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Erythrocyte, plasma and total blood volumes of pigs from birth through six weeks of age

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ERYTHROCYTE, PLASMA AND TOTAL BLOOD VOLUMES OF PIGS FROM BIRTH THROUGH SIX WEEKS OF AGE

by

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I. INTRODUCTION

The regulation of blood volume is a complex mechanism in the animal body as evidenced by its physiological functions of maintaining the temperature, composition and volume of the interstitial fluid as well as continuously supplying nutrients to and removing waste products from all tissues and organs of the body. In the healthy adult animal the blood volume is maintained quite constant from hour to hour as well as from day to day. The presence of physiological changes such as growth, or pathological changes such as anemia, influences the regulatory mechanisms in such a way that the blood volume of an animal may not remain constant.

Information to date has shown that the porcine species provides a good experimental animal for hematological and cardiovascular studies. The literature is lacking in basic information concerning physiological changes in blood volume during the first few weeks of life as well as information concerning blood volume changes that occur during disease processes early in the life of this animal.

Baby pigs are born with what has been termed a "normal" hemogram. If they are dependent upon their dam for their only source of nutrients, they become definitely anemic by the time they reach seven to 14 days of age.

This anemia has been termed "physiological anemia" or "baby pig anemia." The criteria used to arrive at a diagnosis of baby pig anemia are decreased numbers of erythrocytes or concentration of hemoglobin in a unit volume of a peripheral blood sample. Since the concentration of erythrocytes and thus hemoglobin is not the same throughout the cardiovascular system, the total volume of blood and its component parts and the classical parameters of erythrocyte numbers and hemoglobin concentration will provide more complete data for evaluating postnatal anemia of pigs.

The term "physiological anemia" has been used to denote a concept described below concerning the anemia seen in baby pigs. Most pigs are born with a normal quantity of hemoglobin present in their body. That is to say, they have an adequate amount present to carry sufficient oxygen to meet the demands of the tissues. During the first week of life most pigs double their birth weight and during the second week they triple it. This means that after only seven days of life the baby pig has increased his tissue needs for oxygen approximately 100 per cent and therefore the carrier substance for oxygen, hemoglobin, must also be increased 100 per cent. If the baby pig's erythropoietic system is not functionally capable of increasing the hemoglobin at the same rate as the other body tissues are increasing, then a physiological anemia would be present.

If, however, the baby pig is capable of producing hemoglobin and erythrocytes in proportion to the increase in other tissues, then the term "physiological anemia" would be a misnomer and the anemia would be due to some other cause such as a lack of an essential nutrient.

It has been well documented that a deficiency of iron is responsible for at least a portion of the anemia seen in baby pigs. Hematological values obtained from peripheral blood, however, tend to fall below normal during the rapid growth phase of the first few weeks of life even when adequate amounts of iron are supplied to the animal.

The objectives of this study were to compare the blood volumes as well as the classical hematological parameters of iron deficient pigs with pigs that received an exogenous source of iron. These comparisons were designed to accomplish the following aims:

1. To ascertain whether or not the baby pig is capable of producing erythrocytes and hemoglobin in proportion to the increase in total body mass during the first six weeks of life.
2. To ascertain the relative proportion of total erythrocyte volume and plasma volume to total body mass during the first six weeks of life.
3. Verification of the classical hematological parameters used to evaluate anemia in baby pigs.

4. Correlation of the classical hematological parameters with erythrocyte, plasma and total blood volumes during anemia.

Methodology has been given considerable emphasis in this dissertation. One cannot proceed with obtaining results and information regarding physiological aspects of blood volume and its regulation without becoming deeply involved in technics. It has been stated by Gregersen and Rawson (1959, p. 307) that, "Methodology has been, and still is, of first importance to anyone interested in determining blood volume or in evaluating results reported by others."

After defining the original objectives and beginning the project, two additional objectives related to methodology were established. These were validation of a method for determining plasma volume in swine and the influence of blood group antibodies on Cr^{51} labeled erythrocyte survival time in the circulatory system of swine. Studies involving these objectives are also reported in this thesis.

