight range in which individual variation is greatest. The blood volume was determined on these birds twice, with six or seven days elapsing between determinations. This lapse of time was allowed in order that all of the dye would be lost from the blood stream, although Gibson and Evans (1937) showed that repeated as well as continuous determinations can be made using the dye method. Also it was desired that the chickens would completely recover from the withdrawal of blood in the previous determination.

The results of these two determinations show a coefficient of correlation of $r = 0.93$, indicating a close agreement between the two

values obtained. There was, as was expected, some variation between the two values. Approximately one-half of the birds showed a decrease in blood volume and the rest of them showed a slight increase in blood volume at the time of the second determination.

Females - Total blood volume -- The blood volume of 113 normal New Hampshire females was determined. Inspection of a scatter diagram of blood volume plotted against body weight indicated that a straight line could not be used to express the data correctly. This was evident since a straight line showed that a chicken weighing approximately 200 grams contained no blood whatsoever. Also, the values for the blood volume of adult hens obtained by interpolation from the straight line were much larger than the values obtained by actual blood volume determination on hens of the same weight. One of the motives for fitting curves to non-linear data is the elimination of the inaccuracies which non-linearity of regression may introduce. Because these inaccuracies were introduced by attempting to fit a straight line to the data, it was decided to use a curvilinear regression and to calculate a curve which would express the data accurately. It was found, by calculation and plotting the values, that a second degree parabola type of curve best fitted the scatter diagram of the data at hand. The equation for this type of curve is $Y = a + bX + cX^2$ (Snedecor 1946).

The regression curve showing the relation between the values for the blood volume and body weight of the females is shown in Figure 1. The values for the constants used in calculating the points on this line are; $a = -6.2$; $b = 0.11973$; $c = -0.00002$. The standard error for the estimate of Y is 14.3 cc., the limits of which are indicated on Figure 1 by the broken lines.

