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A Determination of the Blood and Plasma Volume of Dairy Cattle

A Study of Blood and Plasma Volume During
Growth, Pregnancy, and Lactation

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PREFACE

The great differences in the productive ability of dairy cattle which to all obvious external appearances such as size and conformation might be expected to yield comparable quantities of milk and fat, is one of the most perplexing observations of dairy cattle breeders. While many attempts have been made to find external indications of the productive ability of dairy cattle, experience has shown that the Babcock fat test and the milk scale are the most reliable tools by which productive ability may be gauged.

Within recent years a renewed effort has been made to find the physical basis responsible for the differences in the producing ability of dairy cows. In some of these studies anatomical measurements of the external form have been compared with the size and conformation of the internal organs in an effort to determine some external indication of production. Such studies are based upon the supposition that there is a relation between the anatomy of the dairy cow and the size and secretory activity of the udder. By this method the sacrifice of the animal becomes necessary.

In the study of the problem at this station, the physiological processes involved in the hormonal stimulation of the growth of the udder and the secretion of milk are being given major consideration although the anatomy is not neglected.

In connection with the secretion of milk, the volume of circulating blood is of considerable interest. It is believed that studies of the volume and composition of the blood in living animals during growth, pregnancy and lactation will give an insight into one phase of the physiological differences of dairy cattle which will help to explain the variation in productive ability.

As a preliminary to such a study, a method has been adapted by which the blood volume of living cattle can be determined with a reasonable degree of accuracy.

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ABSTRACT

The dye injection method for the determination of the blood and plasma volume in the living animal has been adapted to dairy cattle. In 119 determinations by this method the bovine was found to have a blood mass of 6.76 per cent of the body weight. Young and growing dairy cattle were found to have 6.25 per cent of blood by weight. Lactating cows were found to have a blood mass of approximately 8.11 per cent of body weight, while non-lactating cows were found to have 6.38 per cent of blood by weight. The plasma was found to make up about 60 per cent of the blood volume and showed very little variation.

In comparison to the "drain out" method of determining blood volume on cows of similar condition and weight, it was observed that the dye injection method gave values approximately 40 to 47 per cent higher.

A Determination of the Blood and Plasma Volume of Dairy Cattle

A Study of Blood and Plasma Volume During Growth, Pregnancy, and Lactation

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Studies dealing with the physiology of milk secretion, and the phenomena related to this characteristic process in the mammal, invariably focus attention on the function of the blood. In this connection the circulating volume of blood and plasma is of considerable interest. One of the major functions of the blood is to transport nutritive materials so necessary for all physiological activity. It constitutes the internal medium, according to Claude Bernard's expression, in which the cells of the body live and with which they react.

From a physiologic consideration of milk secretion, one is impressed with the activity which is demonstrated by the mammary gland in its ability to draw freely upon the constituents of the blood stream. The mammary gland, in its synthetic ability, possesses the remarkable power of converting these nutrients into milk. Changes in the composition of blood which has passed through the mammary gland system, indicating a removal of the precursors of milk from the blood stream, have been summarized by Meigs, Blatherwick and Cary (1919). This information concerns only the concentration of the blood constituents. It does not give total values for the blood constituents (amino acids, glucose, lecithin, water and ash) which are the potential precursors of milk.

The volume as well as the concentration of nutrients of the blood stream must play an active part in the interpretation of the physiological activity of the mammary gland. In addition, the true significance of changes in the circulating tissue, the blood, can be understood only when the actual blood volume is also considered.

The study of blood volume and blood composition in the living animal gives an insight into some of the processes taking place under various physiological conditions. In the dairy cow we are particularly interested in these phenomena associated with growth, pregnancy and lactation. Observations covering these states, when conducted on live animals, under definite physiological conditions, will do much to estab-

*The data presented in this paper formed part of a thesis presented by H. A. Herman in partial fulfillment of the requirements for the Degree of Master of Arts in the Graduate School of the University of Missouri, 1931.

lish the limits of the normal and also the influence of various physiologic processes.

In the realm of milk secretion many researches have been conducted and many more are in progress at the present time, which attempt to explain the physical basis responsible for the difference in the producing ability of dairy cows. In many instances, anatomical measurements are looked to as a means of throwing light on this subject. While of interest in studies of growth, change of form, and in the correlation of body parts, these routine anatomical measurements on both living and dead animals tell little or nothing of the physiological differences in the producing animal, which are responsible for the difference in productive ability. Studies concerned with the factors responsible for the growth, development, and the initiation of milk secretion in the mammary gland are bringing forth new evidence on the physiology of milk secretion.

The functioning of the mammary gland in its designed purpose, however, is a problem strictly physiological. Does a high producing cow have a richer supply of nutrient available to the udder? Is this supply, if present, due to an increased plasma and blood volume, or is it due to an increased concentration of nutrients available to the mammary gland from the blood stream? Is there increased secretory activity of the endocrine glands?

The observations to date indicate the blood to be fairly constant in its composition, and varying only in case of distinct physiological disturbances or pathological conditions. Without a knowledge of blood volume, changes in composition may be more apparent than real. Is a change in concentration associated with a change in plasma or cell volume? When means of attaching values or estimating the significance of these and associated factors are known, we can better interpret the processes concerned in milk secretion.

The composition of the blood of the bovine has been studied by a great many investigators. Abderhalden (1911) gives a complete analysis of the blood, blood serum, and blood corpuscles of the common domestic animals, including the bovine. Individual constituents of the blood in all classes of dairy animals have been investigated more or less during the past decade. To our knowledge, however, no attempt has been made to determine the blood volume of living dairy cattle.

Blood volume has been a subject of investigation in other animals. In man it is sometimes considered a necessary part of the clinical procedure in diagnosing diseases of the blood. Indirect methods, though not fault free, have proved of value as a guide in the interpretation of physiological disturbances and normal functions of the blood. Since the blood volume is considered a significant factor in the physiology of milk

secretion, it seems highly desirable to obtain, as accurately as possible, this value for the bovine.

It is the purpose of this paper to present the adaptation of a method suitable for the indirect determination of blood volume in dairy cattle. Data collected by this method on growing animals, mature animals of various weights, breeds and ages, are given. Available data on lactating, dry and pregnant animals in the Missouri Experiment Station dairy herd are also included, though this study is far from complete.

REVIEW OF LITERATURE

Blood Volume Determinations

Methods of determining the total amount of circulating blood have been the subject of many controversies among investigators. That the volume of blood was a point of interest and investigation to the physiologists of the early nineteenth century is pointed out in the writings of Hayem (1822), Valentin (1838), and Plesch (1909). Many methods have been tried in an effort to measure the blood volume. The major portion of the knowledge concerning total blood volume, its variations, and its regulation has come as a result of attempts to apply various methods of measurement. A general survey of the blood volume investigations reported in the literature up to 1921 are reviewed in a very complete manner by Erlanger (1921).

This review indicates a most careful and comprehensive study, a critical analysis, and an unbiased opinion concerning all methods of determining blood volume. For a more complete discussion of the various methods, the reader is referred to the original paper. The various methods will be pointed out in this paper only as a matter of historical interest and in matters pertaining to the dye injection method which has been used in this study, for determining the blood volume of dairy cattle.

In general, methods for the determination of the total blood volume may be divided into two types, (a) *direct methods* and (b) *indirect methods*. The chief characteristic difference of these two methods is that the direct methods invariably demand the sacrifice of the animal, whereas the indirect methods seek to determine the blood volume *in vivo* and are thus of much value to the physiologist.

Direct Methods for Blood Volume Determinations

The "Drain out" Method.—It is of interest to note that practically all of the blood volume values to date for the bovine have been of the direct type. The "drain out" method is the best known example of this type of determination in the bovine.

The "drain out" method is simple. By this method the animal is weighed at slaughtering time, stunned, and bled by the ordinary "sticking" process. The blood which flows from the incision is collected and weighed. The time allowed for drainage of the blood varies considerably. Trowbridge, Moulton, and Haigh (1915), in using this method, state that collection of blood was continued until all dripping from the carcass had ceased. Others have advocated the collection of only that blood which streams out freely when the animal is "stuck" at the time of slaughter. Collection stops when the blood begins to drip. Some workers have adopted the plan of collecting all of the blood possible throughout the entire process of slaughter. (Haecker 1920).

Results obtained by the "drain out" method, even if relative in nature, are subject to considerable variation. The time of coagulation of the blood covers a fairly wide range and is often governed by the extent to which the flowing blood comes into contact with body tissues and fluids. Variations are due, as a rule, to incomplete drainage, lack of uniformity in bleeding, formation of clots throughout the body and the collection of body fluids other than blood during drainage.

In a separate section of this paper, data obtained by the "drain out" method in the slaughter of dairy animals are presented. Though not to be compared with the indirect methods of blood volume determination, data collected by this method are interesting because of their relative value. It is sufficient to say that direct blood volume data are of little value in a study of the physiology of milk secretion. In every instance the animal is sacrificed and the investigator is barred from carrying out repeated determinations on the same animal under different physiological conditions.

The "Wash Out" Method of Total Blood Volume Determination.—The "wash out" methods generally speaking are those which seek to determine the total blood volume by actually collecting all of the blood in the body by hemorrhage, washing out the circulatory system and leaching the macerated tissues. The blood volume is then estimated by determining the degree of dilution of the washings and blood through a comparison of the concentration of some one of its constituents with the concentration of that constituent in an undiluted sample of blood from the same animal. Erlanger (1921) points out the fact that this method was first used by Lehman and Ed. Weber in 1850. They collected the blood of two executed criminals and then washed out the circulation with water.

This method was modified by Welcher (1854) and is widely quoted in text books on physiology. This method was largely responsible for the establishment of the value that blood mass in man is approximately

one-thirteenth of body weight. This value has been questioned in later studies and does not agree any too closely with values by other methods. However, Welcher's method has often been a criterion for testing the value of determination by indirect methods. The plan set by Welcher was to collect all of the blood possible by hemorrhage, wash out the circulation with water, leach out the macerated tissues, and use the hemoglobin as a means of determining colorimetrically the relative concentration of the original blood and washings. In the case of small animals the body was merely ground up and the blood washed out after a sample of blood had been drawn.

Welcher's early method has been criticized because of difficulties inherent to the method, both extra- and intra-vascular clotting was responsible for a loss of hemoglobin, tissue extracts containing myohemoglobin as well as hemoglobin of the blood, and turbidity of the washings caused error and difficulty in matching of colors. The change in concentration of hemoglobin is perhaps the greatest drawback to this entire procedure.

Malassez (1874) attempted to improve Welcher's method. By his plan the vessels were washed out and the tissues leached with an "artificial serum" containing gum arabic, sodium chloride and sodium sulphate. He made the comparison as to relative amounts by counting the red blood cells. He also took precaution to prevent coagulation of the drawn blood.

Various investigators have added suggestions for the improvement of the direct method. Erlanger (1921) gives a discussion of these in his original paper. In general no crucial test is available for determining the accuracy of the direct method. This same statement may be applied to *every method of total blood volume determination that has been suggested.*

Indirect Methods of Total Blood Volume Determinations

The indirect method of blood volume determination is of particular interest to the physiologist. With modern day technique of intravenous injection it is possible to obtain blood volume values under various physiological states without sacrifice of the animal. In a study of the physiologic processes concerned in milk secretion it is the only method of much value, since the *in vivo* determination may yield useful knowledge concerning the animal under observation. An indirect method for determination of total blood volume seems to have been suggested first by Valentin (1838). His method employed distilled water as a diluent.

The Water Dilution Method.—The general plan set forth by Valentin has formed a basis for many methods since developed. The method employed in all indirect blood volume determinations is the introduction into the blood stream of a known volume or mass of some

accurately detectable substance, and after allowing time for thorough mixing, a sample of blood is drawn and the amount of introduced substance per unit of blood determined. From this figure the total blood volume is calculated.

Weber and Lehmann (1850), utilizing the method suggested by Valentin concluded that the blood constituted about one-eighth the body weight of man. Vierordt (1858) experimented on animals. He determined the amount of blood pumped from the heart in one second and multiplied this by the number of seconds required for a complete circulation, concluding that in man the blood constitutes one-thirteenth of the body weight. Quincke (1877) employed the indirect method recommended by Malassez (1874). Red blood cell counts were made in cases of pernicious anemia. A known amount of blood with a known cell count was transfused, following which the patient's red blood cells were again counted. From these data the total blood volume was calculated.

Sodium Chloride Dilution Methods.—Sodium chloride dilution of the blood by intravenous injection of salt solution as a means of blood volume determinations was used by Sander and Kronecker (1881). They substituted for blood an equal volume of sodium chloride solution and determined the degree to which it diluted the hemoglobin. This method, as did Valentin's method of dilution with distilled water, proved to be none too satisfactory. Errors due to the rapid rate of disappearance of water and salt solution from the blood stream were apparent. Sherrington and Copeman (1893) diluted the blood with 0.75 per cent sodium chloride solution. In the case of the rabbit they injected an amount of the solution equal to about one-third of the blood volume in the space of 15 seconds, and determined the degree of dilution 30 seconds later, using the specific gravity as an index. These workers do not claim a very high degree of accuracy for the method, and their estimated volumes show a wide range of variation. Kottmann (1906) applied the sodium chloride method to man. He injected about 3.5 c. c. of isotonic salt solution per kilogram of body weight and determined the dilution by means of a special hematocrit value obtained before and after injection. Plesch (1909), in man, injected a larger quantity of sodium chloride, 5 c. c. per kilogram of body weight, and determined the degree of hemoglobin dilution, the hemoglobin concentration being measured in a Plesch colorimeter. Using much the same method as Plesch, de Crinis (1917) determined the grade of dilution of the plasma.

Sodium chloride dilution methods have not given very consistent results. Aside from technical errors, the accuracy of this method depends upon (1) the complete mixing of the blood and salt solution, (2) the negligible escape of the solution from the blood stream during injection

and post-injection periods. Erlanger (1921) gave a critical discussion of the accuracy of sodium chloride methods and pointed out that in general the escape of sodium chloride from the circulation had been underestimated.

Other Dilution Methods.—Dilution by substances other than sodium chloride for total blood volume determination has been attempted. Loewy (1920) injected isotonic glucose (500 c. c.) in man and used the resulting dilution of the sodium chloride as an index of blood volume. This method is criticized because of the use of Bang's micro-method of sodium chloride determination which was used by Loewy and also because of the rapid disappearance of the injected solution from the circulation. Malassez (1874) suggested the use of blood serum as a diluent. Erlanger (1921) pointed out that this method might be of value where compatible serum was available, for serum has the advantage over most substances in that it leaves the circulation slowly. Robertson and Bock (1919) injected a 6 per cent solution of gum acacia in saline solution and determined the blood volume by using the hemoglobin dilution as an index. This method offers an advantage over other reported dilution methods in that gum solutions leave the circulation quite slowly.

Since substances introduced into the circulation as diluents tend to increase arterial pressure with possible consequences on the blood volume, Malassez (1874) suggests that the animal be bled first and the amount of bleeding be replaced by an equal amount of serum. Nelson (1909) using this method on two rabbits found values agreeing closely with the wash-out method. Harris (1920) has attempted to determine the total blood volume by dilution with Bayliss' gum-saline solution. His plan was to bleed the animal while transfusing a vein at the same rate with gum-saline solutions. The hemoglobin dilution gave an index to the blood volume.

Injection of Foreign Substances.—Indirect methods of total blood volume determination have been attempted by the intravenous injection of many foreign substances in addition to those named in this discussion. Von Behring (1912) has used antitoxin. The dilution of the antitoxin was determined biologically. Schurer (1911) injected foreign serum and studied its dilution quantitatively by precipitin reactions. Meek and Casser (1918-1919) used gum acacia as an intravenously injected colloid, and determined gravimetrically at the end of three minutes the amount of gum acacia in a known volume of blood. The results obtained by these workers closely approached those obtained by the dye injection method as employed by Keith, Rowntree and Geraghty (1915). McQuarrie and Davis (1920) have injected a non-coagulable, highly refractive substance, gum acacia and gelatin solution and noted the change in the refractive

index as the result of dilution. By the use of this method they found about 9.76 c. c. of blood per 100 grams body weight in the dog. In the rabbit they report an average of 6.49 c. c. of blood per 100 grams body weight. They also state that hemolysis, lipemia and cholemia do not affect the accuracy of their methods. Lee and Whipple (1921) have injected hemoglobin to determine the volume of circulating plasma. They estimated their values to be fairly indicative of the total plasma volume, but stated that in their opinion this method gave no direct information concerning the erythrocyte or hemoglobin values.

Hemorrhage Methods.—Hemorrhage methods of blood volume determinations were suggested by Vierordt (1854). The general theory is advanced that the reduction in the number of erythrocytes that occurs during the first few hours after hemorrhage is entirely relative and due to the replacement of the blood drawn. Douglas (1906), Dreyer and Ray (1910), and Keith (1919) have used and attempted to improve this method. In general the method is uncertain. Erlanger pointed out that the method can be employed only on animals that have not been bled before, and that this is indicative of the unstable position of the equilibrium point of blood regeneration.

The Carbon Monoxide Method.—This method was introduced by Grehant and Quinquaud (1882) who attempted to determine the blood volume in the dog by adding to the air inhaled a measured volume of carbon monoxide. The amount of carbon monoxide inhaled was less than that causing an oxygen want. The proportion of hemoglobin remaining combined with oxygen was then determined by gasometric methods.

This method was applied to man by Haldane, and Lorrian Smith (1899-1900). Improvements in the technique have been made by a number of investigators including Douglas (1906), Zuntz and Plesch (1908), and Van Slyke and Salvensen (1919). More recently Van Slyke and Robscheit-Robbins (1927) have adapted a method suitable for the gasometric determination of small amounts of carbon monoxide.