II. REVIEW OF LITERATURE

A. Definition of Blood Volume

Blood volume has been defined by Gregersen and Rawson (1959) as the sum of the volume of cells and plasma inside the circulatory system. This definition has been followed in this thesis. It should be stated that of all test substances used for measuring blood volumes, no single one measures total blood volume. Each measures either plasma volume or cell volume.

There are three methods commonly used today from which a numerical estimation of total blood volume can be arrived. These are as follows:

1. Individual measurement of both cell volume and plasma volume and the subsequent addition of these two parameters.
2. Measurement of cell volume and the packed cell volume, which gives the proportion of cells in the blood sample, and the appropriate calculation from these two parameters.
3. Measurement of plasma volume and the packed cell volume and the appropriate calculation from these two parameters.

Because of the varying results due to different technics, the terminology used in this thesis will be that of naming the method as well as the test substance used for any blood volume determination.

B. Technics for Measurement of Blood Volumes

The historical development of blood volume measurement at various periods is given by Erlanger (1921), Rowntree et al. (1929), Peters and Van Slyke (1932), Reeve (1948), Gregersen (1946, 1951), Sjostrand (1953) and Brown et al. (1957). A recent review by Gregersen and Rawson (1959) is quite detailed and complete.

One of the first technics for estimation of blood volume was the procedure described by Welcker (1854) involving exsanguination and flushing out of the cardiovascular system. This procedure has been referred to as the wash-out method and according to Gregersen and Rawson (1959) was the earliest approach to a quantitative estimate of blood volume where the results have stood the test of time in comparison with cell labeling technics.

Another early method involved the labeling of hemoglobin with carbon monoxide (Grehand and Quinquand, 1882). The determination of carbon monoxide is a science within itself and has been discussed by Van Slyke and Salveson

(1919).

From the mass of literature on methods and procedures for measuring blood volume, Gregersen and Rawson (1959) have selected certain publications and arranged them under various categories in chronological order as a synopsis of methodology. The list is, by their own admission, not a complete list of the work in the field, but it does show the general trend in evolution of technics, when they were introduced, modified or improved and the periods of time during which they have been in common use.

The methods in current use are all based on the dilution technic. This technic involves the introduction of a known quantity of a test substance into a fluid chamber of unknown quantity. Upon complete and uniform dispersion of the test substance, a sample of the fluid containing the test substance is removed from the chamber and the concentration of the test substance is determined. Obviously, the greater the volume of the chamber, the more dilute the test substance becomes.

Broadly speaking, there are four basic requisites for the dilution technic. They are:

1. Uniform distribution of the test substance within the fluid chamber.
2. Accurate determination of the concentration of the test substance.

3. Interpretation of the time-concentration relationship of the test substance within the fluid chamber.
4. Correction for uneven distribution of plasma and cells in the circulation.

Many of the reports in the literature, especially in the early years of use of the dilution technic, did not take the above factors into consideration, thus presenting many controversies and growing pains. The research evidence now on hand provides satisfactory answers to the problems. The precautions that are necessary and tests required to demonstrate the validity of observations in various species under normal and abnormal conditions can at least be defined for the test substances commonly used today. Most reports dealing with blood volume measurements take into consideration the first three of the above outlined requisites. The fourth one is still abused in much of the present literature especially in reports dealing with blood volume measurements in animals.

There are three dilution methods in common use today for measuring plasma volume. They are the T-1824 dye, radio-iodinated albumin, and chromic chloride methods.

The T-1824 dye dilution technic is probably the most universally accepted method of measuring plasma volume

today and possibly one of the most abused methods. It has been shown (Allen et al., 1953b) that the spectral absorption curve of T-1824 is not the same in plasma from all species. Thus the dye should be standardized for each species. The spectral characteristics of various dyes, including T-1824, are different in aqueous and plasma solutions. A study of this aspect by Lindhard (1926) disclosed large errors in the plasma dye determinations of some early investigators who used water standards. Even with this information available since 1926, reports have appeared in the last five years in which dye standards were diluted in water and used to compare with plasma samples containing dye (Stahl and Dale, 1958). A concise summary of the basic aspects of the T-1824 technic has been published (Gregersen, 1944). The fact that the basic concepts published by Gregersen were correct and that the dilution volume of T-1824 does represent the true plasma volume has been verified by many subsequent studies. Simultaneous determinations of the volume distributions of T-1824 and the antigens, bovine albumin, bovine globulin and the polysaccharide SIII (Gregersen et al., 1945; Gregersen et al., 1950) as well as similar tests with T-1824 and albumin I^{131} (Franks and Zizza, 1958; Gibson et al., 1946) and hemoglobin (Allen et al., 1953a) all yield essentially the same value for plasma volumes, providing the latter is calculated from

extrapolation of the time-concentration curve on a semi-log plot.