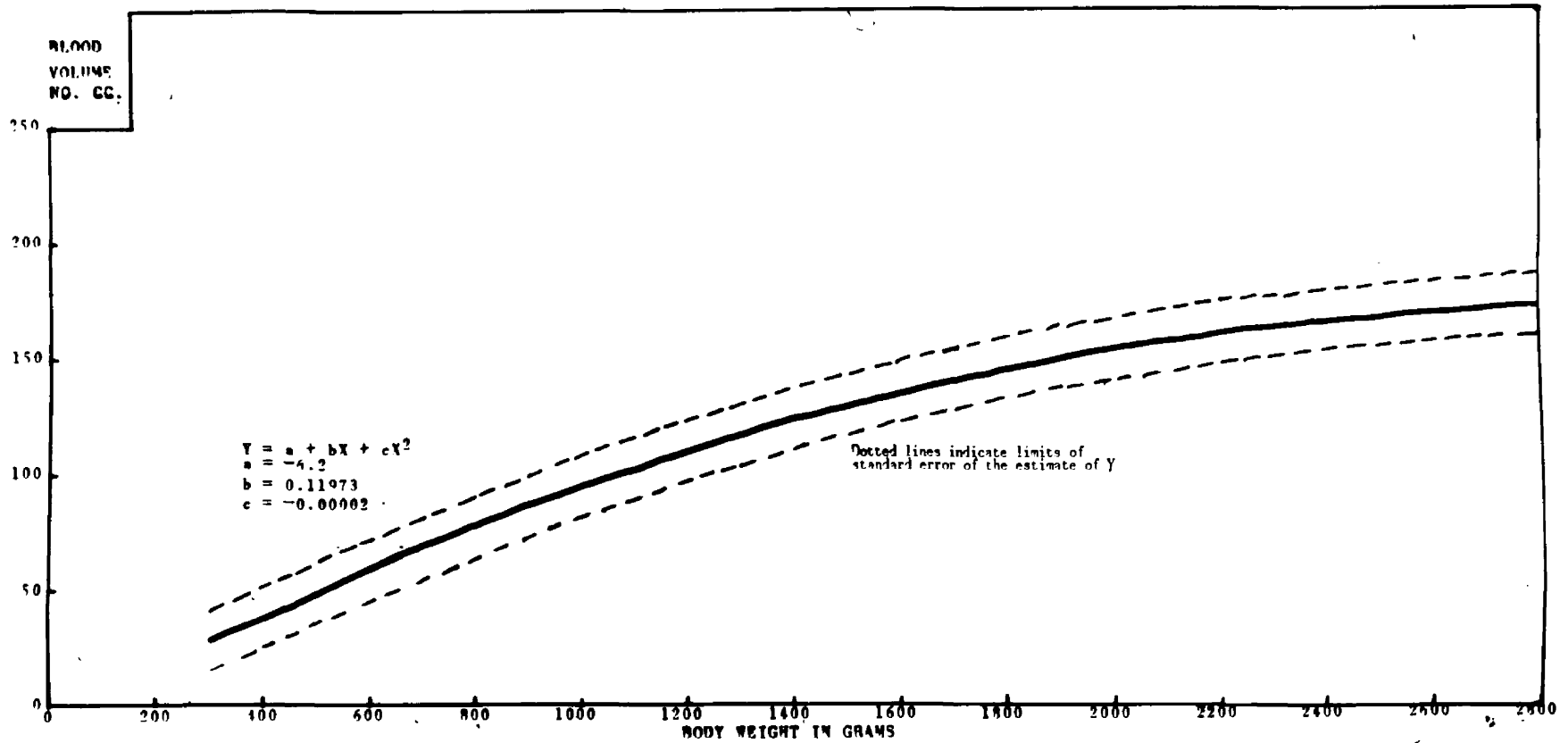


Figure 1. The relationship between total blood volume and body weight of normal New Hampshire females

The blood volume for females weighing approximately 400 grams was found to be 44 cc. or 10.6 percent of the body weight. The volume increases in almost a straight line relationship up to a weight of 1200 grams, when the blood volume is approximately 108 cc. Above this weight the blood volume begins to level off and additional increments of weight are accompanied by relatively smaller increments of blood.

It was observed that within this range, 400 to 1200 grams, individual variation was less than in birds of the heavier weights. Variations in blood volume at weights above 1200 grams are undoubtedly due to variations in the deposition of fat in the carcass of the bird. Up to 1200 grams most of the weight gains of the bird are due to general body tissues which are rather highly vascularized. At weights above 1200 grams a higher percentage of the weight increases is due to the deposition of fat in the muscles as well as in the viscera. Fatty tissue is not as highly vascularized as is muscle tissue. Thus weight changes due to increases of fat would not be accompanied by as much increase in blood volume as when weight increases are due to general body growth.

Individual chickens vary in the deposition of fat and so when this deposition begins, individual variation in blood volume would increase. Also because some chickens grow to a heavier weight than others before beginning to deposit fat individual variation would be greater.

The curve, as calculated from the values of blood volume in females, indicates that at a weight only slightly above the weights encountered in this study the blood volume would actually decrease. This is undoubtedly not the case. What is probably true is that above the weights used in this study, increases in weight are accompanied by

only slight increases in blood volume. Thus the curve above this point is very flat and because of the increases in blood volume at the lighter weights, the formula for the curve causes it to descend at weights above 2990 grams. If hens above this weight are used, the entire formula must be recalculated and the curve redrawn. Although this curve seems to hold for the birds studied, it is not known at this time whether it will hold true for breeds which mature at different weights from New Hampshires.

Males - Total blood volume -- The blood volume of 110 New Hampshire males was determined, and inspection of a scatter diagram of the blood volume plotted against body weight led to the conclusion that the relationship between the two values was also curvilinear. Thus the same equation was used for calculating the regression line for total blood volume in males as was used in females. Figure 2 shows the regression line calculated by this formula and illustrates the relationship between blood volume and body weight of the males. The values for the constants are; $a = 1.98$; $b = 0.10138$; $c = -0.0000025$. The standard error for the estimate of Y is 23.48, the limits of which are shown by the broken lines in Figure 2.

The males used for these blood volume determinations ranged in weight from 300 to 4000 grams and the respective blood volumes ranged from 48 cc to 365 cc. The blood constitutes 11.2 percent of the body weight in the smaller males and 9.2 percent at the heavier ones.