The carbon monoxide method is based on the fact that carbon monoxide reacts with hemoglobin to form carbon monoxide hemoglobin at the expense of an equivalent amount of oxyhemoglobin. The carbon monoxide method has been severely criticized because the role of myohemoglobin is still unsettled and renders the interpretation of data unsatisfactory.

In a discussion of physiological changes with environment, Barcroft et al (1922) reported that the carbon monoxide failed to give results properly because it indicated marked changes in blood volume with changes in environmental temperature. This observation was later

corroborated by work carried on in a glass respiration chamber in the Cambridge Physiologic Laboratories. The results showed that as environmental temperature increased this method showed an *increase* of blood volume. Thus it is evident that in all studies involving CO methods the environmental temperature must be considered. Further difficulties have been pointed out in this method by Barcroft and Barcroft (1924), who demonstrated that the blood of the splenic spaces failed to keep pace with the peripheral blood in carbon monoxide saturation. There seems to be wide variations in the blood volume reported on similar individuals by the carbon monoxide method when used by various investigators. Erlanger (1921), in his summary of data collected by this method, showed ranges from 2.6 to 8.8 per cent for normal men and women. The carbon monoxide method, if handled with well regulated technique, certainly has its value, but it does not seem to give such consistent results as the dye injection method, which will be discussed later.

The carbon monoxide method and the dye injection method are much used for total blood volume determination at present.

Other indirect methods have largely given way to these two, which seem to be fitted better for the purpose. There is much controversy as to the superiority of each of these methods. The carbon monoxide method, because of its technical difficulties, does not seem nearly so adaptable to total blood volume determinations in the dairy cow as the dye injection method. Doubtless by suitable means the carbon monoxide method could also be adopted for this purpose. However, the dye injection method is simple, it can be used on cows under average barn conditions, and does not require the laboratory apparatus and highly developed technique so necessary for successful employment of the carbon monoxide method.

The Dye Injection Method.—The dye injection method for the determination of plasma volume and blood volume was introduced by Keith, Rowntree and Geraghty (1915). This method involves a new principle.

(a) A known amount of a slowly absorbable, non-toxic dye is introduced directly into the circulation. (b) The dye remains in the plasma sufficiently long for the two to become well mixed. (c) The concentration of dye in the plasma is determined, after a suitable mixing period, by colorimetric comparison with a suitable standard mixture of dye and serum. From the extent of the dilution of the dye by the plasma, the plasma volume is determined. (d) Hematocrit values are obtained and total blood volume computed on the basis of plasma volume as determined directly, and the cell volume as shown by the hematocrit.

By the use of this method it was shown that for normal men the plasma volume was approximately 5 per cent of the body weight or 42 to 56 c. c. of plasma per kilogram of body weight. The total blood volume was found to equal about 8.8 per cent of the body weight with 79 to 99 c. c. blood per kilogram. These investigators have pointed out that duplicate determinations on normal subjects were found to yield approximately the same results. Loss of blood by hemorrhage or an increase in blood volume due to infusion with physiologic saline solution have been found to be fairly accurately reflected. Numerous *in vitro* experiments also show a high degree of accuracy for the method.

The dye injection method as set forth by Keith, Rowntree and Geraghty has been widely used. It has, however, been improved somewhat by Hooper, Smith, Belt and Whipple (1920), who advocated an isotonic solution of potassium or sodium oxalate as an anticoagulant. These workers show by numerous experiments that dry oxalate causes a shrinking of the cells and introduces an error in hematocrit values. To avoid this difficulty they add 2 c. c. of 1.6 per cent sodium oxalate to each 10 c. c. of blood. This is approximately isotonic with the blood. Rowntree, Brown and Roth (1929) have carried out further studies concerning the shrinkage of corpuscles when the dry oxalate technique is used as compared to wet oxalate, and reported that the cell volume was reduced about 14 per cent in the case of dog's blood. They further state that the shrinkage of the erythrocytes of the dog is much greater than those of man, under parallel conditions. In 93 duplicate "wet and dry" oxalate hematocrit determinations on the human, a higher cell volume averaging 3.4 per cent (of the blood in the tube) was found for the wet oxalate method. Another question of considerable interest in this connection is whether or not the shrinkage of the cells causes a relative increase in plasma and an undue dilution of the dye. This, if true, would give excessive values for blood plasma.

Using the dry-oxalate technique, Rowntree, Brown and Roth (1929) show no significant differences in plasma values for normal subjects as compared to the wet-oxalate as an anticoagulant. Since the evidence is not entirely clear on this point they have refrained from any corrections for plasma volumes. The adoption of the "wet-oxalate" method by practically all who use the dye method renders such a correction unnecessary it would seem.

A 1.6 per cent solution of potassium oxalate has been used exclusively in all blood volume determinations on dairy cattle presented in this paper.

The choice of a dye has been a matter of discussion among advocates of the dye method. A dye suitable for this purpose must be non-toxic,

not readily taken up by body tissues or permanently stored in the body, must remain in the circulating plasma for several minutes after injection, and also be suitable for colorimetric comparison in high dilution. In general, the vital dyes have been found satisfactory. Dawson, Evans and Whipple (1920) reported observations on more than 60 dyes injected into the blood stream. They found approximately 30 dyes possessing the qualities essential for use in the estimation of plasma volume. Most of the vital red dyes were found to be satisfactory. These investigators preferred certain blue dyes (blue azo dye—T1824) which they claimed left the blood stream more slowly and could be more easily compared in a colorimeter than the red dyes. In general though they have employed vital red dyes, which favorably compared with the original vital red dye used by Rowntree et al (1915). Whipple and his associates have used brilliant vital red quite extensively in their studies. Harris (1920) suggested Congo-red as a substitute for vital red, claiming for it a greater availability and a slower disappearance from the blood stream. It has since come into general use and Rowntree, Brown and Roth (1929) state, "In our own studies we are now employing Congo-red almost exclusively. Long experience with both vital red and Congo-red led us to believe they may be used interchangeably. In fact, Congo-red probably yields a color slightly more satisfactory from the standpoint of colorimetric readings, but its chief advantage consists in the fact that it is readily available."

One of the greatest technical difficulties of the dye method, as well as all indirect methods, is the determination of the proper mixing time to be allowed after the dye has been injected and before the sample for colorimetric comparison of the dye-tinged plasma is drawn. Two factors are concerned. (1) Allowance of adequate time for thorough mixing of the dye with the blood; (2) drawing of sample for the determination of the extent of dye dilution by the blood before any appreciable amount of dye has left the circulatory system. Therefore, the optimum time for making the determination is of paramount importance.

The dye injection method has been used successfully on all laboratory animals. Values obtained by this method have been reported by many investigators. Keith, Rowntree and Geraghty (1915), and many others have applied the method to the human. This paper will attempt to show that this method may also be of value to the physiologist engaged in a study of domestic animals.

The Combined Dye and Carbon Monoxide Method for the Determination of Blood Volume.—Lamson and Nagayma (1920) doubted the accuracy of the dye method for total blood volume determination and developed a method by which they believed the "true blood volume"

could be estimated. They used the dye method to obtain plasma volume and the carbon monoxide method to obtain the erythrocyte volume and combined the two results. Smith, Arnold and Whipple (1921) compared the dye, the carbon monoxide, and the Welcher methods on dogs. They concluded that the combined method could be used to estimate "the absolute blood volume".

Erlanger (1921) stated that Lamson and Nagayma fail to present any new evidence indicating weaknesses inherent in either the dye or carbon monoxide methods which would favor their being combined as a means of determining total blood volume. Rowntree, Brown and Roth (1929) heartily disagree with Smith et al (1921) as to the necessity of combining the two methods. They cite the statements of both Lamson and Whipple, who expressed their belief, based on animal experimentation, that the dye method yields the most reliable information obtainable with reference to plasma volume. "Lamson stated that if a single test of blood volume is to be employed at all, the dye method is as good as any, if not actually the best in existence."

The Anatomy and Physiology of the Blood Vascular System of the Bovine

In a study of the blood volume of dairy cattle a knowledge of the anatomy and physiology of the circulation is essential. The application of a desirable method for blood volume determination requires a knowledge of many associated factors concerning the circulation. In a consideration of the time necessary for adequate mixing of the dye and blood, the speed of circulation and velocity of flow are of importance. Likewise, the path of circulation and the supply of blood to the various organs of the body are factors concerned.

In general, veterinary text books give only limited information directly concerning the physiology of circulation and physical constants of the blood of the bovine. Feeling the need of more information directly applicable to the blood volume investigations in hand, data concerned with the speed of circulation and pulse rates have been obtained. Determinations of the specific gravity of both blood and plasma have been made also.

The Blood Vascular System of the Bovine.—The blood vascular system of the bovine need not be discussed here at length. The vascular system consists of the heart, the arteries, the capillaries, and the veins. These structures form a continuous network of branching tubes which convey the blood from the heart, through the arteries and capillaries to all parts of the body. The circulatory system is divided into two divisions. That division carrying the venous blood from the right side of the heart through the lungs to the left side of the heart is known as the

pulmonary circulation. That division of the circulation concerned in the passage of blood, thus purified, through the body and back to the right side of the heart is the *systemic circulation*. The systemic circulation is divided into several divisions which serve to supply the various body organs and systems with a continual blood supply. Branches of this system are responsible for the blood supply of the skeleton, muscles of the skeleton, and accessory appendages. Other branches or systems are concerned with the passage of blood through the splanchnic regions. These are, namely (a) *circulation of the digestive tract*; (b) *the hepatic-portal system*; (c) *the renal-circulation*; (d) *the splenic circulation*; and (e) *the mammary circulation*, one of much interest in milk secretion studies.

The arterial and venous systems and the manner in which various organs of the body, including the mammary gland, are supplied with blood is shown in Fig. 1.

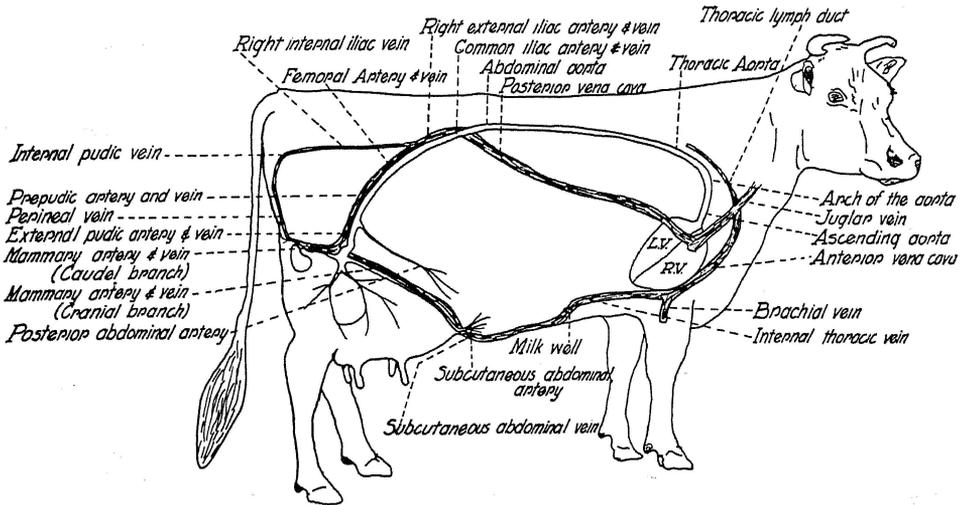


Fig. 1.—Schematic circulatory system of dairy cow. Showing large veins and arteries and general scheme of the blood supply to the mammary gland.

The venous system is of particular interest in blood volume determinations. The arteries of the dairy cow, as a rule, are fairly deeply imbedded and inaccessible. The *jugular vein*, quite close to the skin, passes downward and posteriorly, lying parallel to the trachea. It is readily accessible for vein-puncture and has been used, particularly for young dairy cattle, as a point of dye injection in blood volume determinations. Blood from the posterior regions of the body is returned to the heart by the posterior vena cava and by the subcutaneous abdominal veins which continue as the *anterior vena cava*. The jugular vein and the anterior vena cava return most of the blood from the anterior region of the body to the heart. Blood from the mammary gland is returned to

the heart by at least three branches of the venous system: *the external pudic, the subcutaneous abdominal* (or "milk veins"), and the *perineal*. The subcutaneous abdominal veins (or "milk veins"sm) with from one to two smaller medial radicles, emerge from the anterior basal border of the udder as a continuation of the anterior or cranial mammary vein. The "milk veins" proceed anteriorly with numerous branchings, are quite close to the skin, and do not enter the abdominal wall until near the xiphoid cartilage. The subcutaneous abdominal veins enter the thoracic cavity to join the *thoracic veins* of their corresponding sides. Lying so near the surface the subcutaneous abdominal vein (Fig. 2) proved to be an ideal point of injection of the dye for blood volume determination. For convenience, it is to be preferred over the jugular vein as will be pointed out later.

While not of immediate value in blood volume determinations, the abundant supply of blood to the mammary gland is of great importance in a physiological study of milk secretion. We are interested in blood volume chiefly in the role which it plays in this connection.

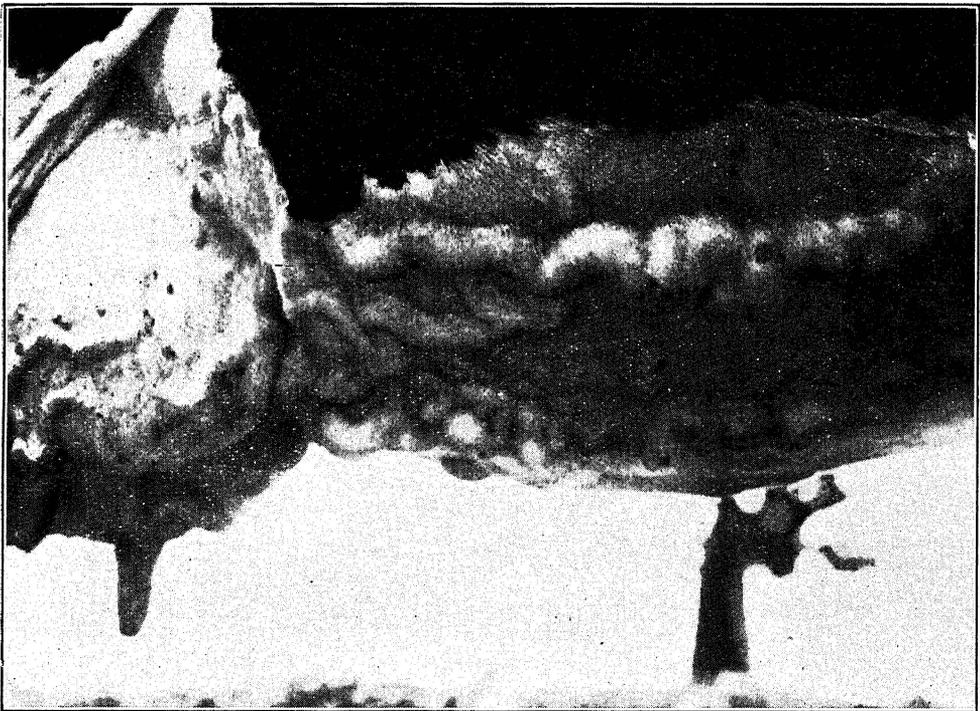
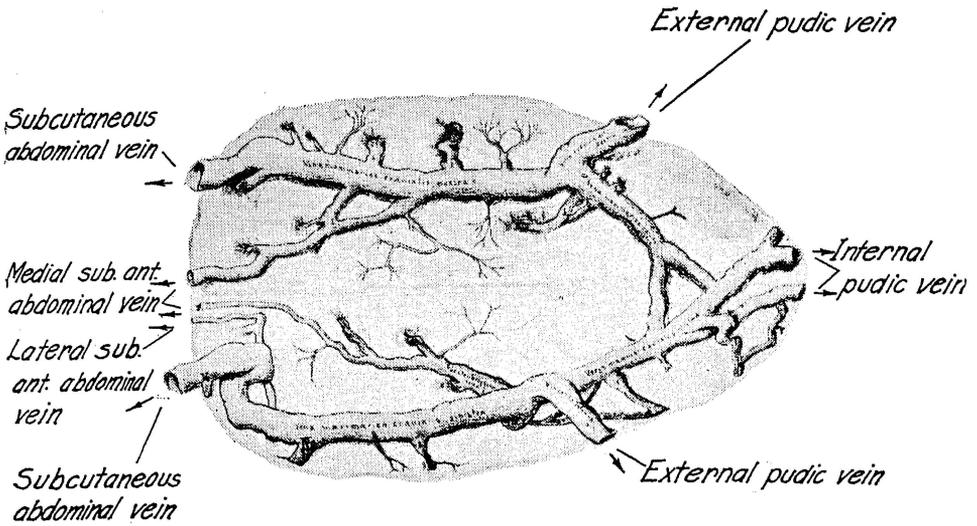
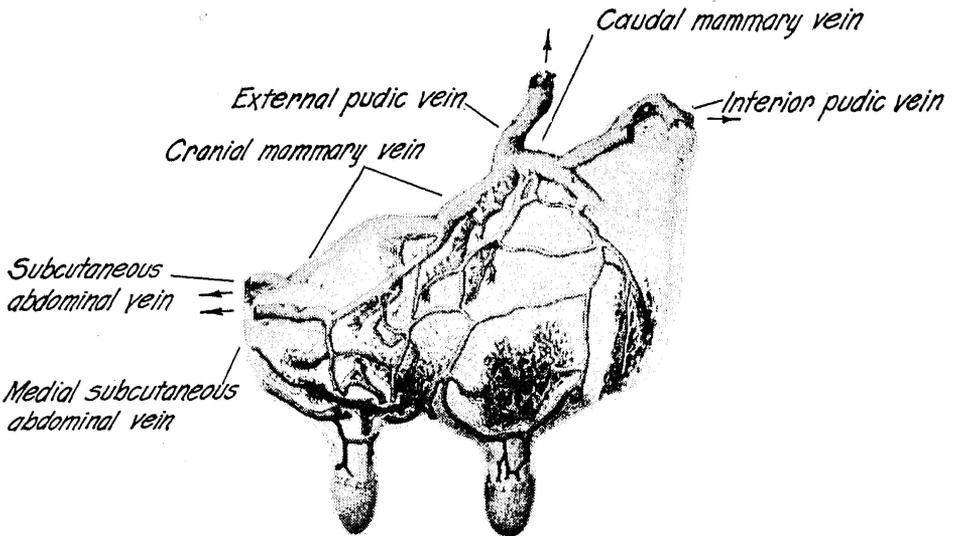


Fig. 2.—Subcutaneous abdominal mammary veins. Showing their nearness to the surface and large size in the dairy cow. The arrow points to the usual place of injection of the dye-solution employed in blood volume determinations on mature cows.