Allen (1953) devised a simple paper pulp extraction technic for removing T-1824 dye from serum. The extraction of the dye from the serum and the subsequent determination of its concentration eliminates errors from hemolysis, lipemia and from changes in inherent plasma color. The technic eliminates the consideration of factors which affect the spectral absorption of T-1824 in plasma including species differences. Campbell et al. (1958) made certain modifications which speed up the elution.

None of the test substances used for determining plasma volume by the dilution technic are perfect indicators of the volume chamber to be measured because of the fact that a portion of the test substance is always being lost from the volume chamber at any given time. During the period of time necessary after injection of the test substance for complete mixing and uniform distribution there occurs some loss of the test substance. In order for the calculation of the volume of the fluid chamber to be accurate and valid, the loss occurring between the time of injection and the time of sampling must be taken into account. Graphic analysis of a time-concentration curve on a semi-log plot and back extrapolation of the disappearance curve is now the accepted means of arriving at the

initial concentration of the test substance (Gregersen and Rawson, 1959). The initial concentration, or that amount of test substance that would have been present if the test substance were completely mixed at the time of injection, is the value needed for calculation of plasma volume. The validity of graphic analysis depends upon the assumption that the loss rate during the mixing period is correctly estimated from the slope of the disappearance curve. Evidence to support this assumption for the dye T-1824 has been presented for the dog (Allen and Gregersen, 1953), the cow (Reynolds, 1953), and the pig (Talbot and Swenson, 1963a). There is evidence that T-1824 overestimates plasma volume in the rabbit (Zizza and Reeve, 1958). The reasons are not clear but unequal distribution and loss may be factors. The work points out the need for validation in each species in which the dye is going to be used.

Gibson et al. (1946) were evidently the first to estimate plasma volume with the I^{131}-albumin technic. This method has been widely used. Recognition of the limitations of the method has not always been made. Unaccounted for free I^{131} in preparations of I^{131}-albumin (Franks and Zizza, 1957) will lead to false estimates of plasma volumes as will absorption of iodinated protein by certain types of glassware (Reeve and Franks, 1956). The development of an immune reaction following injection of a foreign

protein into some species also may lead to false estimates (Gregersen and Rawson, 1959).

A method for measuring plasma volume that utilizes radioactive chromic chloride was introduced by Gray and Sterling (1950). They showed that when a saline solution of chromic chloride is injected intravenously, the cationic, trivalent chromium is bound to the extent of 98 per cent by the plasma proteins and thus may be used for plasma volume measurements. Subsequent investigations have verified the original results (Gray and Frank, 1953; Small and Verloop, 1956).

Technics for measuring erythrocyte volumes are currently limited to two methods, radioactive chromium and radioactive phosphorus, although other methods have been used in the past. Radioiron as a cell label is laborious and no longer widely used. Radioactive potassium has been used (Yalow and Berson, 1951) but is limited by its short biological half-life.

Radiochromium is probably the most widely used test substance for estimation of erythrocyte volume today. The original technic was first described by Sterling and Gray (1950). Radiochromium is now available as $Na_2Cr^{51}O_4$ with a high specific activity. The physical half-life of Cr^{51} is 27.8 days and while it decays for the most part by "K" capture, 10 per cent of its total emission is in the form

of a 0.32 MEV gamma ray which makes measurement in any properly shielded well counter quite feasible.[1] Ebaugh et al. (1953) and Necheles et al. (1953) have shown that chromium binds with the globin portion of hemoglobin and that it is eluted from globin at a constant rate of one per cent per day. Hollingsworth (1954) subsequently demonstrated that when the chromated cell eventually becomes destroyed, the chromium does not label other cells. With no significant loss of the tagging material during the first 24 hours, back extrapolation of a time concentration curve is not necessary, thus giving the radiochromium method a distinct advantage over other methods. The addition of ascorbic acid to a mixture of blood and radiochromium reduces the chromium that has not already tagged erythrocytes from the anionic hexavalent form to the cationic trivalent form (Read, 1954). The cationic trivalent chromium formed after reduction by ascorbic acid could be used to estimate plasma volume but Read and Gilbertsen (1957) have demonstrated that the technic is not valid.