Although the relationship between blood volume and body weight is curvilinear, the curve in Figure 2 appears to be nearly straight. There is almost as much increase in blood volume per weight increase at the lighter weights. This differs considerably from the females, which

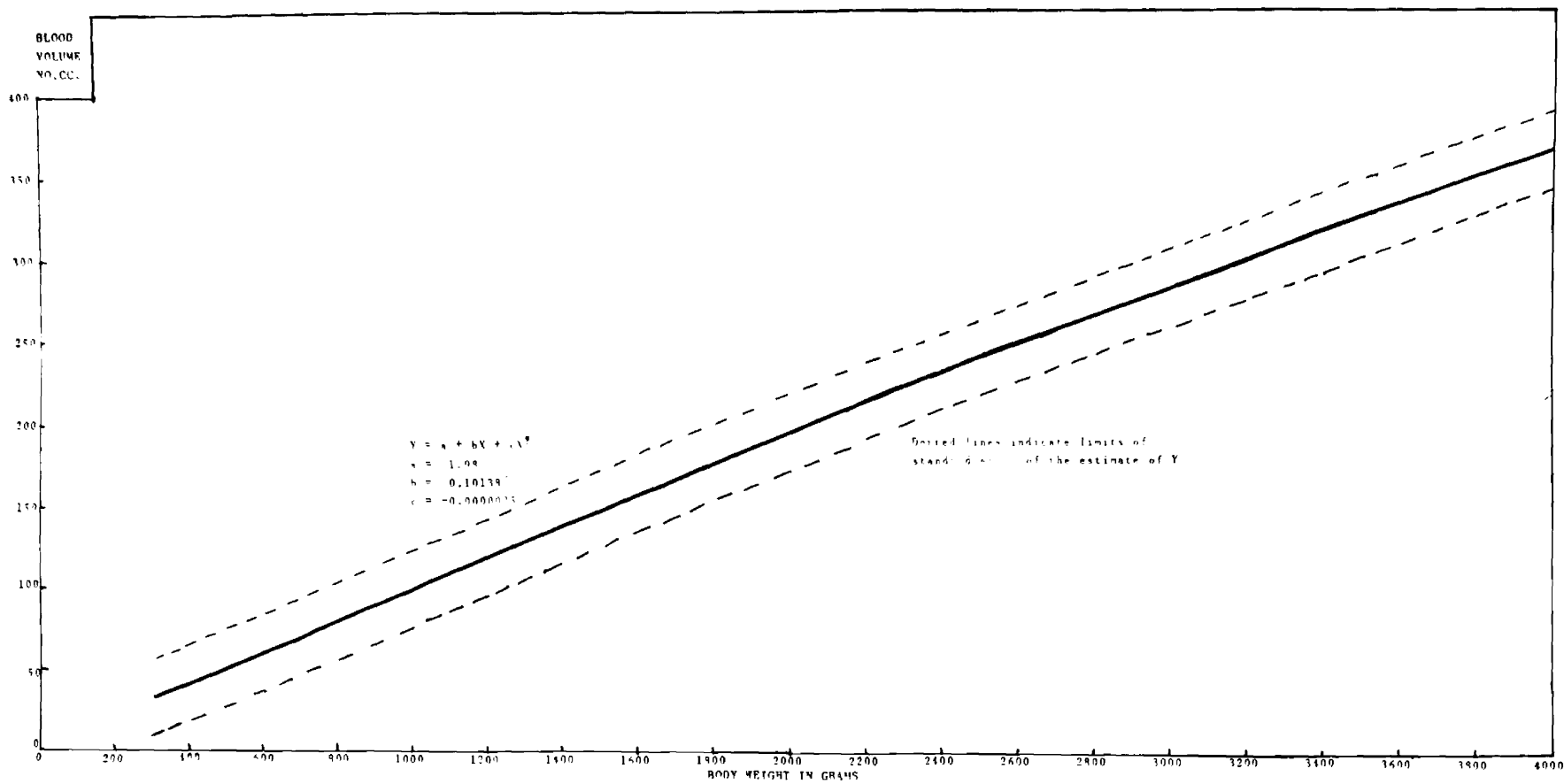


Figure 2. The relationship between total blood volume and body weight of normal New Hampshire males

showed a leveling of the curve above 2000 grams. Throughout the life of males, weight increases consist of increases in general body tissue and not fat, thus explaining this sex difference. The males do not deposit nearly as much fat as the females in either tissue or viscera and the blood maintains more nearly the same percentage of the body weight throughout their lives.