Basal Veins of the Udder (Glättli)

Fig. 3.—Veins of the Udder (Glättli). The udder is supplied with a large number of veins. It will be noticed above that there are frequent anastomotic arcs. The cranial mammary veins proceed anteriorly as the subcutaneous abdominal veins and aid in the return of blood from the mammary gland to the heart.



Veins of the Udder (Glättli)

Fig. 4.—Basal veins of the Udder (Glättli). The subcutaneous abdominal veins or "milk veins" emerge from the anterior basal border of the mammary glands and run anteriorly to the xiphoid cartilage where they enter the abdominal wall, thus forming the "milk wells". The numerous medial and lateral branches of cranial mammary veins are also shown above.

EXPERIMENTAL PROCEDURE

The Speed of Circulation in the Bovine

It is a matter of interest in connection with blood volume determination to have an approximate idea of the time necessary for the blood to make a complete circuit of the vascular system, that is, starting from any given point to determine how long it will take for a particle of blood to arrive again at the same place. It must be borne in mind, however, that there are many different paths open to the flow of blood. The time for a complete circuit, therefore, will vary with the circuit actually followed. There is a rather limited amount of information available concerning the speed of circulation in the larger domestic animals. For dairy cattle this value seemed to be lacking so far as direct determinations are concerned.

The experiments on the speed of circulation which have been reported for other animals are valuable in indicating the rapidity with which substances introduced into the blood stream are spread over the body.

The circulation time may be determined by injecting an easily distinguishable salt, such as potassium ferrocyanide, and determining its first appearance in the vein on the opposite side of the body. Smith (1921) cites the work of Hering who developed this method and reported that in a horse with pulse frequency of 42 the average complete circuit is performed in 31.3 seconds; equivalent, according to this observer, of about 28 beats of the heart. In the rabbit with a pulse frequency of 168 per minute, the time occupied in completing the round of the circulation was 7.79 seconds, or about 28 beats of the heart. In the dog 16.7 seconds were reported, or 26.7 heart beats. Stewart (1894) devised the method of electrical conductivity for ascertaining the duration of circulation and reported that in a dog weighing 13.3 kilograms the average circulation time in the spleen was 10.95 seconds, in the kidney 13.3 seconds, and in the lungs 8.4 seconds.

Lian and Barras (1930) applied to the study of the measurement of speed of circulation in man the use of a 5 per cent solution of fluorescein. They injected 2 c. c. of this solution in a vein of the elbow and then punctured the vein at the same place in the other arm to collect samples. The blood is collected at 10 second intervals in small glass tubes, containing 95 per cent alcohol, from 10 to 180 seconds after injection. The tubes are shaken and placed in a rack for a few minutes. If the tubes are then inspected on a dark background, in a room to which no sunlight is admitted, the fluorescence will be readily observed. The first appearance in the vein opposite the point of injection is considered as a com-

plete circulation. They found the speed of circulation between the two upper limbs of the normal human to be thirty seconds.

Determination of Time of Circulation in the Dairy Cow.—The method of Lian and Barras was applied to five dairy cows by injecting 15 c. c. of 5 per cent fluorescein in a physiologic saline solution. The pulse rate was determined by feeling the posterior tibial artery on the medial surface of the tibial region eight to ten inches above the hock joint.

The procedure in brief was to (a) secure the pulse rate; (b) insert 16 gauge hypodermic needles into both the right and left subcutaneous abdominal veins; (c) inject 15 c. c. of 5 per cent fluorescein in physiologic saline, using a well greased syringe, into one of the subcutaneous abdominal veins; (d) during injection time and every five seconds following injection samples were collected in small tubes containing 95 per cent alcohol, collection being continued for 90-120 seconds after injection; (e) the samples were shaken and allowed to stand several minutes; (f) samples were examined in front of a dark background and in dim light for fluorescence. The first appearance of the fluorescein in the mammary vein opposite injection was considered as having undergone a complete circulation. The results, as obtained on a limited number of animals, are as follows.

TABLE 1.—THE SPEED OF CIRCULATION THROUGH THE BODY OF THE COW BY WAY OF THE MAMMARY GLAND

Herd No.	Breed	Pulse (average of three trials)	Seconds required for circulation
515	Holstein	68	50
511	Holstein	65	45
510	Holstein	60	55
512	Holstein	58	60
290	Holstein	63	50
Average		62.8	52

Though few in number these data show that about 52 seconds are required for the blood to make one complete circulation of the body. The pulse rate was found to be about 63 frequencies per minute for mature cows. Observations on young and growing animals showed the pulse to be much faster, ranging from 80-95 frequencies per minute for animals under three months of age and 65-80 per minute for animals from three to 18 months of age.

Obviously much more data is needed concerning the speed of circulation in dairy cattle. In general it has been found that a number of factors affect the pulse rate and consequently the speed of circulation.

The larger species tend to have a much lower pulse rate than the smaller. The variations with age are quite pronounced, the young animals having a much higher pulse rate than the mature. Excitement, exercise, and low temperature tend to speed up the rate of circulation. Theoretically, complete mixing of the injected dye would be completed more quickly in young animals than in old.

The Specific Gravity of the Blood and Plasma of Dairy Cattle.—It is often desirable to express the relation between blood mass and body mass. Erlanger (1921) favored this method of expressing results. Often times, however, blood values are expressed in terms of cubic centimeters per kilogram of body weight or in cubic centimeters per square meter of body surface. In this study it was decided to express values both in terms of blood mass and also in terms of volume. Since all determinations are computed in terms of cubic centimeters the results must be multiplied by the specific gravity of the blood or plasma to convert it to grams of body weight.

The specific gravity of the blood may vary with the water intake of the body. The plasma has also been shown by Barbour and Hamilton (1926) to be subject to considerable variation. Appel and Farr (1928) show that the specific gravity of the serum may give an index to water metabolism.

Smith (1921) states that the specific gravity of the blood varies in different animals. In the horse, ox, and pig, it averages about 1.060. In the sheep an average of 1.050 to 1.058 has been reported. He also states that Hoppe-Seyler has found the specific gravity of the plasma of the horse to be 1.027 to 1.028 and the corpuscles 1.105. In the human, whole blood usually varies between 1.054 and 1.060 in specific gravity. In new born infants the values may be higher, about 1.066. The plasma has a specific gravity of 1.0237 to 1.0276 and the corpuscles approximately 1.088, according to Bodansky (1927). In general 1.056 has been arbitrarily used as the specific gravity of blood by many investigators. Erlanger (1921) used this figure in expressing the relation between blood weight and body weight.

Since comparatively few values concerning the specific gravity of the blood and plasma of the bovine are available, further determinations were deemed necessary. The specific gravity of the blood or plasma may be determined by the use of a pycnometer, by the use of the falling drop method, as suggested by Barbour and Hamilton (1926) or by the use of a chainomatic balance devised to test specific gravity of liquids. The falling drop method is suited to micro-amounts. The pycnometer method of course requires larger amounts of blood, as does the chainomatic balance type of specific gravity test.

The pycnometer method was used in all specific gravity determinations on the whole blood of dairy cattle. The procedure was to use pycnometers of 10 c. c. and 20 c. c. capacity. The capacity was determined on the basis of distilled water at 21 degrees Centigrade by weighing on an analytical balance. All weighings of the blood-filled pycnometers were made with the contents at 21 degrees Centigrade, which practically obviated the necessity of temperature correction. Sodium citrate (0.1 gm. per 10 c. c. blood) was used as an anticoagulant and the necessary corrections for this factor have been made in the results shown in Table 2. Since there might be some variation in the specific gravity of blood coming away from the mammary gland and that passing through the jugular vein, determinations have been made on samples drawn from both veins. These results are shown in Table 2.

TABLE 2.—THE SPECIFIC GRAVITY OF THE WHOLE BLOOD OF THE BOVINE
(Pycnometer Method)

Samples drawn from mammary vein		
Number of Animal	Specific Gravity	Remarks
569H*	1.0506	Female
290H	1.0491	"
510H	1.0481	"
509H	1.0487	"
557H	1.0466	"
578H	1.0491	"
814J	1.0502	"
125J	1.0510	"
164J	1.0451	"
266H	1.0482	"
804J	1.0461	"
Average of samples from mammary vein	1.0482	
Samples drawn from jugular vein		
154J	1.0529	Female
190J	1.0524	"
266H	1.0514	"
290H	1.0488	"
509H	1.0465	"
510H	1.0503	"
512H	1.0513	"
428G	1.0420	"
Leto A	1.0492	Male
Huish G	1.0522	"
Caesar H	1.0524	"
Mercury J	1.0600	"
Avanelle J	1.0590	"
Average of samples from jugular vein	1.0514	
Average of all samples	1.0499	

*Letter designates breed.

The average specific gravity of whole blood drawn from the mammary vein of the mature dairy cow was found to average 1.0482 for the eleven samples upon which this determination was made. An average specific gravity of 1.0514 was found for thirteen samples drawn from the jugular vein. The average specific gravity of blood as drawn from both veins was 1.0499. It will be noticed in Table 2 that the blood of males was higher than that of females, several of the samples approaching 1.059. The limited data would not permit the drawing of any significant conclusions from these variations. Jones (1891) reported that the specific gravity of blood varied with age and sex; that it is diminished after eating and increased after exercise; diurnal variations are also reported and a wide variation in individuals has been observed. As a result of the observations reported in Table 2 and 3 a value of 1.05 has been used to convert the blood volume values of the dairy cow into weight units and a value of 1.0268 has been used in converting plasma volume to similar terms.

The specific gravity of the plasma was tested by the use of a Becker specific gravity chainomatic balance. The plasma was obtained by centrifuging the whole blood. Samples were drawn from the jugular vein and determinations made immediately. About 30 c. c. of blood were used which yielded 12-15 c. c. of plasma. Sodium citrate (0.1 gm per 10 c. c. of blood) was used as an anticoagulant and the necessary corrections have been made in all of the observations reported in Table 3.

TABLE 3.—SPECIFIC GRAVITY OF THE BLOOD PLASMA OF THE BOVINE
Determined by Becker Specific Gravity Chainomatic Balance (Temp. 21 degrees C.)

No. of Animal	First Reading	Second Reading	Third Reading	Average Specific Gravity of Plasma
		(Corrected readings)		
577	1.0299	1.0296	1.0293	1.0296
820	1.0261	1.0266	1.0262	1.0263
340	1.0268	1.0271	1.0271	1.0270
127B	1.0301	1.0301	1.0301	1.0301
557	1.0332	1.0334	1.0329	1.0331
828	1.0246	1.0244	1.0241	1.0243
266	1.0292	1.0294	1.0293	1.0293
579	1.0241	1.0239	1.0242	1.0240
427	1.0275	1.0268	1.0275	1.0272
569	1.0241	1.0245	1.0246	1.0244
810	1.0224	1.0226	1.0224	1.0224
589	1.0262	1.0264	1.0263	1.0263
341	1.0281	1.0280	1.0279	1.0280
290	1.0276	1.0273	1.0274	1.0274
509	1.0266	1.0267	1.0261	1.0264
521	1.0259	1.0253	1.0254	1.0255
512	1.0261	1.0230	1.0266	1.0252
511	1.0252	1.0254	1.0257	1.0254
Average of 18 samples				1.02677

The Determination of the Plasma and Blood Volume of the Bovine by the Dye Injection Method

The blood volume of the bovine may be measured by the use of the dye injection method by a procedure similar to that used on smaller animals.

The method outlined by Whipple et al (1920) has been used in the determination of the blood volume of dairy cattle in the University of Missouri herd. This method is fully described by Hooper, Smith, Belt and Whipple (1920) and few changes have been found necessary in its application to the bovine.

The Technique of the Dye Method as Used for Dairy Cattle.—The procedure used in the blood volume determination of dairy cattle is essentially the same in principle as that outlined by Whipple et al (1920) for the dog. Certain changes due to the size of the animal, however, have been made.

1. A 16 gauge, sterile hypodermic needle is inserted in the subcutaneous mammary vein or the jugular vein of the animal. No compression of the vein is necessary if the subcutaneous abdominal vein is used, but if the jugular vein is used the compression of the vein is necessary before inserting the needle and can be obtained by the use of a "choke-rope" about the neck of the cow. After the needle is fixed in the vein and the blood is flowing freely, 17 c. c. of blood are collected in a graduated tube containing 3 c. c. of 1.6 per cent potassium oxalate solution. The total of 20 c. c. of blood and oxalate solution is mixed by agitation and the tube stoppered.

2. Meanwhile, one operator should have filled a well greased syringe, graduated to deliver an accurate amount of dye. Approximately 3 mg. of vital red or Congo-red per kilogram of body weight is injected. The dye is made up in a 1.5 per cent solution for injection purposes.

3. Approximately five to six minutes after the injection of this dye a clean needle is again inserted into the jugular or mammary vein on the opposite side of the body, and another 17 c. c. of blood are drawn into a tube graduated at 20 c. c. and containing 3 c. c. of 1.6 per cent potassium oxalate. The contents are well mixed and the tube is stoppered.

The collected blood samples are taken into the laboratory immediately and measured in an accurately calibrated 25 c. c. graduated cylinder. Often it is impossible to draw approximately 17 c. c. of blood with any degree of accuracy. If 15-19 c. c. of blood are drawn into a tube containing 3 c. c. of 1.6 per cent oxalate solution the entire content may be measured and the necessary correction for oxalate dilution made. Allowing the blood to run directly into a graduated tube from the in-

serted needle was found to be more satisfactory than attempting to draw any given amount with a syringe. The struggles of the animal will throw the needle out of the vein much more easily if a syringe is engaged. A piece of sterile rubber tubing, 4-6 inches in length, attached to the inserted needle, was found to greatly facilitate collection of samples. The syringe is engaged directly to the needle in dye injection. This can be easily accomplished in mammary vein injections, and fairly readily where the jugular vein is used, provided the animal has become quiet following the insertion of the needle.

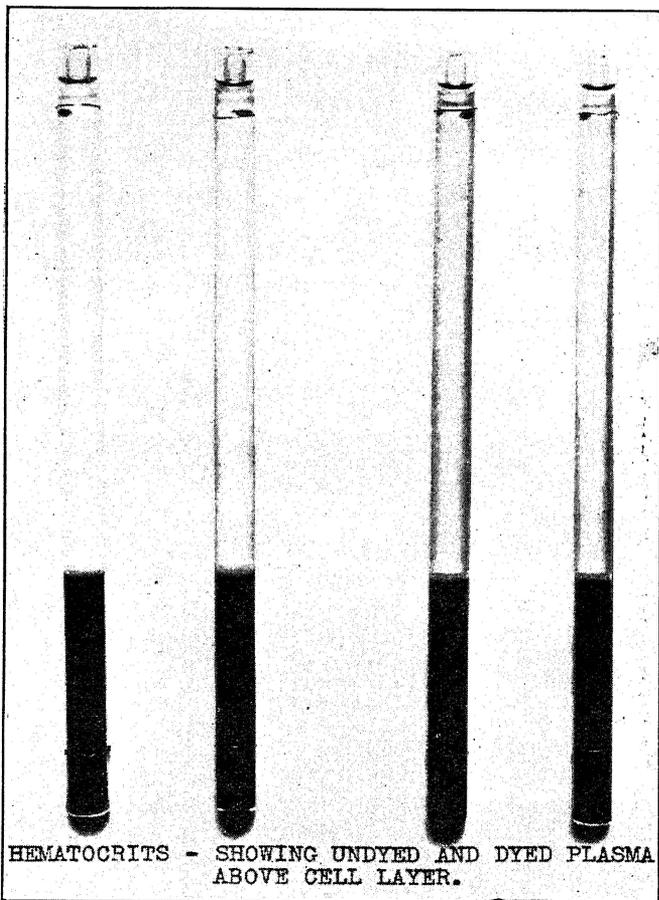


Fig. 5.—Hematocrit values were obtained by the use of a tube of the type shown above. The difference in color of the dyed and un-dyed plasma is shown. A layer of leucocytes on top of the red corpuscles is also apparent.

4. After accurately measuring the collected blood and mixing thoroughly, hematocrit tubes are filled in duplicate and a few drops of mineral oil added to prevent evaporation and to minimize errors due to the loss of carbon dioxide. A hematocrit tube 12-14 cm. in length and made of 3 mm. bore glass tubing was found to be very satisfactory in this respect (Fig. 5). The hematocrit tubes are centrifuged for 45 minutes at a speed of 2800 revolutions per minute. The height of the cell column and the total length of the combined cell and plasma column are obtained by measurement. The volume of cells is expressed in per cent.