The second most widely used method of estimating circulating red cell volumes utilizes radioactive phosphorus. The initial use of P^{32} involved a time consuming

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[1]Abbott Laboratories Radio-Pharmaceuticals. Rachromate. Lithographed. Oak Ridge, Tennessee. 1960.

process of synthesizing tagged red cells in a donor by injecting the phosphorus, then removing and injecting the red cells into the subject to be studied (Hahn and Hevesy, 1940). Later, Nylin (1945) simplified the technic by showing that erythrocytes could be labelled with a satisfactory degree of stability by merely incubating them for a short time with inorganic P^{32}. Since then several investigators (Anderson, 1942; Reeve et al., 1953b; Huang and Bondurant, 1956) have used P^{32} labelled cells for erythrocyte volume determinations. Reid and Orr (1950) have presented the technics that are commonly cited in current reports utilizing the P^{32} method.

The greatest portion of the literature reports dealing with blood volume presents total blood volume data that have been derived from determination of either plasma volume or cell volume alone. From either parameter and the packed cell volume the total blood volume has been computed. It is quite evident that calculations made in this manner are based upon the assumption that the average cell percentage in the blood vascular space is the same as the cell percentage in the blood sample from which the packed cell volume determination was made. It has been recognized for many years that the concentration of red cells is not the same in all parts of the circulatory system. One would expect that the average or overall cell percentage would be

less than the venous cell percentage since the cell/plasma ratio is lower in the capillaries and small vessels than in the larger arteries and veins. That is to say, the PCV is lower in the capillaries and small vessels than in the large arteries and veins. In man, plasma volume-PCV estimates of blood volume are higher than cell volume-PCV estimates (Gregersen and Rawson, 1959). Reeve and Veall (1949) were evidently the first to calculate a factor to correct for the discrepancy between true measured total blood volume and total blood volume calculated by using the packed cell volume. This factor has been termed the F_{cells} factor and is calculated from the ratio of the overall cell percentage to the venous cell percentage. The F_{cells} factor has been reported to be 0.91 in adult man (Gray and Frank, 1953; Chaplin *et al.*, 1953). F_{cells} factors for several other species have also been reported. O'Brien *et al.* (1957) reported 0.90 for the goat, Zizza and Reeve (1958) reported 0.89 for the rabbit, Wang (1959) reported 0.74 for the rat, and Gregersen *et al.* (1959) reported 0.83 for the monkey. Studies on the canine species have been quite variable. The normal dog with an intact spleen has been shown to have F_{cell} values varying from 0.87 to 1.10 (Gregersen, 1953) depending upon the fraction of red cells present in the spleen. The F_{cells} value in the splenectomized dog has been reported to be constant at

0.88 and not altered by large changes in blood volume or packed cell volume (Reeve et al., 1953a; Rawson et al., 1959; Baker and Remington, 1960). The same variability in F_{cells} demonstrated by the non-splenectomized dog has also been shown in the normal anesthetized cat while the splenectomized cat presents a constant F_{cells} value of 0.78 (Farnsworth et al., 1960). These workers also presented evidence that species differences in the F_{cells} value may be related to body size. Chien (1960), working with dogs, showed that while the sympathetic nervous system influences erythrocyte and total blood volume values, it has no influence on the F_{cells} factor. The profound influence of the spleen in the dog has been demonstrated by Sliwinski and Lilienfield (1958) who showed that under pentobarbital anesthesia the ratio of splenic packed cell volume to large vessel packed cell volume was 1.96 ± 0.12. Evidence presented in 1961 (Fudenberg et al.) shows that in man the spleen does not have the function of a significant blood reservoir as it does in the dog. They found identical F_{cell} values for intact and splenectomized patients. Under pathological conditions manifested by splenomegaly, however, they did find the F_{cells} value to be significantly increased in direct proportion to the degree of splenic enlargement indicating an increased concentration of erythrocytes in the enlarged spleen. This evidence would

point to a definite species difference which may be related not only to physical differences in the ratio of splenic size to total body size but also to functional differences between species. Future investigations are necessary to examine this aspect in species other than dog, cat and man.