At weights below sexual maturity, the blood volume for males and females is very close to the same value. Inspection of the scatter diagrams showed that individual variation in both sexes was also low at these lighter weights. These individual variations increase at heavier weights reaching a greater magnitude for males than for females. This is evident from the values for the standard error for the estimate of Y, which is 23.48 for males and 13.4 for females. From these figures it would appear that there is a much larger variation in males than in females. However, when the larger volume of blood in the males is considered, the coefficient of variability is approximately equal between the two sexes (which is 41.6 for males and 33.8 for females.)

From the preceding discussion it is evident that there is a sexual dimorphism in the blood volume of normal New Hampshire chickens. This difference is not evident in young chickens below 800 grams but becomes evident above this weight. The blood volume in males increases more rapidly and over a much longer period of time than it does in females.

Females - Corpuscular volume -- As shown in Figure 3, the red and white blood cells comprise slightly less than 30 percent of the total blood of the females. This percentage remains almost constant throughout the weight range studied. Thus it would appear that neither weight nor age has much effect on the corpuscular composition of the blood of females. The females studied varied not only in weight but also in age from young chickens to adult hens. This observation is in accord with that of Juhn and Domm (1930), who found very little change in erythrocyte count in Brown Leghorn females from hatching time to 490 days of age. It would follow from this that any increase in blood volume observed in females is an increase in plasma volume with a corresponding increase in corpuscular volume. Also in the heavier weights studied, although the blood comprised a smaller percent of the body weight, the corpuscular volume of the blood remains the same.

Males - Corpuscular volume -- The relationship between corpuscular volume, as a percentage of total blood volume, and body weight in males is shown in Figure 3. The scatter diagram is included with the freehand curve drawn from it to give an indication of the individual variation found. It will be seen from the curve that the percentage of red and white cells remains fairly constant at about 30 percent until a weight of 1800 grams has been attained. Between this weight and 2800 grams the percentage increases rapidly to about 45, after which the curve again levels off and rises only slightly throughout the rest of the weight range studied. It would appear from this that a part of the increase in blood volume of males over females is due to this increase in corpuscular volume.