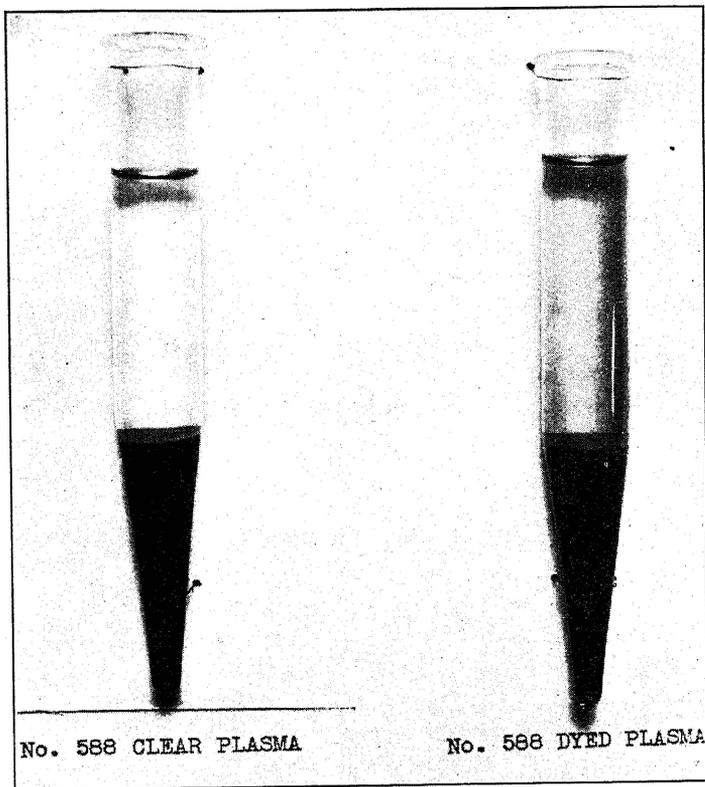


Fig. 6.—This view shows the dyed and un-dyed plasma above the cell layer. Ten cubic centimeters of bovine blood provides sufficient plasma for carrying out plasma volume determinations.

Next, 10 c. c. samples of blood, as taken before and after injection of the dye, are pipetted into 15 c. c. centrifuge tubes. These tubes are stoppered, properly labelled, and centrifuged for 25-30 minutes at 2500

revolutions per minute. The plasma thus obtained is used for colorimetric comparison of the dye concentration in the plasma (Fig. 6).

5. Three cubic centimeters of the dye-colored plasma are pipetted off and diluted in a small tube with 6 c. c. of 0.9 sodium chloride. This unknown is then read in a colorimeter against a standard described by Whipple (1920) as follows:

"1. Seventy-five hundredths cubic centimeter of 1.5 per cent vital red is pipetted into a 200 c. c. volumetric flask which is then made up to the mark with distilled water.

"2. Five cubic centimeters of this aqueous dye solution are then diluted with 5 c. c. of clear dye-free plasma (obtained from the first sample of blood drawn from the animal) and 5 c. c. of 0.9 per cent sodium chloride, making in all 15 c. c. of standard dye solution."

6. Against the standard solution the above unknown is compared in a colorimeter and expressed in per cent. The setting of the standard is always given first, thus a reading of 20:30 is expressed as 66.66 per cent. This value is referred to as R for convenience in computation.

The Calculation of the Plasma and Blood Volume.—The plasma and blood volume may be calculated by the use of a formula suggested by Whipple et al (1920). This formula is expressed as follows:

$$\text{"Plasma volume (in c. c.)} = \frac{26666.67DC}{R} \quad (1)$$

$$\text{Blood volume} = \frac{\text{Plasma volume} \times 100,}{\text{Plasma per cent}} \quad (2)$$

D is the number of cubic centimeters of 1.5 per cent dye injected into the animal; C is the necessary correction for oxalate dilution (as explained below); and R is the colorimetric reading expressed in per cent of the standard.

The correction for oxalate solution is made on the following basis:

Suppose 3 c. c. of isotonic oxalate are used as an anticoagulant for 17 c. c. of blood drawn into a tube graduated at 20 c. c. A hematocrit tube of 20 cm. length would contain 3 cm. column of oxalate, all of which will be in the plasma column. Hence the correction for the plasma will be:

$$\text{Plasma column} - \frac{\text{Total length of tube} \times 3}{\text{No. c. c. oxalated blood}} = \text{Plasma column corrected} \quad (3)$$

Then the correction for the total column of blood will be

$$\text{Total length} - \frac{\text{Length} \times 3}{\text{No. c. c. oxalated blood}} = \text{Blood column corrected for oxalate dilution} \quad (4)$$

According to Whipple et al (1920), "The formula for plasma volume may be derived as follows: The standard for color comparison contains 0.75 c. c. of 1.0* per cent dye in 200 c. c. of fluid, or 1 c. c. in 266.66 c. c. Then D c. c. of 1 per cent dye (the amount injected) will impart the same color intensity to 266.6667 D c.c. of fluid. If, however, the color

intensity is $\frac{R}{100 C}$ (that is, the colorimeter reading in per cent corrected

for the dilution of the plasma by the oxalate solution), the number of cubic centimeters of fluids equals

$$\frac{(266.6667D) (100 C)}{R} \text{ or } \frac{26666.67 DC.}{R}$$

Plasma per cent refers to the percentage of the whole blood which the plasma constitutes, and is obtained by dividing the *corrected plasma column* present in the hematocrit by the *corrected total length of the entire column* of cells and plasma in the tube.

Examples of Calculations Necessary for Computing the Blood Volume of a Dairy Cow.—This example will serve to illustrate the calculation of blood volume. The following arrangement of data and manner of recording results is typical of all the determinations on dairy cattle reported here. It will be noticed that colorimetric readings are expressed in readings at different depths as a check. All hematocrit determinations are made in duplicate. Often as a manner of testing the agreement of results, samples collected at four and six minutes after injection were obtained. Both samples are used here to illustrate the close agreement.

Typical Data as Obtained in a Normal Determination of the Blood Volume of a Dairy Cow.—Herd Number 553. Weight in lbs.—1326. Date 12-19-30. Dye used—100 c. c. 1.6 per cent vital red. Vein Used—Subcutaneous Abdominal.

HEMATOCRIT VALUES

Sample No.	Tube No.	Total Column (mm.)	Cell Column (mm.)	Oxalated Plasma Column (mm.)	Plasma Per cent
I Before injection.---	31	127	34	93	68.9
	(21.9 c. c. drawn) --	32	126	34	93
II 4 min. after injection	33	126	35	91	67.8
	(22.1 c. c. drawn) --	34	129	37	92
III 6 min. after injection	35	126	35	91	67.5
	(20.8 c. c. drawn)--	36	126	35	91
Average of six					67.86

*For our purpose a 1.5 per cent solution of dye has been employed.

COLORIMETER READINGS ABOVE SAMPLES

	Standard (mm.)	Unknown (mm.)	Per cent of Standard
4 min. after-----	20	27.5 .2	72.7 {
(average of 5 readings) --	15	20.1 .2	74.6 { Ave. 73.7
6 min. after-----	20	27.9 .2	71.6 {
(average of 5 readings) --	15	20.6 .2	72.8 { Ave. 72.2

Calculations.—After the readings of the hematocrits have been made the first step is to correct for the dilution by the oxalate solution. Using the formula given on a preceding page, the calculations for sample I, hematocrit tube 31, follow:

$$9.3 - \frac{12.7 \times 3}{21.9} = 7.56, \text{ or } 75.6 \text{ mm, corrected plasma column} \quad (3)$$

$$\text{Then, } 12.7 - \frac{12.7 \times 3}{21.9} = 10.96 \text{ or } 109.6 \text{ mm, corrected height of total column} \quad (4)$$

$$\text{Therefore, } \frac{75.6}{109.6} = 68.9 \text{ per cent plasma in Sample I,}$$

then $100 - 68.9 = 31.1$ per cent cells.

All of the hematocrit values are corrected by the described steps and an average of the plasma percentage is obtained.

Calculating the plasma volume of the above sample:

$$\frac{26,667 \times 100}{73.7} \frac{7.45}{9.20} = 29,300 \text{ c. c. plasma} \quad (1)$$

$$\text{Then } \frac{29,300 \times 100}{67.86} = 43,177 \text{ c. c. blood for the sample drawn four minutes after injection} \quad (2)$$

The sample No. II drawn six minutes after injection is thus found to be:

$$\frac{26,667 \times 100}{72.2} \frac{7.29}{9.10} = 29,588 \text{ c. c. plasma.} \quad (1)$$

and

$$\frac{29,588 \times 100}{67.86} = 43,600 \text{ c. c. blood.} \quad (2)$$

There is a difference of 413 c. c. or 0.95 per cent, which is considered a very good check in a determination of this type. The average of the two determinations would be 43,388 c. c. of whole blood.

To obtain the blood mass, simply multiply the number of cubic centimeters by 1.05. Then $43,388 \times 1.05$ is equal to 45,557 grams of blood or 45.557 kilograms. One kilogram is equal to 2.2 pounds, so 2.2×45.557 equals 100.2 pounds.

Therefore, $\frac{100.2 \times 100}{1326} = 7.55$ per cent of body weight.

Discussion and Criticism of the Dye Injection Method

The dye injection method, as with all blood volume methods, has been the subject of some criticism. The proper time after injection for the drawing of the samples and the rate of disappearance of the dye from the blood stream immediately following the injection are most perplexing problems. In the adaptation of the method to the bovine additional data dealing with some of the technical problems under criticism have been obtained. These data will be presented and discussed in detail in their bearing on the subject.

Adsorption of the Dye by the Corpuscles of the Blood of the Bovine.—Linhard (1926) reported serious errors in the dye method due to adsorption of the dye by the stroma of the red corpuscles. In a series of *in vitro* experiments excess values for plasma as compared with the hematocrit were observed. Linhard explained this wide discrepancy on the basis that the dye was taken up by the red corpuscles. Rowntree, Brown, and Roth (1929) repeated the experiments of Linhard and *failed* to find adsorption of the dye when Congo-red and vital red were employed in concentrations twenty to forty times greater than that used by Linhard. Keith, Rowntree and Geraghty (1915), and Hooper, Smith, Belt and Whipple (1920) showed a very close agreement between plasma volume as obtained by the dye method *in vitro* and the actual plasma volume measurement. Rowntree, Brown and Roth (1929) conclude that the discrepancy in results as reported by Linhard might be traced to the dye he used. They raise the question as to whether or not different lots of dye vary in their adsorptive properties. Rowntree et al (1929) failed to recover Congo-red or vital red from cells after repeated washing of samples used for *in vitro* studies. They claim the loss in this manner is entirely negligible.

Linhard (1926) suggested that there might be considerable difference between the corpuscles of various species in their adsorptive ability. From his data it would appear that the corpuscles of the bovine exhibit a most extraordinary adsorptive power in this respect.

From a repetition of Linhard's experiments on bovine blood and the close agreement of *in vitro* methods, it was shown that there is no sig-

nificant adsorption of the vital red or the Congo-red employed in this laboratory.

The evidence for these statements is pointed out in the following experiments:

Experiment I.—Blood samples were drawn from a cow five minutes after the injection of the usual amount of Congo-red (3 mg. per kg. of body weight) for blood volume determinations. Samples drawn before injection of the dye were used as controls in this procedure.

(a) Two tubes of 20 c. c. of blood each were centrifuged, the plasma pipetted off and the cells washed with 75-100 c. c. of physiologic saline solution.

(b) The cells were hemolyzed with distilled water, and extracted with a small quantity of ether. The insoluble stroma was thrown down by further centrifuging.

(c) The stroma or residuum was washed several times with distilled water. Color was not present and no visible difference in color was apparent between stroma of the dyed and the un-dyed samples. The addition of a few drops of 1 N. hydrochloric acid failed to show a blue color, which would mark the presence of Congo-red.

Experiment II.—(a) Two tubes of blood before and after the injection of vital red were obtained in a normal blood volume determination on four cows. The usual amount of dye was employed in each instance.

(b) All of the tubes were centrifuged, the plasma pipetted off and the cells hemolyzed with distilled water and ether.

(c) The insoluble stroma was thrown down by centrifuging and washed from three to five times with distilled water.

(d) A color difference was not noted between stroma after injection of vital red and the controls taken before administration of the dye.

Experiment III.—Eight samples of blood, of 10 c. c. each, were procured.

(a) One cubic centimeter of 1.5 per cent Congo-red was added to each of three tubes, and to four of the remaining tubes 1 c. c. of 1.5 per cent vital red was added. One tube was kept free of dye as a control.

(b) The solution of blood and dye was well mixed, centrifuged, the plasma removed, and the cells hemolyzed.

(c) There were slight traces of color in all of the tubes to which both Congo-red and vital red had been added. Repeated washings failed to remove all of this color and a few drops of normal hydrochloric acid added to the tubes containing Congo-red gave a slightly dark color to the stroma indicating the presence of slight amounts of Congo-red. This concentration of dye was more than eighty times that employed by Linhard.

Experiment IV.—(a) The procedure of Experiment III was followed, using four tubes of 10 c. c. of blood each. To each was added 5 c. c. of 1.5 per cent Congo-red solution. To four additional tubes of 10 c. c. of blood each, 0.5 c. c. of 1.5 per cent vital red was added and the tubes similarly treated.

(b) On hemolyzing the blood, adding ether and centrifuging, color could not be extracted after six washings with distilled water. The stroma was not colored in any of the tubes. The addition of normal hydrochloric acid to the tubes to which Congo-red had been added failed to give a blue color. In this experiment 7.5 mg. was used in 10 c. c. of blood. More than forty times the amount employed by Linhard, who used 0.3 c. c. of 1 per cent dye in 200 c. c. of ox-blood.

These data would indicate a lack of adsorption of the dye by the stroma of the red corpuscles of the bovine when normal amounts of dye are used. Examination of the stroma in samples obtained from different cows in making normal blood volume determinations has further shown this to be the case.

In Vitro Determinations.—A close agreement between the use of the dye method *in vitro* and actual measurement of the plasma volume has been repeated by Keith, Rowntree and Geraghty (1915) and Hooper, Smith, Belt and Whipple (1920). Their experiments were conducted on oxalated dog's blood. Linhard (1926) reported considerable error due to the dye being adsorbed by the cells, which gave an increased plasma volume by the dye method *in vitro*. In view of these reports, the following experiments have been repeated, using bovine blood. These data tend to show a fair degree of accuracy for the dye method as evidenced by the following protocols.

Experiment I. Blood volume in vitro.—A total of 867 c. c. of blood were drawn from the jugular vein of a normal calf just before slaughter. The blood was collected in a clean flask containing 150 c. c. of 1.6 per cent sodium oxalate. This blood was thoroughly mixed. Two and one-half cubic centimeters of 1.5 per cent vital red were added. The contents were poured into a flask and thoroughly mixed by rotation and inversion for about six minutes. At the end of this time 10 c. c. were pipetted into a dry 15 c. c. centrifuge tube, and two hematocrit tubes were also filled. The tube of blood was centrifuged for 30 minutes at 2500 revolutions per minute and at a later whirling the hematocrit tubes were centrifuged for 45 minutes at 2800 revolutions per minute. The hematocrit value of eight tubes was found to average 40 per cent cells. Two cubic centimeters of dye-tinged supernatant fluid were mixed with 4 c. c. of 0.9 per cent NaCl and read against a standard prepared as follows: 0.75 c. c. of 1.5 per cent vital red was accurately brought up to 200 c. c. with distilled water in a volumetric flask. Five cubic centimeters of this standard dye solution were mixed with 5 c. c. of the clear dye-free oxalated plasma and 5 c. c. of the 0.9 per cent saline solution. Against this standard the above diluted sample of dye-colored plasma was read in a colorimeter. This reading was found to give 105.4 per cent.

With this concentration of dye the number of cubic centimeters of oxalated plasma in the total of 1017 c. c. of blood would be

$$\frac{26666.67 \times 2.5}{105.4} = 632.5 \text{ c. c. determined volume} \quad (1)$$

From the hematocrit it was found that the plasma equals 60 per cent of the total volume in 1017 c. c. Therefore,

$$.60 \times 1017 = 610.2 \text{ c. c. plasma (by hematocrit).}$$

$$632.5 \text{ c. c.} - 610.2 \text{ c. c.} = 22.3 \text{ c. c. difference, or } 3.65 \text{ per cent error.}$$

Experiments 2-6 Inclusive. Blood volume in vitro.—Following the procedure as outlined in Experiment I, *in vitro* determinations were made on a number of samples of blood. These samples were all drawn from the jugular vein and except in two cases the wet oxalate technique was employed. Dry oxalate was used as the anticoagulant in two determinations. The amounts of dye used varied for different samples in which Congo-red and vital red were compared. The tests were carried out immediately after drawing the blood. The results are shown in Table 4.

TABLE 4.—BLOOD VOLUME IN VITRO

Sample No.	Blood Volume	Dye Used (1.5% sol.)	Hematocrit	Plasma Volume		
				Calculated	Determined	Error on Determination
	<i>c. c.</i>	<i>c. c.</i>	<i>per cent</i>	<i>c. c.</i>	<i>c. c.</i>	<i>per cent</i>
1	1017	2.5 vital	40.0	610.2	632.5	3.65
2	500	1.2 Congo	42.5	287.5	291.8	1.50
3	200	0.2 Congo	42.4	115.2	123.6	7.40
4	200	0.2 Congo	44.2	111.6	125.0	11.90
5	390	0.4 vital	46.1	215.6	209.6	-3.0
6	390	0.4 Congo	44.8	215.2	203.1	-5.6

No systematic error in these *in vitro* experiments was encountered. Colorimetric readings were found to agree more closely when vital red was used.

The Concentration Curve of the Dye Following Injection.—One of the most perplexing problems connected with the dye injection method is the determination of the optimal time, following injection of the dye, that samples for colorimetric comparison should be drawn. Briefly, two factors are concerned: (a) there must be sufficient time following injection for thorough mixing of the dye with the blood, and (b) the sample must be drawn before there is an appreciable loss of dye from the blood stream.

Erlanger (1921) presented nine mixing curves constructed from the data obtained by various workers. These data include values obtained by the use of several indirect methods. The concentration curves showed considerable variation during the first ten minutes following injection. Harris (1920) advocates that samples be drawn two and one-half minutes after injection in the human. He also concluded that mixture was complete in the dog 80 seconds after injection. Dawson, Evans and Whipple (1920) and Hooper, Smith, Belt and Whipple (1920) favor a mixing period of two to four minutes and claim that six to eleven per cent of the dye is eliminated from the circulation within 20 minutes after injection. They also concluded that four to five per cent of the dye is eliminated from the fourth to the tenth minute after injection. Rowntree, Brown and Roth (1929) advocate the collection of samples from three to six minutes after injection in the case of the human. They determined the concentration of the dye from three to six minutes following injection in 46 subjects, including 20 normal persons, 17 patients with polycythemia vera and nine patients with various types of anemia. The results were recorded in percentage variation from the concentration of the dye after two minutes, which arbitrarily was taken as 100 per cent. There was practically no difference in the readings after three, four and six minutes. Smith (1925) using brilliant vital red in determinations on dogs concluded that about 12 per cent of the dye left the circulation the first hour and possibly four per cent between the fourth and tenth minute after injection.

The actual time necessary for the dye and blood to become thoroughly mixed has never been fully determined. A comparison of the dye concentration at varying intervals after injection gives an insight into the time required for mixing and the rate of disappearance. To become well mixed with the entire mass of blood the injected dye must traverse even the slowest parts of the circulation at least once. When mixing is complete samples from any part of the body should show approximately the same dye concentration.

Concentration of the Dye at Various Intervals Following Injection in the Dairy Cow.—A study of the dye concentration from four to ten minutes following injection has been made in the dairy cow. The mature cow has a much lower heart rate than the human, or the small laboratory animal, upon which blood volume determinations are usually made. The speed of circulation is considerably slower than that of the human or the dog; therefore, it would seem that a slightly longer time is necessary for thorough mixing of the dye in the blood of the bovine. As described earlier in this paper, the circulation time in the mature bovine has been found to be about 52 seconds. A comparison of the resultant blood volumes as determined from samples drawn at four, six, and eight minutes after injection of the dye has been made.

The dye concentration was arbitrarily taken to be 100 per cent four minutes after injection and the blood volume as determined for the six and eight minute samples was expressed in percentages of the four minute

TABLE 5.—DYE CONCENTRATION IN BLOOD DRAWN AT VARYING INTERVALS AFTER INJECTION
(Expressed in percentage variation of blood volume)

Herd No.	Mixing Period of Dye in Blood						
	4 min.	6 min.	Per cent	d	8 min.	Per cent	d
	<i>c. c.</i>	<i>c. c.</i>			<i>c. c.</i>		
569	45,600	45,950	100.76	+0.76	46,900	102.85	+2.85
569	39,300	35,900	91.34	-8.66	35,600	90.58	-9.42
517	28,400	30,900	108.80	+8.80	27,300	96.12	-3.88
517	22,100	23,400	105.88	+5.88	24,750	111.99	+11.99
517	25,800	25,800	100.00	0.00	25,400	98.44	-1.56
515	27,300	28,000	102.56	+2.56	29,000	106.22	+6.22
426B	10,550	11,800	111.84	+11.84	11,700	110.00	+10.00
826	7,000	7,350	105.00	+5.00	7,000	100.00	0.00
127B	11,940	11,160	93.46	-6.54	11,260	94.30	-6.70
127B	10,900	11,400	104.58	+4.58	11,650	106.88	+6.88
826	6,100	6,260	102.62	+2.62	6,440	105.57	+5.57
826	8,090	8,340	103.09	+3.09	8,275	102.20	+2.20
127B	11,400	12,150	106.57	+6.57	12,150	106.57	+6.57
127B	12,150	13,500	111.11	+11.11	13,220	108.80	+8.80
811	13,700	13,300	97.08	-2.92	13,100	95.62	-4.38
811	14,900	15,700	105.36	+5.36	16,700	112.08	+12.08
811	11,700	11,200	95.72	-4.28	12,500	106.84	+6.84
811	14,800	15,500	104.72	+4.72	16,200	109.45	+9.45
811	15,600	15,600	100.00	0.00	16,600	106.41	+6.41
Mean calculated blood volume in per cent*			102.6	±0.80		103.6	±0.91
Standard deviation of distribution			5.2			5.9	

*The concentration of the dye for the four-minute period is arbitrarily taken as 100 per cent.

sample. In 19 determinations thus compared it was found that the blood volume determined from the average sample drawn at six minutes after injection was 102.6 ± 0.8 per cent of the average four minute sample. The average sample drawn eight minutes after injection was found to be $103.6 \pm .91$ per cent of the four minute sample (Table 5). These variations cannot be considered statistically significant.

These values are expressed in terms of total blood volume and not on the comparison of the dye concentration alone. Besides showing a fair agreement between dye concentration in the samples drawn four, six and eight minutes after injection these data indicate a reasonable degree of accuracy in duplicate determinations. A comparison of 31 samples drawn at six and eight minute intervals after injection showed the dye concentration to be slightly less in the eight minute sample. The blood volume, determined by the eight minute sample, was 102.9 ± 0.9 per cent of the total volume as determined from the six minute sample. From these studies, it would seem that four to eight minutes would be the optimal time after injection of the dye for drawing the samples in the bovine. The general plan has been to draw samples at four and six minutes after injection of the dye and average the two results. If only one sample was drawn, six minutes after injection of the dye was chosen as the optimal time.

Comparison of the Samples Drawn from Different Veins.—Rowntree, Brown and Roth (1929) show a close agreement in the blood and plasma volume as determined by simultaneous samples drawn from the right and left arms. Linhard (1926) claimed that exercise was necessary to obtain proper distribution of the dye throughout the body. Rowntree et al (1929) pointed out that Linhard drew samples from the superficial vessels of the legs and that vein-puncture in these regions is often impossible within the prescribed time. This they believed was sufficient to vitiate his results. Thompson, Alper and Thompson (1928) by means of vital red, studied the effect of posture upon the velocity of blood flow in man and found that a longer time was required for blood to move from a vein in the arm to a vein in the foot, or the reverse in the standing position than in the recumbent position.

Blood volume determinations on dairy cattle reported in this paper have been made with the animals standing in the stall in the normal manner. The determinations were made at approximately the same time each day on various animals. The body weight was secured at the time of the determination of the blood volume.

A comparison of samples drawn as simultaneously as practicable shows that there is an even distribution of dye throughout the circulatory system of the cow at the normal time (four to eight minutes) of drawing

samples. The evidence for this statement is shown in Table 6. The subcutaneous abdominal vein was the point of injection in all of these determinations. Usually four to six minutes had elapsed before the samples were drawn.

TABLE 6.—COMPARISON OF BLOOD AND PLASMA VOLUME AS DETERMINED FROM SAMPLES SIMULTANEOUSLY DRAWN FROM THE JUGULAR AND MAMMARY VEINS

Herd No.	Volume			
	Blood		Plasma	
	Jugular	Mammary	Jugular	Mammary
	<i>c. c.</i>	<i>c. c.</i>	<i>c. c.</i>	<i>c. c.</i>
290H	51,000	51,900	32,600	33,100
290H	46,500	46,500	29,000	28,800
515H	47,117	48,179	29,559	30,225
515H	53,800	55,400	32,600	33,600
509H	51,400	53,600	31,300	32,600
508H	47,100	46,600	31,400	31,050
510H	42,300	41,300	25,000	24,400

Choice of Dye for Dairy Cattle.—From the earlier discussion of dyes suitable for blood volume work it has been pointed out that vital red, brilliant vital red and Congo-red are commonly used. In determination of the blood volume of the bovine both vital red and Congo-red were used in this investigation. Vital red was found the more satisfactory. Close colorimetric readings are possible. There is a ready matching of colors and no interference due to the color of the plasma. The dye was made up in a 1.5 per cent solution, using freshly distilled water. Vital red (HR Ehrlich) was employed (National Aniline and Chemical Company).

Congo-red (the sodium salt of benzidindiazobinaphthylamin-sulphonic acid) has been used to a moderate extent in blood volume determination in dairy cattle. In general it has not given as accurate colorimeter readings as vital red. Linhard (1926) experienced so much difficulty with Congo-red that he declared it of no value in blood volume studies. The straw colored plasma of the mature bovine seems to interfere with proper color matching when Congo-red is used. In young animals, where the plasma is normally fairly light in color, Congo-red gives values seemingly as satisfactory and agreeing as closely as those obtained when vital red is used. Congo-red was normally used in 1.5 per cent solution.

In using these red dyes it is important that precaution be taken to prevent hemolysis. Even slight hemolysis renders accurate colorimetry impossible.

All dye solutions were autoclaved at least 30 minutes and allowed to cool before injection. If a new dye solution was not made up every few days the solutions on hand were always autoclaved again before being used. The flasks were kept stoppered with gauze wrapped cotton plugs and aseptic precautions observed.

The Technique of Dye Injection.—The amount of dye injected must be sufficient to give the plasma a color deep enough for colorimetric comparison. After several trials using various amounts of dye it was found that approximately three mg. of dye per kilogram of body weight gave the most satisfactory results. The use of larger amounts showed no advantage. In general this amount agrees quite closely with that used for laboratory animals (Smith 1925). For large mature cows the usual plan was to inject 100 c. c. of 1.5 per cent dye. This amount closely approximates three mg. per kilogram for a cow weighing 1200 to 1300 pounds.

The dye was injected from a calibrated Luer syringe. In general a 100 c. c. syringe fitted with a reducing cap to accommodate a 16-gauge needle was found to be very satisfactory. Syringes of 20 to 50 c. c. capacity were used for the smaller animals.

Dye injection can best be accomplished when the subcutaneous abdominal vein is used. When mature cows are used the injection of dye is easily accomplished. Usually there is little struggle and all of the dye can be injected without leakage. It is extremely difficult to inject 100 c. c. of dye into the jugular vein without some struggles and often a failure results due to the needle being thrown out of the vein. When the jugular vein was employed it was necessary to use considerable care in securing the animal's head, or unsuccessful injection resulted. In this work the jugular vein was employed as the point of injection for blood volume determinations on young dairy cattle.

The Fate of the Dye in the Animal Body.—The dye injected intravenously is slowly eliminated from the circulation. Smith (1925) using brilliant vital red in studies on dogs showed a gradual disappearance of the dye from the blood stream. Using various amounts of dye, he showed that the speed of elimination was quite rapid the first few hours after injection. After six hours it was shown that the rate of elimination was considerably decreased. The rate of elimination slows down, according to Smith, when the phagocytes reach equilibrium with the dye-tinged plasma. Sometimes the plasma remains colored for days after the dye is injected.

The rate of elimination of the dye was studied in two dairy cows, and the concentration of dye present at varying intervals after injection

is shown in Fig. 7. These data show that in dairy cattle the rate of elimination follows a course similar to that of the dog.

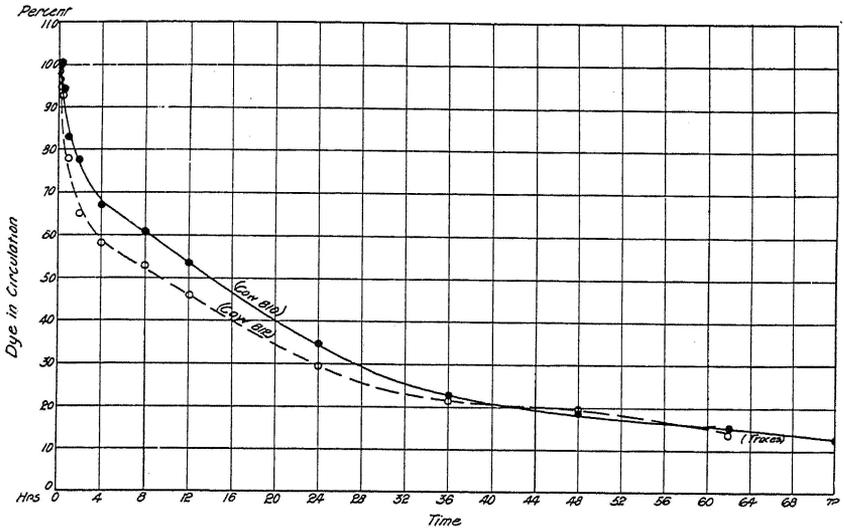


Fig. 7.—The rate of elimination of intravenously injected vital red.

Taking it for granted that concentration of the dye was 100 per cent four minutes after injection the colorimeter readings have been expressed in terms of the actual per cent of dye present at various intervals (Fig. 7). In cow No. 810, a Jersey weighing 970 pounds, 175 c. c. of 1.5 per cent vital red were injected. In cow No. 812, also a Jersey weighing 895 pounds, 100 c. c. of 1.5 per cent vital red were injected. Despite the difference in amounts of dye injected it is shown that the rate of disappearance of the dye is quite similar for both animals. Very little loss of dye is apparent the first few minutes following injection. Two to four per cent of the dye disappeared in the first 12 minutes after injection. In one hour after injection about 18-20 per cent of the dye is eliminated from the blood stream.

The eliminated dye does not appear in the urine or feces of the dairy cow. Smith found that about 25 per cent of the dye appeared in the bile of the dog within 24 hours and that it did not appear in the urine. In the dog, Smith found that much of the dye, on leaving the blood stream, is stored in the tissues, particularly in the reticulo-endothelial cells of the liver, spleen, and lymph-nodes. The dye is slowly eliminated by the liver but not by the kidneys. Before being eliminated from the

body much of the dye is taken up and stored by the phagocytes. The phagocytes do not completely remove the dye from the plasma because the phagocytic reaction is a matter of equilibrium between the phagocytes and the dye-colored plasma. It is also found that dye passes into the lymph, which indicates that some lymph might be included in the blood volume as determined by the dye method. Smith says, "This phenomenon doubtlessly explains in part the fact that the dye method gives figures which are larger than the carbon monoxide or direct method of Welcher." The amount of lymph, if any, included in blood volume determinations on the dairy cow would be very small since only about three per cent of the dye leaves the blood stream six to twelve minutes after injection.

PRESENTATION OF DATA

Blood and Plasma Volume of Dairy Cattle

The blood and plasma volume of dairy cattle, as determined by the dye injection method, in approximately 120 cases are presented herewith. These data are based on a study of 54 growing animals and 65 cows of approximately mature age. There are included animals of the Holstein, Jersey, Ayrshire and Guernsey breeds. The ages vary from one week to maturity. The weight ranges from approximately 82 to 1600 pounds.

Dairy cattle of all ages and particularly lactating and non-lactating cows are subject to considerable variation in weight and fullness of the body. For convenience of study the animals have been grouped as follows: (a) growing dairy cattle from 2 to 127 weeks of age, (b) non-lactating, approximately mature cows, and (c) lactating cows of approximately mature age. The normal blood and plasma values for each group are given in Tables 7 and 8.

Due to the differences in the average blood volume of the three groups, each will be discussed separately.

Blood Volume of Growing and Mature Dairy Cattle.—The blood volume of growing dairy cattle from two weeks to approximately two years of age has been studied in 54 determinations (Table 7). The animals comprising this group are largely of the Holstein and Jersey breeds. The body weight ranged from 82 to 895 pounds, with an average of 500 pounds. There is considerable variation in the values obtained for animals in this group. When the blood is expressed in terms of body weight an average of 6.25 per cent was obtained. This is somewhat lower than that found for mature cows.

The ratio of blood weight to body weight in the extremely young dairy animal is very high. In three determinations on animals under

seven weeks of age (Herd No. 427G and 572H) the blood was found to constitute from 11.9 to 15.7 per cent of the body weight. The data are too limited to draw any definite conclusions. It is of much interest, however, to note that blood volume studies in infants have shown much higher values for blood and plasma than is found in the normal adult.

Darrow, Soule and Buckman (1928) studied blood and plasma volume in infants and children up to 11 years of age. They found a rise in plasma volume from 50 to 62 c. c. per kilogram during the first year of life, and a return to 50 c. c. after the fourth year of life. When compared with body surface, the blood volume of the child is less than that of the adult.

Lucas and Dearing (1921) using the dye method, found an average blood volume of 147 c. c. per kilogram for infants from one to two and one-half years of age. These values would give a blood mass of approximately 15.5 per cent of the body weight or nearly twice that reported by Keith, Rowntree and Geraghty (1915) for the adult.

In view of these findings it would appear that the normal dairy calf a few weeks of age has a blood volume proportionately much higher than that of older animals. The values obtained in these preliminary studies of young animals are quite suggestive and show the need of further study. When expressed in terms of surface area the average blood volume of calves under seven weeks of age is approximately 4,675 c. c. per square meter. This is slightly higher than the average for the entire group (Table 7 and Fig. 13), but is much lower than the average for mature cows (Table 8 and Fig. 15). From 113 to 149 c. c. of blood per kilogram of body weight was obtained. This is much higher than the average for all growing and mature animals. The plasma per cent was found to be about 7.7 per cent of body weight. The cell volume was slightly higher in extremely young animals than in growing or mature animals. Animal No. 572H at six weeks of age was observed to have a cell volume of 55 per cent of the total blood volume. The growing animals as a group (including the three extremely young calves) were found to have a much lower blood volume than the mature animals (Table 7 and 8). It will be noted (Fig. 8) that the blood volume of the mature dairy animal constitutes a much higher percentage of the body weight than was found in growing animals. In animals from 200 to 900 pounds of weight the blood mass was found to be about 5.81 per cent of the body weight, in the non-lactating cow this was observed to be 6.38 per cent, and in the lactating cow an average of 8.11 per cent was obtained (Fig. 9).