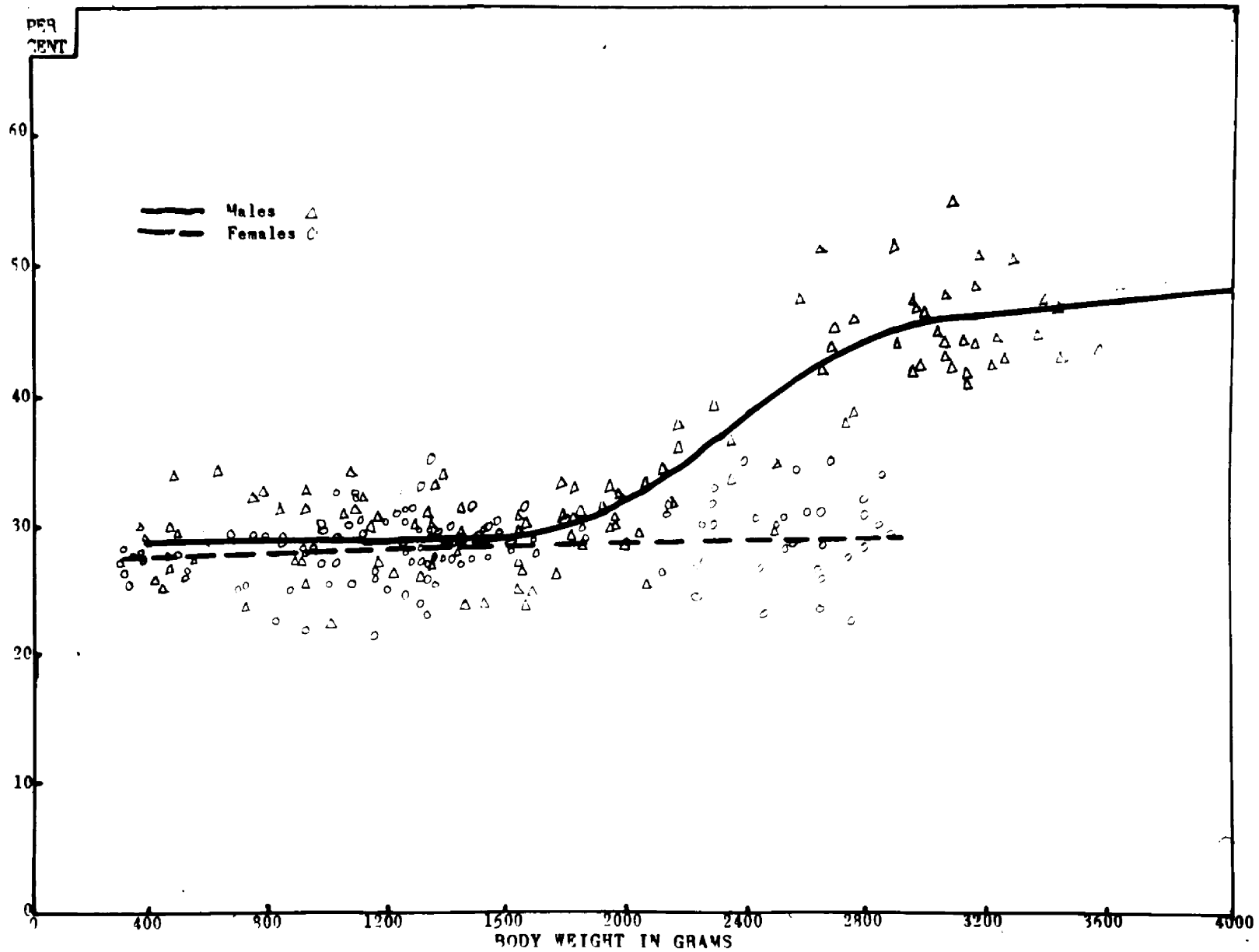


Figure 3. The relationship between corpuscular volume as a percentage of total blood volume and body weight of normal male and female New Hampshire chickens

The change in corpuscular volume which occurs at about 1800 grams is probably influenced as much by age as by weight. However, the exact age of the males in this study was not known and the effect of age must be deduced from body weight. The results of the study by Juhn and Domm previously referred to showed that the erythrocyte count in Brown Leghorn males increased considerably at the time of sexual maturity and was maintained at this higher level throughout the period covered by their observations.

The sex differences in corpuscular volume as disclosed by this study are rather interesting. At the lighter weights the percentage of red and white blood cells present in the blood is almost the same in males and females. Below 1600 grams the blood of the males contains about two percent more red and white blood cells than that of the females. Above this weight the difference begins to be very noticeable. The percentage for males increases rapidly to one and one-half times its former value and for females remains almost constant throughout the rest of the weight range. Additional study is needed on this point to determine whether this change in males is influenced most by sexual maturity, aging, or body weight.

Blood volumes of treated chickens -- There were available at the time of this study a number of chickens undergoing a test on a diet containing thiouracil and thyroprotein. This treatment is being tested by this laboratory as a means of increasing fat deposition. So far as is known no studies have been made on the effect of these chemicals on blood volume. Since the birds were available, it was decided to determine the blood volume of a representative sample of these birds to determine what effect the drugs might have on blood volume.