Rowntree, Brown and Roth (1929) found a higher degree of correlation between blood volume and surface area than between blood vol-

TABLE 7.—BLOOD VOLUME AND PLASMA VOLUME IN GROWING DAIRY CATTLE

Date	Herd Number	Age	Weight	Weight	Surface Area	Plasma					Cells by Hematocrit	Blood			
						Total Volume	Per Kilo-gram of Body Weight	Per Square Meter	Plasma Weight	Total Volume		Per Kilo-gram of Body Weight	Per Square Meter	Blood Weight	
									Body Weight						Body Weight
3-6-31	427G	<i>weeks</i> 2	<i>lbs.</i> 82	<i>kg.</i> 37.1	<i>sq. m.</i> 1.136	<i>c. c.</i> 3,290	<i>c. c.</i> 88.46	<i>c. c.</i> 2,985	<i>per cent</i> 9.06	<i>per cent</i> 32.5	<i>c. c.</i> 4,875	<i>c. c.</i> 131.08	<i>c. c.</i> 4,289	<i>per cent</i> 13.75	
3-28-31	427G	5	95	43.0	1.234	2,990	69.38	2,422	7.10	38.8	4,890	113.48	3,962	11.90	
3-28-31	572H	6	135	61.2	1.500	4,090	66.79	2,726	6.84	55.4	9,160	149.59	6,105	15.70	
3-6-31	588H	20	235	106.5	2.049	5,760	54.03	2,810	5.53	39.4	8,520	79.93	4,157	8.40	
2-7-31	511H	15	265	120.2	2.191	5,050	42.01	2,303	4.30	45.7	9,300	77.37	4,242	8.11	
11-15-30	605H	23	310	140.6	2.393	6,710	47.72	2,804	4.89	36.8	10,000	71.12	4,178	7.91	
2-7-31	582H	23	319	144.7	2.432	6,140	42.43	2,524	4.35	43.0	10,780	74.49	4,432	7.80	
7-10-30	825J	53	323	146.5	2.449	3,960	27.03	1,616	2.77	40.2	6,326	43.18	2,583	4.56	
7-14-30	825J	54	327	148.3	2.466	4,446	29.97	1,802	3.07	38.8	7,270	49.02	2,948	5.23	
7-17-30	825J	54	331	150.1	2.482	5,030	33.51	2,026	3.43	36.9	7,980	53.16	3,215	5.56	
7-10-30	826J	47	344	156.0	2.537	4,690	30.06	1,848	3.08	34.8	6,650	42.62	2,621	4.47	
7-3-30	826J	54	344	156.0	2.537	4,150	26.60	1,635	2.72	33.7	6,266	40.16	2,469	4.21	
7-14-30	826J	47	344	156.0	2.537	4,986	31.96	1,965	3.27	29.7	7,116	45.61	2,804	4.78	
7-7-30	826J	46	344	156.0	2.537	5,480	35.12	2,160	3.60	33.2	8,215	52.66	3,238	5.51	
12-13-30	340A	36	350	158.8	2.562	6,560	41.30	2,560	4.23	48.6	12,800	80.60	4,996	8.49	
10-5-30	426G	37	351	159.2	2.566	7,040	44.22	2,743	4.53	34.9	10,800	67.83	4,208	7.12	
12-6-30	828J	47	363	164.7	2.615	6,330	38.43	2,420	3.94	38.4	10,300	62.53	3,938	6.55	
12-13-30	351A	39	390	176.9	2.722	7,855	44.40	2,885	4.55	37.0	12,350	70.37	4,573	7.27	
10-11-30	426G	38	392	177.8	2.729	7,620	42.85	2,792	4.39	35.2	11,750	66.08	4,305	6.92	
12-6-30	831J	41	393	178.3	2.734	6,060	33.98	2,216	3.48	36.6	9,565	53.64	3,498	5.61	
11-1-30	426G	40	401	181.9	2.764	6,980	38.37	2,525	3.93	37.5	11,175	61.43	4,043	6.45	
11-15-30	603H	28	405	183.7	2.780	6,975	37.96	2,508	3.89	34.5	10,625	57.83	3,821	6.07	
10-7-30	825J	64	424	192.3	2.852	7,455	38.76	2,613	3.97	36.0	16,625	86.45	5,829	6.36	
10-11-30	826J	63	425	192.8	2.856	8,070	41.85	2,825	4.29	33.7	12,150	63.01	4,254	6.61	
12-6-30	829J	45	449	203.7	2.945	7,035	34.53	2,388	3.54	42.3	12,175	59.76	4,134	6.27	
11-15-30	600H	42	449	203.7	2.945	7,755	38.07	2,633	3.90	32.1	11,400	55.96	3,870	5.87	
11-15-30	602H	32	457	207.3	2.974	6,600	31.83	2,219	3.26	36.7	10,475	50.53	3,522	5.29	
11-8-30	826J	66	463	210.0	2.996	7,715	36.73	2,575	3.76	37.1	12,270	58.42	4,095	6.12	
11-15-30	597H	47	464	210.5	3.000	7,665	36.41	2,555	3.73	37.7	12,300	58.43	4,100	6.13	
10-5-30	575H	37	471	213.6	3.025	9,440	44.19	3,120	4.53	35.1	15,650	73.26	5,173	6.70	
12-6-30	602H	35	495	224.5	3.110	6,820	30.37	2,192	3.11	40.3	11,400	50.77	3,665	5.34	
6-19-30	127J	59	540	244.9	3.265	7,140	29.15	2,184	2.99	40.0	11,316	46.20	3,465	4.80	
7-3-30	127J	61	545	247.2	3.282	7,285	29.47	2,219	3.02	31.0	10,548	42.66	3,213	4.48	
7-1-30	127J	61	545	247.2	3.282	7,415	29.99	2,259	3.07	35.3	11,453	46.33	3,489	4.87	
7-10-30	127J	62	555	251.7	3.316	8,033	31.91	2,422	3.27	32.8	11,900	47.27	3,588	4.95	
7-14-30	127J	64	565	256.3	3.350	8,740	34.10	2,608	3.49	35.3	13,500	52.67	4,029	5.51	
10-5-30	325A	47	600	272.2	3.464	8,025	29.48	2,316	3.02	41.8	13,800	50.69	3,983	5.32	

12-13-30	596H	56	611	277.1	3.499	10,320	37.24	2,949	3.81	44.0	18,400	66.40	5,258	7.00
10-11-30	820J	96	632	286.7	3.566	7,275	25.37	2,040	2.60	42.2	12,600	43.94	3,533	4.60
10-11-30	127J	74	682	309.3	3.721	14,750	47.68	3,963	4.88	36.8	21,770	70.38	5,850	7.37
10-25-30	127J	76	695	315.2	3.761	8,600	27.28	2,286	2.79	35.6	13,360	42.38	3,552	4.44
12-13-30	127J	82	737	334.3	3.887	12,240	36.62	3,148	3.75	37.9	19,700	58.92	5,068	6.21
11-1-30	593H	64	754	342.0	3.937	11,830	34.59	3,004	3.54	36.5	18,600	54.38	4,724	5.70
6-19-30	811J	39	759	344.3	3.951	8,215	23.86	2,079	2.44	38.2	13,285	38.58	3,362	4.05
7-1-30	811J	127	760	344.7	3.954	8,540	24.77	2,159	2.54	36.2	13,367	38.77	3,380	4.07
7-3-30	811J	128	763	346.1	3.963	9,645	27.86	2,433	2.85	38.8	15,767	45.55	3,978	4.78
7-7-30	811J	129	763	346.1	3.963	7,168	20.71	1,808	2.12	39.2	11,800	34.09	2,977	3.75
7-10-30	811J	130	763	346.1	3.963	8,290	23.95	2,091	2.45	44.5	15,300	44.20	3,860	4.70
7-14-30	811J	130	763	346.1	3.963	9,200	26.58	2,321	2.72	42.9	16,100	46.51	4,062	4.88
11-1-30	592H	73	767	347.9	3.975	11,020	31.67	2,772	3.24	38.8	18,000	51.73	4,528	5.42
11-8-30	820J	101	771	349.7	3.987	8,995	25.72	2,256	2.63	39.6	14,850	42.46	2,724	5.42
12-13-30	821J	98	808	366.5	4.092	11,400	31.10	2,785	3.19	42.0	19,650	53.61	4,802	5.66
12-13-30	350A	115	885	401.4	4.306	12,925	32.19	3,001	3.30	57.5	30,400	75.73	7,059	7.98
11-8-30	591H	89	895	406.0	4.334	10,300	25.36	2,376	2.60	42.5	18,000	44.33	4,153	4.64
Average of all animals (54)			501.81	227.6	3.063	7,557	36.40	2,459	3.72	38.5	12,495	59.96	4,035	6.25

TABLE 8.—BLOOD VOLUME AND PLASMA VOLUME IN MATURE COWS
Lactating Cows

Date	Herd Number	Weight	Weight	Surface Area	Plasma				Cells by Hematocrit	Blood			
					Total Volume	Per Kilo-gram of Body Weight	Per Square Meter	Plasma Weight		Total Volume	Per Kilo-gram of Body Weight	Per Square Meter	Blood Weight
								Body Weight					Body Weight
		<i>lbs.</i>	<i>kg.</i>	<i>sq. m.</i>	<i>c. c.</i>	<i>c. c.</i>	<i>c. c.</i>	<i>per cent</i>	<i>per cent</i>	<i>c. c.</i>	<i>c. c.</i>	<i>c. c.</i>	<i>per cent</i>
12-6-30	191J	891	404.2	4.323	16,300	40.32	3,770	4.13	44.1	29,133	72.07	6,739	7.55
1-2-31	163J	940	426.4	4.454	19,825	46.49	4,451	4.76	37.6	31,750	74.46	7,128	7.87
1-2-31	165J	895	406.0	4.334	24,300	59.85	5,606	6.13	36.6	38,400	94.58	8,860	9.94
2-14-31	537H	1,328	602.2	5.415	28,725	47.70	5,303	4.88	33.0	42,950	71.32	7,930	7.46
1-7-31	537H	1,355	615.0	5.468	32,650	53.08	5,971	5.44	35.0	50,300	81.38	9,198	8.61
12-19-30	537H	1,357	615.8	5.472	30,700	49.85	5,610	5.11	31.9	45,125	73.27	8,246	7.72
12-2-30	537H	1,386	628.4	5.535	21,800	34.69	3,938	3.55	36.1	34,100	54.26	6,160	5.68
12-2-30	290H	1,345	610.0	5.443	28,311	46.41	5,201	4.75	36.9	44,950	73.68	8,258	7.72
1-10-31	290H	1,387	628.8	5.535	32,850	52.24	5,934	5.35	36.1	51,450	81.82	9,295	8.61
11-8-30	290H	1,400	635.0	5.567	29,366	46.24	5,275	4.74	37.3	46,833	73.75	8,412	7.72
12-19-30	290H	1,408	638.8	5.586	33,200	51.97	5,943	5.33	33.5	50,000	78.27	8,950	8.26
1-30-31	290H	1,389	629.6	5.541	32,000	50.82	5,775	5.20	41.9	55,125	87.55	9,948	9.17
2-14-31	290H	1,334	605.4	5.417	28,900	47.73	5,335	4.82	38.0	46,675	77.09	8,616	8.07
3-28-31	290H	1,335	606.0	5.422	30,066	49.61	5,545	5.09	40.9	51,933	85.69	9,578	8.82
12-2-30	515H	1,322	599.8	5.391	27,257	45.44	5,056	4.66	38.8	44,200	73.69	8,198	7.81
3-21-31	515H	1,335	606.0	5.422	30,431	50.21	5,612	5.15	40.6	48,507	80.04	8,946	8.39
1-10-31	515H	1,340	608.0	5.433	33,100	54.44	6,092	5.58	39.4	54,600	89.90	10,049	9.46
2-14-31	515H	1,356	615.4	5.470	28,800	46.79	5,265	4.80	37.2	45,900	74.59	8,391	7.83
2-14-31	509H	1,256	569.6	5.242	30,375	53.32	5,794	5.46	33.1	45,475	79.84	8,675	8.36
3-28-31	509H	1,270	576.0	5.271	31,150	54.07	5,909	5.54	34.1	47,375	82.24	8,987	8.60
1-30-31	509H	1,270	576.0	5.271	30,950	53.73	5,871	5.50	38.0	50,025	86.84	9,490	9.09
2-14-31	508H	1,250	567.0	5.225	31,100	54.85	5,952	5.62	35.1	48,000	84.65	9,186	8.86
1-10-31	508H	1,255	569.0	5.235	34,650	60.89	6,618	6.23	40.4	58,100	102.10	11,098	10.76
12-2-30	508H	1,260	572.0	5.250	28,100	49.12	5,352	5.04	39.7	46,600	81.46	8,876	8.60
3-28-31	508H	1,350	612.0	5.453	31,283	51.11	5,736	5.23	33.3	46,966	76.74	8,612	8.04
1-30-31	510H	1,264	574.0	5.261	27,350	47.64	5,198	4.87	39.6	45,400	79.09	8,629	8.29
3-28-31	510H	1,274	578.0	5.282	29,633	51.26	5,610	5.25	39.7	49,266	85.23	9,327	8.92
12-10-30	510H	1,306	592.0	5.353	24,625	41.59	4,600	4.26	40.9	41,625	70.31	7,776	7.41
3-6-31	510H	1,306	592.0	5.353	26,116	44.11	4,875	4.51	32.0	38,450	64.94	7,182	6.80
12-19-30	510H	1,318	599.0	5.387	24,625	41.11	4,571	4.22	36.7	41,625	69.49	7,726	7.41
2-7-31	553H	1,290	585.0	5.318	22,750	38.88	4,277	3.98	43.5	40,300	68.88	6,888	7.22
3-6-31	553H	1,298	590.0	5.342	24,675	41.82	4,619	4.29	35.8	38,400	65.08	7,188	6.84
3-6-31	569H	1,375	624.0	5.513	29,606	47.44	5,370	4.86	35.8	46,150	73.95	8,371	7.76
2-22-31	569H	1,420	644.0	5.611	20,587	31.96	3,669	3.27	43.7	36,512	56.69	6,507	5.94
1-2-31	557H	1,407	637.0	5.577	30,050	47.17	5,388	4.82	41.9	51,650	81.08	9,261	8.53
3-21-31	512H	1,391	630.0	5.543	22,483	35.68	4,056	3.65	40.3	37,733	68.07	6,807	6.25
1-2-31	512H	1,400	635.0	5.567	25,350	39.92	4,553	4.09	38.1	40,950	64.48	7,355	6.79
12-2-30	266H	1,210	549.0	5.132	32,900	59.92	6,410	6.14	39.0	54,000	98.36	10,522	10.30
12-19-30	266H	1,237	560.0	5.189	26,400	47.14	5,087	4.82	35.7	41,100	73.39	7,920	7.71
1-10-31	511H	1,222	553.0	5.153	34,400	62.20	6,675	6.36	32.1	50,575	91.45	9,814	9.62
1-2-31	549H	1,206	547.0	5.121	24,250	44.33	4,735	4.54	40.9	41,025	75.00	8,011	7.90
Average of all animals (41)		1,291	585.64	5.314	28,097	48.125	5,283	4.929	37.66	45,103	77.479	8,467	8.11

TABLE 8 (CONTINUED).—BLOOD VOLUME AND PLASMA VOLUME IN MATURE COWS
Non-lactating Cows

Date	Herd Number	Weight	Weight	Surface Area	Plasma				Cells by Hematocrit	Blood			
					Total Volume	Per Kilo-gram of Body Weight	Per Square Meter	Plasma Weight		Total Volume	Per Kilo-gram of Body Weight	Per Square Meter	Blood Weight
								Body Weight					Body Weight
1-2-31	125 J	<i>lbs.</i> 1,053	<i>kg.</i> 479.0	<i>sq. m.</i> 4.754	<i>c. c.</i> 23,650	<i>c. c.</i> 49.37	<i>c. c.</i> 4,974	<i>per cent</i> 5.07	<i>per cent</i> 37.5	<i>c. c.</i> 37,950	<i>c. c.</i> 79.22	<i>c. c.</i> 7,982	<i>per cent.</i> 8.35
2-7-31	125 J	1,085	492.0	4.826	20,275	41.20	4,201	4.22	43.9	36,150	73.47	7,490	7.69
1-2-31	164 J	895	406.0	4.334	17,575	43.28	4,055	4.43	36.2	27,550	67.85	6,356	7.15
2-7-31	164 J	918	416.4	4.396	15,200	36.50	3,457	3.74	42.6	26,500	63.64	6,028	6.68
1-2-31	199 J	1,056	479.0	4.754	14,100	29.44	2,965	3.02	39.8	23,450	48.95	4,932	5.14
12-17-30	199 J	1,086	492.0	4.826	19,150	38.92	3,968	3.98	37.7	30,700	62.39	6,361	6.57
11-8-30	197 J	869	394.2	4.263	9,500	24.09	2,228	2.47	40.4	15,950	40.46	3,741	4.23
11-8-30	157 J	900	408.2	4.347	11,970	29.32	2,753	3.00	46.3	22,300	54.63	5,129	5.67
12-31-30	424 G	1,200	544.0	5.105	23,200	45.44	4,544	4.37	37.1	36,900	67.83	7,228	7.15
12-31-30	426 G	1,230	558.0	5.179	22,500	40.32	4,344	4.13	40.9	38,050	68.18	7,346	7.18
1-10-31	509 H	1,407	638.2	5.577	31,950	50.06	5,728	5.13	39.0	52,500	82.26	9,413	8.65
12-2-30	509 H	1,440	653.0	5.655	26,350	40.35	4,659	4.13	38.6	43,150	66.07	7,630	6.92
12-19-30	509 H	1,440	653.0	5.655	28,700	43.95	5,075	4.50	34.5	43,850	67.15	7,754	7.07
1-14-31	509 H	1,465	665.0	5.713	29,350	44.13	5,173	4.52	39.5	48,525	72.96	8,493	7.70
12-6-30	510 H	1,385	628.0	5.533	26,333	41.93	4,759	4.29	41.6	45,150	71.89	8,160	7.53
12-19-30	553 H	1,326	601.0	5.398	29,400	48.91	5,446	5.01	32.6	43,600	72.54	8,077	7.64
1-2-31	569 H	1,532	694.0	5.851	23,050	33.21	3,939	3.40	42.6	40,300	58.06	6,887	6.25
1-30-31	569 H	1,598	726.3	6.002	25,700	35.38	4,281	3.63	44.0	45,850	63.12	7,639	6.63
7-14-30	517 H	1,285	583.0	5.307	18,000	30.87	3,391	3.16	35.9	28,100	48.19	5,294	5.05
7-1-30	517 H	1,300	590.0	5.342	16,416	27.82	3,073	2.85	36.0	25,666	43.50	4,804	4.41
7-10-30	517 H	1,310	594.0	5.363	14,833	24.97	2,765	2.56	36.7	23,416	39.42	4,366	4.02
7-3-30	517 H	1,342	608.0	5.433	17,433	28.67	3,208	2.93	37.2	27,800	45.72	5,116	4.78
6-19-30	517 H	1,342	608.0	5.433	17,616	29.97	3,242	2.96	39.0	28,866	47.47	5,113	4.98
12-31-30	341 A	1,272	576.0	5.271	18,200	31.59	3,452	3.23	42.0	31,400	54.51	5,957	5.73
Average of all ani- mals (24)		1,239	561.9	5.180	20,852	37.03	4,148	3.78	39.2	34,319	60.81	6,554	6.38

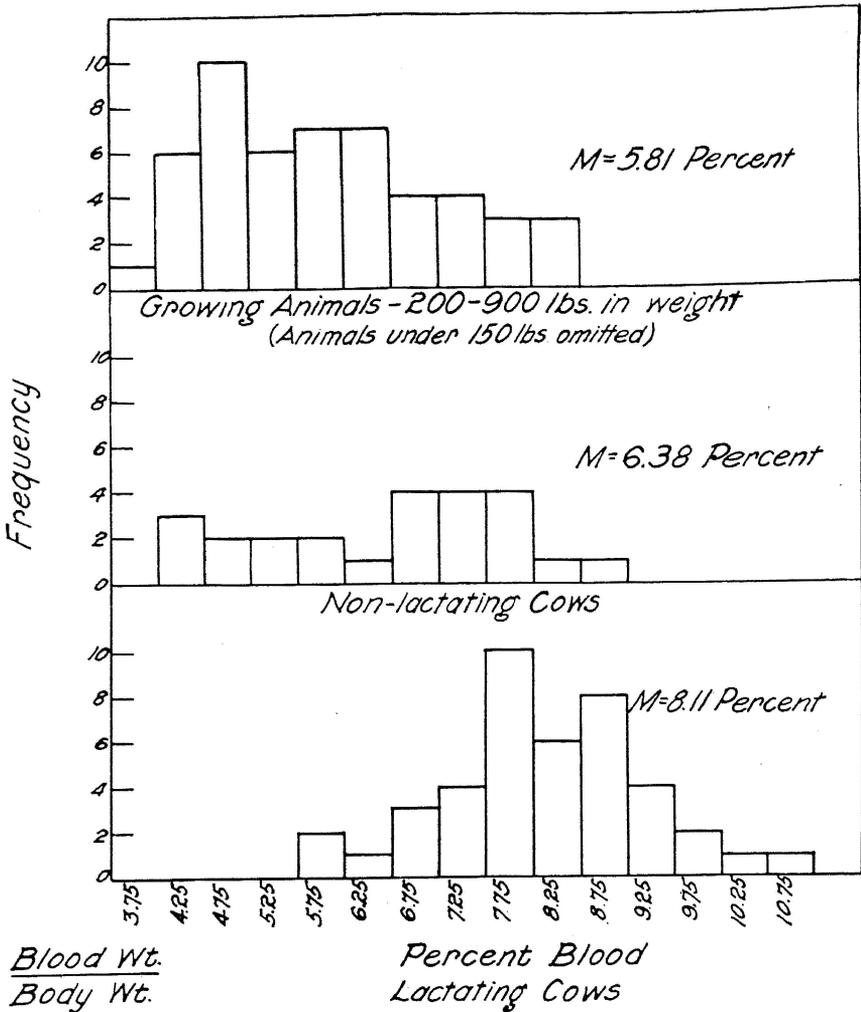


Fig. 8.—A comparison of the blood weight to body weight in dairy cattle.

ume and body weight in the human. There are many ways of expressing blood volume. For the sake of comparison, blood volume has been expressed in relation to surface area as well as to body weight. The surface area of dairy cattle included in this study was computed from the weight by the use of the equation

$$S. A. = 0.15 W^{0.56}$$

derived by Elting and Brody (1926). In this equation S. A. is the surface area in square meters and W. is the weight in kilograms.

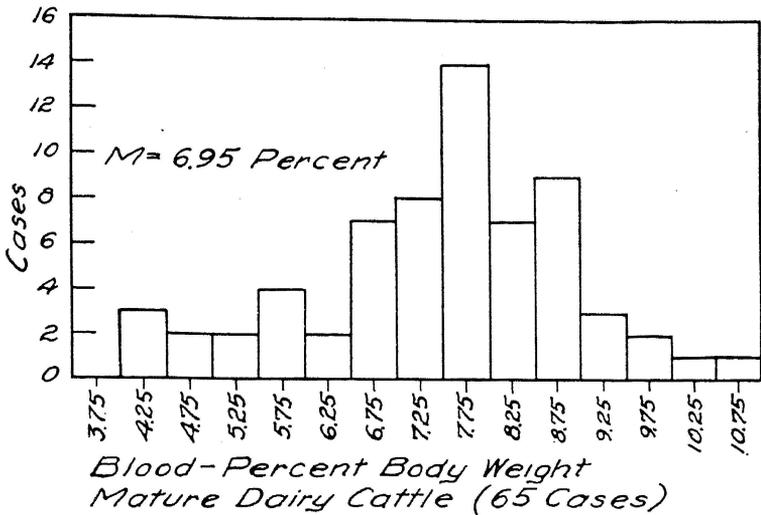


Fig. 9.—The relation between blood weight and body weight of mature dairy cattle. Values for both lactating and non-lactating cows are included.

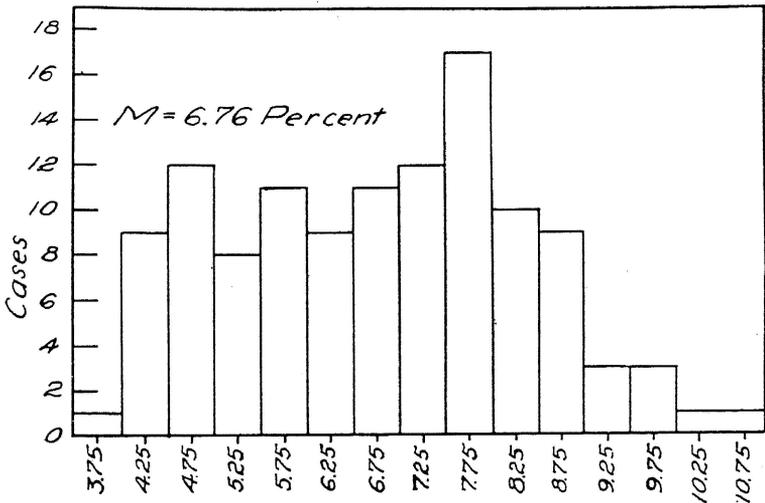


Fig. 10.—The relation of blood weight to body weight of all dairy cattle.

In young dairy cattle an average blood volume of 4,036 c. c. per square meter of body surface was obtained. The average of the mature animals shows approximately 7,768 c. c. per square meter. Growing

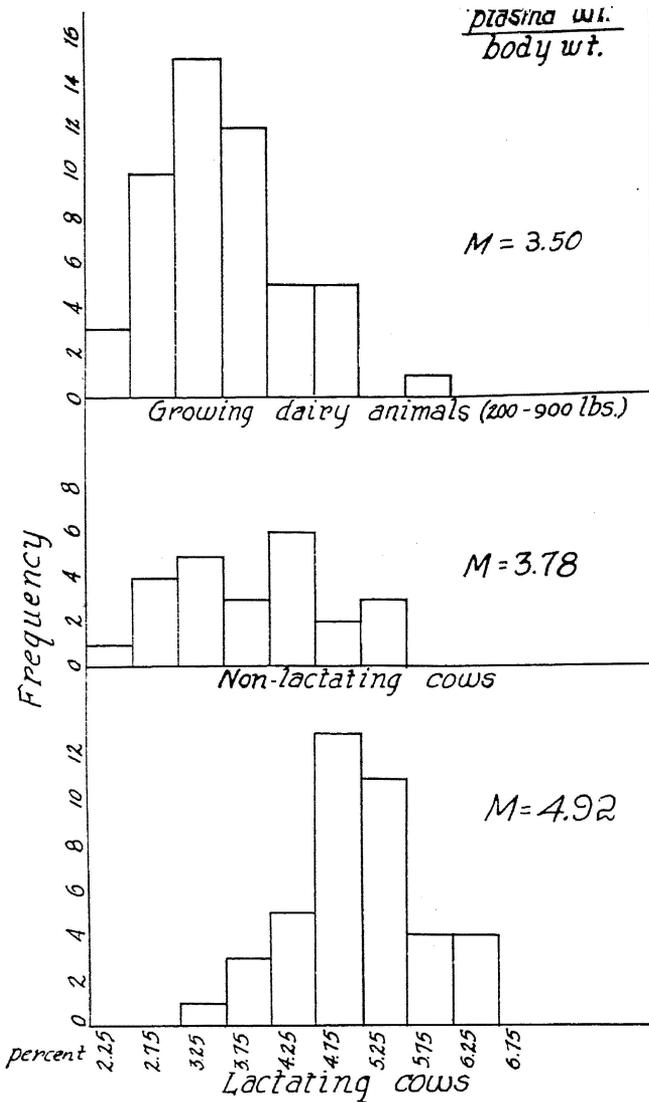


Fig. 11.—The relation of plasma weight to body weight in dairy cattle.

dairy cattle show considerable variation in the amount of blood per square meter. A distribution of the values obtained for young animals is shown in Fig. 13, in which the blood volume per square meter is plotted against the body weight. Similar relationships for mature dairy cows

are shown in Fig. 15. In this group we find the blood volume per square meter to be more uniformly grouped than in the young animals.

A comparison of the blood volume per kilogram of body weight shows an average value of 60 c. c. for the growing animals and 68 c. c. for all mature animals. These relations are shown graphically in Fig. 12 and 14.

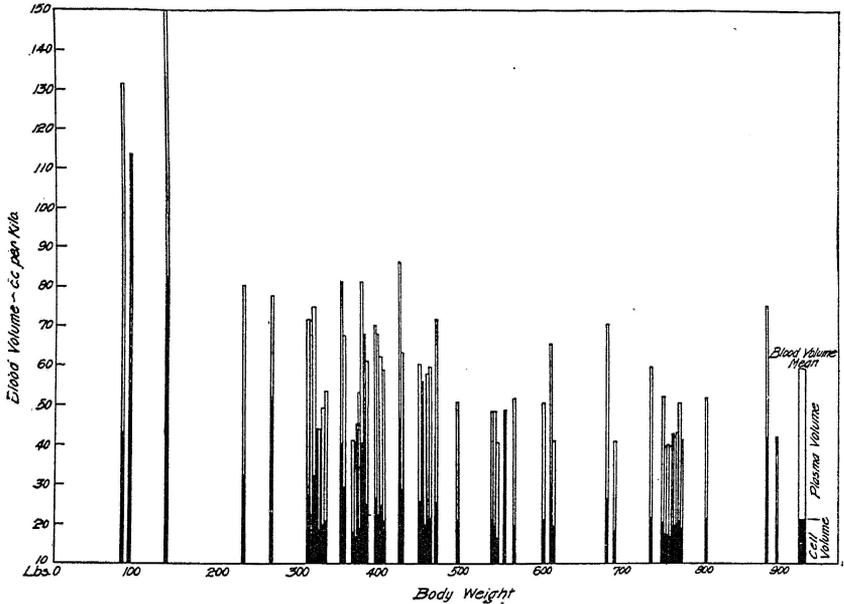


Fig. 12.—The relation between plasma volume, cell volume, and blood volume per kilogram in growing dairy cattle.

The plasma per cent of the blood of growing and mature animals shows no significant difference (Tables 7 and 8). The plasma expressed in terms of body weight, however, shows that mature cows with their larger blood volume have a considerably larger plasma volume also (Fig. 11). The increase in blood volume in mature animals is not due to an increased per cent of plasma but an *actual increase in the entire blood mass*.

The Blood Volume of Lactating and Non-Lactating Cows.—A study of the blood volume of the lactating as compared with the non-lactating cow is of particular interest in milk secretion studies. In Table 8 the data obtained in 41 blood volume determinations on lactating and 24 non-lactating cows are presented.

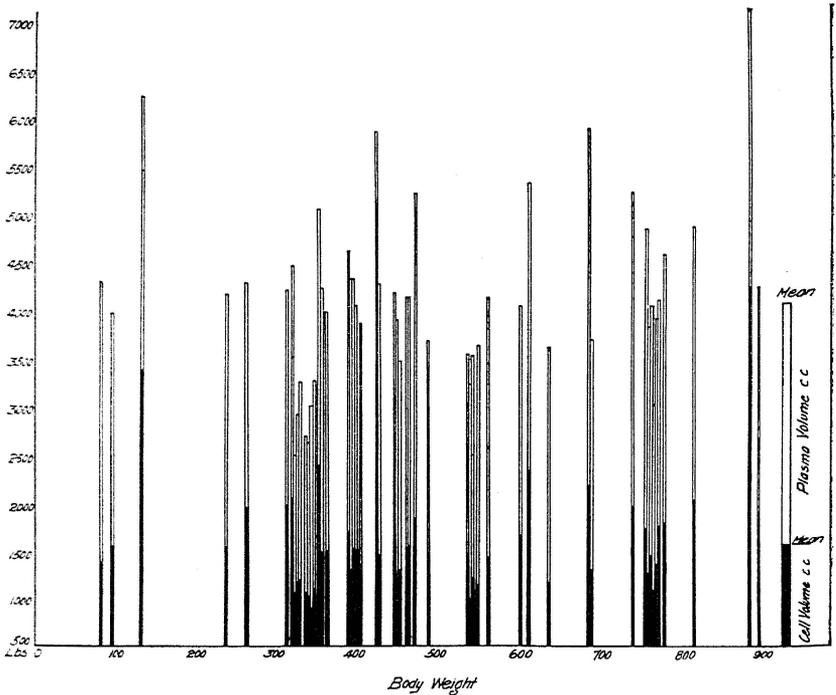


Fig. 13.—The relation between cell volume, plasma volume and blood volume per square meter in growing dairy cattle.

In terms of body weight the blood mass was found to constitute about 8.11 per cent of the total weight of lactating cows. In the dry cows this average was observed to be only 6.38 per cent. This difference can be partly explained as due to the gain in weight of the non-lactating cows. Cows nearing the end of lactation tend to lay on fat which is practically avascular tissue. The extremely poor distribution of data for dry cows (Fig. 8) shows the need of further study before significant conclusions concerning these differences in blood mass can be made.

The dry cows (Table 8) are shown to have an average of 6,554 c. c. of blood per square meter of body surface as compared to 8,467 c. c. per square meter in the lactating cows. The mature cows as a group (Fig. 15) show a much more uniform relation between blood volume and surface area than the growing animals (Fig. 13). This is true even of the limited data on non-lactating cows. The average blood volume per kilogram of body weight for non-lactating and lactating cows is shown in Table 8. The lactating cows show an average increase of 17 c. c. of blood per kilogram as compared to the non-lactating cows.

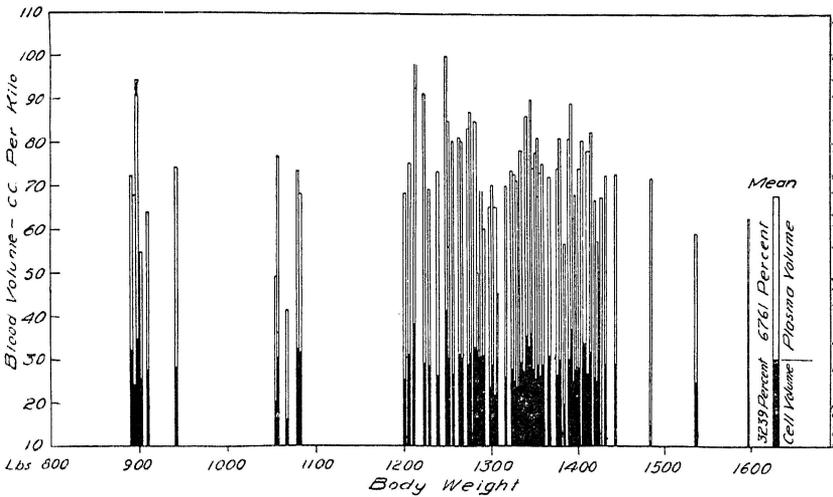


Fig. 14.—The relation between plasma volume, cell volume, and blood volume per kilogram in mature dairy cattle.