The blood volume of the treated chickens was determined by the procedure previously outlined. The blood volume of untreated chickens of the same weight was determined by interpolation from the curves in Figures 1 and 2. The chickens were separated according to sex and interpolation made from the proper curve. By using this method it was found that the average blood volume of the treated females was 17.6 cc. or 14 percent below the normal value and of the treated males 31.4 cc. or 18 percent below the values for normal birds of comparable weights. Although it is realized that this does not take into account the error due to individual variations, still this is a considerable relative decrease in blood volume in relation to body weight. These results would indicate that the treatment causes a greater proportion of the body to be fatty tissue, which is not high in blood capacity. This is in accordance with the general observations on the effect of feeding thiouracil and thyroprotein.

Part II. Studies of Completeness of Bleeding

Since this study established a measure of the amount of blood in the body of normal chickens, it was decided to test different methods of killing chickens for their effect on the completeness of bleeding. The completeness of bleeding can be determined by comparing the actual loss of blood from an individual of a given sex and weight with the volume of blood present in the same bird. This latter figure can be obtained by interpolation from the curves presented in this study.

The simplest way to determine the amount of blood lost is to weigh the chicken before bleeding and again after all bleeding has stopped. The difference between the two weights is the weight of blood lost. To determine whether this method gives an accurate measure of

blood loss, a trial was made in which the loss of blood was determined by catching and weighing the blood. This blood loss was compared with the amount of loss as determined by the change in body weight. The two groups of chickens weighed approximately the same and were killed by similar methods. The average blood loss by the group for which the blood was weighed was 46.8 ± 8.0 percent of the total volume of blood in the body. For the group in which the blood loss was determined by change in body weight the blood loss was 45.3 ± 6.3 percent of the total blood volume in the body. These two figures indicate that the latter method is satisfactory in determining the amount of blood lost.

Three groups of 60 chickens each were killed by three different methods; (1) by severing the juglar vein by a cut through the skin of the throat similar to 'Kosher' killing, although no attempt was made to be sure that the trachea or esophagus was severed; (2) by severing the juglar vein in the conventional manner of inserting the knife into the mouth and also debraining the bird with the knife; (3) by decapitation.

All birds were weighed before killing and again after bleeding had completely stopped. The difference between the two weights was recorded as blood loss. Since the birds had been starved for 12 hours previous to slaughter, little trouble was experienced from loss of weight by fecal material being excreted or by regurgitation during the dying struggles. However, careful watch was kept of the birds and if any such loss occurred the bird was discarded.

The results of the trial comparing the blood loss by the different types of killing are presented in Table 2. These results show that chickens lose between 35 and 50 percent of the blood in the body during

Table 2. The cc. of blood lost per 100 cc. of total blood volume for chickens killed by different methods

Group		Methods of Killing		
		Kosher	Stuck	Beheaded
Males	Treated	46.8 ± 7.8	45.6 ± 3.0	41.6 ± 5.0
	Controls	43.1 ± 5.1	38.7 ± 4.2	35.7 ± 2.4
Females	Treated	48.3 ± 5.4	45.4 ± 5.9	38.9 ± 6.1
	Controls	43.3 ± 6.1	43.6 ± 4.8	40.4 ± 4.6
Males	Treated and Controls	44.8 ± 6.8	41.9 ± 5.1	38.5 ± 5.1
Females	Treated and Controls	45.7 ± 6.4	44.5 ± 5.5	39.5 ± 5.9
Both	Treated	47.6 ± 6.5	45.5 ± 4.7	40.1 ± 5.9
Both	Controls	43.2 ± 6.1	41.1 ± 4.8	37.9 ± 4.6
Average		45.3 ± 6.3	43.2 ± 5.8	39.0 ± 5.6

the bleeding period. There is considerable variation among individuals regardless of the method of killing employed. In all cases the highest percentage of blood was lost by those chickens which were 'Kosher' killed. Killing by beheading seems to be the poorest method of killing, since the loss of blood is relatively the least. The loss of blood by chickens which were killed by the conventional method of sticking and debraining agrees closely with that of the 'Kosher' killed birds.

The difference between the blood loss of the 'Kosher' method of killing and the stuck and debrained method of killing is not statistically significant. However, the differences between the blood loss by either of these methods and the beheaded birds are highly significant statistically at the 1 percent level. Females tend to lose a higher percentage of their blood than males, although the difference is not statistically significant. This higher percentage in the females is probably due to the smaller volume of blood contained by them. This leads to the conclusion that death results as a loss of a percentage of the blood in the body rather than a loss of blood as a percentage of body weight.