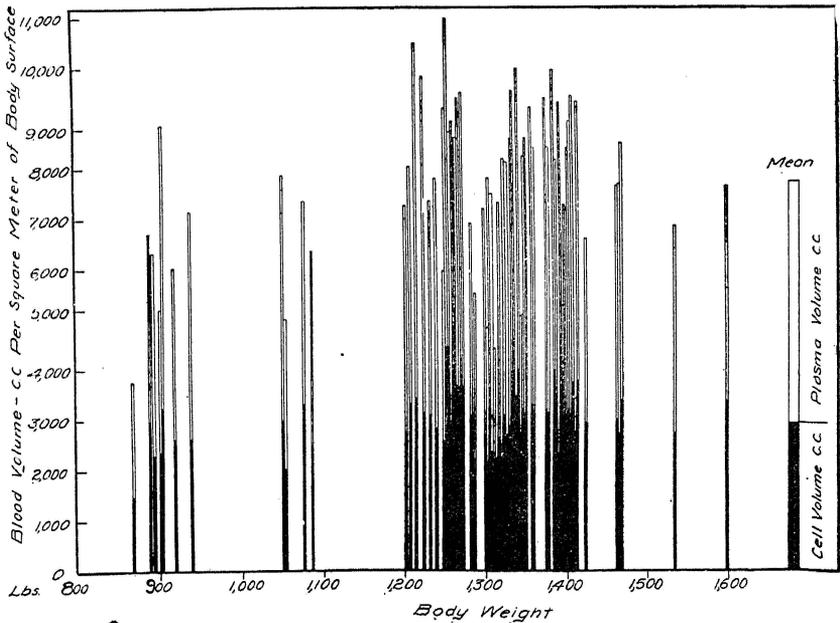


Fig. 15.—The relation between plasma volume, cell volume, and blood volume per square meter of surface in mature dairy cattle.

The dry cows were found to have a plasma percentage of 60.8 as compared to 61.5 in the lactating cows. This point is of interest in view of the fact that certain investigators (Keith, Rowntree and Geraghty 1915) found an increase in the plasma volume during pregnancy. However, Schoenholz (1929) using the carbon monoxide method failed to find a marked increase in the blood volume of pregnant women. Practically all of the dry cows were pregnant at the time blood volume determinations were made. Many of the lactating cows were also pregnant. As a consequence the influence of pregnancy on the blood volume of the dairy cow has not been separately determined.

With an increased blood volume in the lactating dairy cow, the ratio of plasma weight to body weight was observed to be approximately 4.92 per cent. In non-lactating cows the plasma was found on the average to constitute 3.78 per cent of the body weight (Fig. 11).

The Increase in Blood Volume with Increasing Age and Weight.—

It has been shown that the ratio of blood volume to body weight increases during growth and lactation. This indicates that blood volume increases more rapidly than does body weight. When the relation between blood volume and body weight was plotted (Fig. 16) the data appeared to follow a curve of exponential form. This possibility was tested by plotting the values on arithlog paper. It was observed that the points thus plotted deviated but slightly from a straight line.

Using the method described by Brody (1927), an equation of the form

$$B = A_0 e^{kw}$$

was fitted to the data. When the parameters A and k are determined, the equation takes the form

$$B = 6200e^{.0014345w} \tag{5}$$

where B is the blood volume in cubic centimeters, e the base of natural logarithms, and W the body weight in pounds.

These data include animals of the Holstein-Friesian (66 determinations), Jersey (41 determinations) Guernsey (7 determinations) and Ayrshire (5 determinations) breeds. These data include also growing animals from 82 to 895 pounds (54 determinations), mature lactating cows varying from 890 to about 1400 pounds (41 determinations), and mature non-lactating cows varying from 870 to about 1600 pounds (24 determinations). Due to the heterogeneous nature of the data it is realized that the numerical values of the parameters of the equation are not significant. The fact that these data appear to follow a curve of exponential form, however, is believed to be of considerable interest.

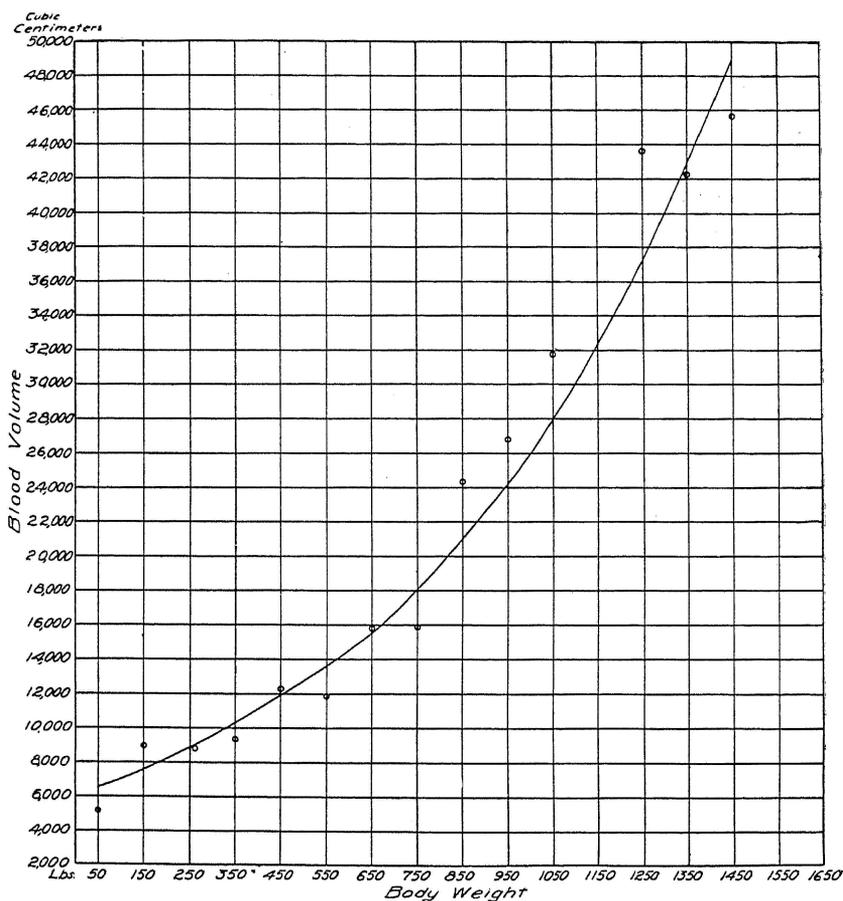


Fig. 16.—The relation between blood volume and an increase in weight and age of dairy cattle.

Individual Fluctuation of Blood Volume and Plasma Volume

The constancy of the blood volume and the plasma volume has not been investigated to any extent. As a part of the determination of the blood volume of normal dairy cows, data have been obtained from weekly to monthly intervals on six mature cows. These data are presented in Table 9. The determinations made at various intervals show a moderate amount of fluctuation of the blood volume. No striking changes in blood or plasma volume seem to be apparent. The determinations made December 10, 1930, on Cow No. 510H are of interest. Four days before calving this animal was observed to have a plasma volume of 26,333 c. c. and a blood volume of 45,150 c. c. The body weight was 1385 pounds.

This animal dropped an 82 pound calf, December 10. A blood volume determination was made four hours after parturition. The plasma volume was found to be 24,624 c. c. and the volume of blood 41,625 c. c. Nine days later the plasma volume was 28,850 c. c. and the blood volume 45,700 c. c.

The variations in plasma volume and blood volume are probably physiologic. In many cases the variation is slight, perhaps due in some instances to errors of the method used. Rowntree, Brown and Roth

TABLE 9.—VARIATION IN BLOOD VOLUME OF INDIVIDUAL COWS

Herd No.	Date	Weight	Cells	Total Plasma Volume	Total Blood Volume	Per cent of Body Weight
				<i>c. c.</i>	<i>c. c.</i>	
537H	12-19-30	<i>lbs.</i> 1357	<i>per cent</i> 31.9	30,700	45,125	7.72
	1-7-31	1355	35.0	32,650	50,300	8.61
	2-14-31	1328	33.0	28,725	42,950	7.46
290H	11-8-30	1400	37.3	29,366	46,833	7.72
	12-2-30	1345	37.0	28,311	44,950	7.72
	12-19-30	1408	33.5	33,200	50,000	8.26
	1-10-31	1387	36.1	32,850	51,450	8.61
	1-30-31	1389	42.0	32,000	55,125	9.17
	2-14-31	1334	38.0	28,900	46,675	8.07
	3-28-31	1335	41.0	30,066	51,933	8.82
515H	12-2-30	1322	38.8	27,257	44,200	7.81
	1-10-31	1340	39.4	33,100	54,600	9.46
	2-14-31	1356	36.2	28,800	45,900	7.83
	3-21-31	1335	40.6	30,431	48,507	8.39
509H	12-2-30	1440	38.6	26,350	43,150	6.92
	12-19-30	1440	34.5	28,700	43,850	7.07
	1-10-30	1407	39.0	31,950	52,950	8.65
	1-14-31	1465	39.5	29,350	48,525	7.70
	1-30-31	1270	38.0	30,950	50,025	9.09
	2-14-31	1256	33.1	30,375	45,475	8.36
	3-28-31	1270	34.1	31,150	43,375	8.60
508H	12-2-30	1260	39.7	28,100	46,600	8.60
	1-10-31	1255	40.4	34,650	58,100	10.76
	2-14-31	1250	35.1	31,100	48,000	8.86
	3-28-31	1350	33.3	31,283	49,966	8.04
510H	12-6-30	1385	41.6	26,333	45,150	7.53
	12-10-30*	1306	41.9	24,625	41,625	7.41
	12-19-30	1318	36.6	28,850	45,700	8.07
	1-30-31	1264	39.6	27,350	45,400	8.29
	3-6-31	1306	32.0	26,116	38,450	6.80
	3-28-31	1274	39.7	29,633	49,266	8.92
553H	12-19-30	1326	32.6	29,400	43,600	7.54
	2-7-31	1290	43.5	22,750	40,300	7.22
	3-6-31	1298	35.8	24,675	38,400	6.84

*Determination of blood volume made four hours after parturition.

(1929) reported considerable variation in the blood volume of the dog over considerable periods of time. Greene and Rowntree (1924) showed that excessive amounts of water administered to the dog caused hemoglobin dilution and an increase in plasma volume. Bock (1921) found the plasma volume fairly constant under varying conditions and believed variation in blood volume to be partly due to changes in the corpuscular content. Rowntree, Brown and Roth (1929) found considerable variation in blood volume due to temperature changes.

Smith (1920) found the dye method suitable for making repeated determinations on the same individual within a very short time.

Repeated determinations on the blood volume of the dairy cow would be of interest in studying the diurnal variation and the effects of water ingestion, etc. With these points more fully understood, one would be in a position to explain the changes which take place under various physiological conditions.

Blood Volume of Dairy Cattle Determined by the Drain-out Method

It is of interest to compare the blood volume as obtained by the "drain-out" method with similar values determined by the dye injection method. Obviously the dye method should give higher results because drainage fails to remove all of the blood from the body as has been pointed out. The blood volume of 14 dairy cows has been determined by the "drain-out method" at the Missouri Station. Through the kindness of E. C. Elting, of the Clemson Agricultural College; P. S. Williams and S. I. Bechdel of the Pennsylvania Station; and H. P. Davis and C. W. Nibler of the Nebraska Station, approximately 98 additional determinations are available for comparison. It is a pleasure to acknowledge our indebtedness to these gentlemen for their cooperation in making possible this comparison.

These data comprise 112 determinations in which the "drain-out" method has been employed. The animals were of various ages, weights, and breeds. Some of the animals were lactating and many were pregnant. The data were grouped according to breed and the ratio of blood weight to body weight compared (Table 10). The per cent of body weight constituted by the blood in lactating and non-lactating cows was compared (Table 10).

The average per cent of blood in terms of body weight was found to be 4.13. The average weight of these cows was found to be 1145 pounds. Using the dye injection method, the average mature cow of 1270 pounds was found to have a blood volume comprising 6.95 per cent of the body weight (Fig. 9).

By the use of the "drain-out" method in 24 determinations the average non-lactating cow was observed to have a blood volume equal

TABLE 10.—THE BLOOD VOLUME OF DAIRY CATTLE DETERMINED BY THE DIRECT METHOD*
(Drain-out Method)

Breed	Lactating	Blood weight	Non-lactating	Blood weight
		Body weight		Body weight
	<i>f</i>	<i>ave. per cent</i>	<i>f</i>	<i>ave. per cent</i>
Holstein	41	4.46	12	3.68
Jersey	18	4.08	8	4.06
Ayrshire	13	4.02	7	3.84
Guernsey	12	4.03	1	3.49
	84	4.25	28	3.82
Total = 112				
Average $\frac{\text{Blood weight}}{\text{Body weight}} = 4.13$ per cent				

*Data from the Nebraska, Pennsylvania, South Carolina and Missouri Agricultural Experiment Stations.

to 3.82 per cent of the body weight. The same method shows the average lactating cow (Table 10) to have a blood mass equal to 4.25 per cent of the body weight. By the use of the dye injection method it was shown that the blood mass of the lactating cow comprises about 8.11 per cent of the body weight, while in the non-lactating cow a value of 6.38 per cent was obtained (Fig. 8 and Table 8).

Haigh et al (1920) using the "drain-out method" found the proportion of the blood to the live weight of the bovine at birth to range from 4.08 to 6.50 per cent. He used two Jersey and two Hereford calves, which were slaughtered at birth.

Compared with blood volume determinations by the dye injection method the "drain-out" method shows the former to give values 47 per cent higher in the case of lactating cows and 40 per cent higher for non-lactating cows of similar weight.

DISCUSSION

The blood never ceases to be of interest in a study of the physiology of milk secretion. It enters into every phenomenon associated with the animal body. The important role played by the blood as a carrier of nutrients and endocrine secretions to the mammary gland is well known.

Mature dairy cattle were found to have a higher per cent of blood than growing animals. The increase in blood volume as body weight increases has been described by the use of an exponential equation.

It is of much interest to find that the blood volume of the dairy cow is fairly large. According to the dye method, the blood makes up about one-twelfth to one-fifteenth of the body weight in mature dairy cows.

The "drain-out" method shows approximately 4.13 per cent (or one-twenty-fourth) of the body weight of a mature cow to be blood. These facts indicate that values obtained by the dye method are not subject to gross error. It is impossible to say just what percentage of the total blood may be eliminated from the body by drainage. Certainly a large amount of blood remains in the smaller vessels.

From these investigations it is evident that the dairy cow has a higher percentage of blood than has been estimated. Writers in the past have more or less arbitrarily considered the blood of the dairy cow to constitute about 5 per cent of the body weight.

Lactating cows were shown to have a higher per cent of blood than non-lactating cows. This can be attributed in part to the tendency of non-lactating cows to lay on fat which is avascular tissue. At the same time considerable involution of the udder takes place. It is a common observation that the udder is greatly reduced in size during the dry period. Furthermore, there is an apparent decrease in the size of the subcutaneous abdominal veins during the dry period indicating a reduced supply of blood in the mammary gland, as compared to the amount of blood present when milk secretion is at its height. Toward the end of pregnancy, the mammary gland undergoes considerably hypertrophy which reaches its height at the time of parturition and persists to a less extent throughout lactation. In the lactating cow the subcutaneous abdominal veins are distended, the vessels of the udder are prominent, and every indication points to a rich supply of blood. The mammary gland of a mature cow may weigh from 15 to 50 pounds. Most mature cows are pregnant at least six to seven months of the normal lactation. The uterus of the pregnant animal undergoes tremendous hypertrophy, particularly in the latter stages. The hypertrophy of these organs perhaps partially explains the greater per cent of blood in the mature dairy cow as compared to young dairy cattle in which neither the udder nor the uterus is functioning.

The ratio of plasma weight to body weight in the bovine has proved to be of interest. The plasma weight of the young animal was found to comprise about 3.5 per cent of the body weight; 3.78 per cent in the non-lactating, and 4.92 per cent in the lactating cow. The plasma constitutes about 60 to 65 per cent of the blood in dairy cattle. It is usually considered a vehicle for transportation of the erythrocytes. In discussing the blood in its role in milk secretion, the plasma is seldom treated separately. However, it is the plasma which distributes the glucose, amino acids and lipoids throughout the body and consequently supplies the mammary gland with the precursors of milk.

SUMMARY AND CONCLUSIONS

1. The dye injection method for the determination of plasma volume and blood volume has been adapted to dairy cattle.

2. In approximately 120 determinations by this method the plasma volume and the blood volume of growing dairy cattle, mature non-lactating, and mature lactating cows have been determined.

3. Growing dairy cattle from 200 to 900 pounds in body weight, in 54 determinations, average approximately 3.5 per cent plasma and 5.81 per cent total blood by weight.

4. Non-lactating cows, in 24 determinations, were observed to have higher plasma and blood volumes than the growing animals, averaging 3.78 per cent plasma and 6.38 per cent blood by weight.

5. Lactating cows, in a total of 41 determinations, were found to have a much higher per cent of blood than growing and non-lactating dairy cattle. The plasma constituted 4.92 per cent and the blood 8.11 per cent of the total body weight in the average lactating cow.

6. Mature cows as a group show more uniformity in blood volume per unit of weight or surface area than growing dairy cattle. Mature animals were found to average approximately 7,768 c. c. of blood per square meter of surface area as compared to 4,035 c. c. per square meter of surface in the growing dairy cattle.

7. When growing, lactating and non-lactating dairy cattle were grouped together, it was found that the increase in blood volume with weight was found to be of exponential form represented by the equation

$$B = 6200e^{-.0014345w}$$

where B is the blood volume in cubic centimeters, e the base of natural logarithms and W the body weight in pounds.

8. A comparison of the blood volume as determined by the dye injection method and the "drain-out" method shows the former to give values 47 per cent higher in the case of lactating cows and 40 per cent higher for non-lactating cows of similar weight.

9. The plasma constitutes from 55 to 65 per cent of the total blood volume of the dairy animal.

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