Throughout this test the thiouracil treated birds lost a slightly higher percentage of blood than the controls. This difference was not great enough to be statistically significant. Since all of the blood volumes for the treated groups were corrected in accordance with the result of the preceding section, this increased loss is no doubt due to the lesser volume of blood in the body of these birds.

As was stated earlier, there was some individual variation in blood loss by all of the methods of killing. The 'Kosher' killed birds exhibited greater variability in blood loss than either of the

other two methods. This is probably because of the greater variation possible in making the cut by this method. Although it is possible to obtain more complete bleeding by this method of killing, it is also possible that a less complete cut may be made and the individual variation greater.

Very little sex difference exists between the standard deviations for blood loss.

SUMMARY

A procedure is given for determining the blood volume of chickens. This is a modification of the technique of Gibson and Evans (1937) and Nitshe and Cohen (1947). These earlier techniques were modified so that only three cc. of blood rather than five or ten cc. needed to be withdrawn from the bird. One cc. of this terminal blood was used for the hematocrit determination and the remaining two cc. were diluted with eight cc. of 0.9 percent saline solution. In this way the macro absorption cells may be used in the spectrophotometer to determine the optical density of the solution. From this optical density, the dilution of the dye in the blood stream can be determined and by use of the formulae presented, the plasma volume and total blood volume in the body can be calculated.

Data are presented in support of this technique in which the volume of blood of in vitro samples agrees closely with the volume as measured. Also presented are the results of repeated determinations on the same chicken, which show close agreement between two determinations.

Regression curves are given for the values of blood volume as determined for 113 New Hampshire females and for 110 males of the same breed. These curves indicate a curvilinear relationship between blood volume and body weight. Comparison of the curves for the two sexes shows that a sexual dimorphism exists in blood volume. The difference becomes apparent at weights above 800 grams and continues throughout adult life.

The blood volume of females increases during growth at weights below 1800 grams, after which the curve for blood volume against body

weight levels off to an almost flat line. In males the blood volume continues to increase in an almost straight line relationship up to weights of approximately nine pounds. The increase in total blood volume in females appears to be an increase in both plasma and corpuscular volume. In males, corpuscular volume increases rapidly at weights above the age of sexual maturity, reaching a value one and one-half times larger than in young chickens.

The changes in blood volume as a result of feeding thiouracil indicate that increases in body weight are due to the deposition of a tissue low in blood content, undoubtedly fat.

Data are presented on the loss of blood as cc. of loss per 100 cc of blood volume by three different methods of killing on 170 birds. These data show that birds which are 'Kosher' killed and birds which are stuck and debrained lose approximately the same amount of the blood in the body. Both of these methods effect significantly more complete bleeding than does decapitation. Males and females lose approximately the same percentage of the blood in the body, which leads to the conclusion that blood loss is dependent on the amount of blood in the body rather than on the body weight of the bird.

CONCLUSIONS

1. Blood volume can be accurately determined by using a relatively simple adaptation of the dye technique.
2. The blood volume of young chicks of either sex is practically the same, comprising approximately 10 percent of the body weight.
3. After sexual maturity there is a leveling off of the curve representing blood volume of females plotted against body weight while the curve for males continues in almost a straight line positive relationship throughout the weights encountered in this study.
4. The volume of red and white blood cells as a percentage of total blood volume remains almost constant in females throughout the weight range of this study. In males, this percentage increases rapidly at weights above sexual maturity age, attaining a value of one and one-half times greater than the value for young chickens.
5. Weight increases during thiouracil treatment are due to tissue low in blood content.
6. Chickens lose between 35 and 50 percent of the blood in the body when bled in the killing operation.
7. 'Kosher' killed birds and birds which are stuck and debrained lose significantly larger percentages of the blood in the body than do birds which have been decapitated.
8. Males and females lose approximately the same percentage of the blood in the body when killed by any of the three methods tested.
9. Death results from blood loss as a percentage of the blood in the body, not as a percentage of body weight.

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