C. Blood Volumes of Swine

A vast amount of literature has been published on blood volume of man and laboratory animals. There are relatively few reports on the pig, and to the author's knowledge none have appeared concerning total blood volume determinations by the simultaneous measurement of Cr^{51} erythrocyte volume and T-1824 plasma volume in the pig.

Hansard et al. (1951) reported blood volume values for 16 swine using the P^{32} labeled erythrocyte-packed cell volume method. Hansard et al. (1953) reported the same data in a more concise form along with data from other farm animals. The youngest pig studied was two weeks of age while the oldest was three years. They noted a progressive decrease in the total blood volume per kg. of body weight from 74 ml. per kg. of body weight at two weeks to 35 ml. per kg. of body weight at three years.

Ramirez et al. (1962) have shown that the total blood volume per kg. of body weight is lower during the first

week of life than at two weeks of age. They found, using the T-1824 plasma-packed cell volume method, a mean of 99 ml. per kg. of body weight at one day of age and 83 ml. per kg. at two weeks of age in 20 pigs.

Bush et al. (1955) used the P^{32} labeled erythrocyte-packed cell volume method and reported a decrease in total blood volume per kg. of body weight with increasing age similar to the data reported by Hansard and co-workers. Their study involved both normal and anemic swine but numerical values are given for only the normal animals and not the anemic ones. No age is given for the swine used but body weights from 10 to 110 kg. are listed. The numerical values for total blood volume, while in general agreement with those reported by Hansard et al. (1951) averaged 15 per cent lower. The animals used were of different breeds, housed at different altitudes and fed different diets which probably explains the discrepancy. Fe^{59} plasma volume values were also estimated by Bush et al. (1955) but the report does not state whether the total blood volume values reported were derived by this technic or are calculated values from the P^{32} erythrocyte volume and packed cell volume values. Both technics are described in the methods portion of the paper.

Jensen et al. (1956) reported a mean plasma volume of 47.7 ml. per kg. and a mean erythrocyte volume of 30.4 ml.

per kg. in 18 normal swine ranging in weight from 8.6 to 97.0 kg. The method used was the Fe^{59} plasma packed cell volume technic. The erythrocyte volume values are therefore calculated values and not measured values.

Bush et al. (1956b) reported the same data along with data obtained from three copper-deficient swine. The mean values for the deficient swine were 72.8 ml. of plasma per kg. of body weight and 15.2 ml. of erythrocytes per kg. of body weight. The mean body weight was 21.6 kg.

In another publication (Bush et al., 1956a), the same data as reported previously by Jensen et al. (1956) and Bush et al. (1956b) are compared with data obtained from nine swine with three different types of experimentally produced anemias.

Working with newborn pigs, McCance and Widdowson (1959) measured plasma volume by the T-1824 plasma technic in seven fasted pigs and seven pigs allowed to suckle. They found that the plasma volume of the suckling pigs increased from 55 ml. per kg. of body weight before suckling to 81 ml. per kg. 24 hours after suckling while the plasma volume of the fasted pigs remained the same over the 24 hour period.

D. Blood Volumes of Other Species

The two most prevalent subjects for blood volume studies have been dog and man. Studies involving small laboratory animals and other domestic animals have been few in number. By no means is all of the literature involving blood volume reviewed in this thesis. Only those articles which the author has deemed appropriate regarding the relationship of results, methodology, or interpretation to the study reported are included.

Many varying reports have been published concerning blood volume values for the dog. As was discussed under F_{cells} though, the discrepancies are primarily related to improper interpretation of methods used and thus incorrect calculation of values. Because of the profound influence that the physiological state of the spleen has on the volume distribution of cells, only those reports which specify the state of the spleen during the study and thus the use of the proper F_{cells} factor or those reports which utilize both a cell volume and a plasma volume determination are discussed here. The data of Baker and Remington (1960), who used T-1824 dye and Cr^{51}, show a mean plasma volume of 52.8 ± 8.7 ml. per kg. and a mean erythrocyte volume of 34.1 ± 9.5 ml. per kg. in 16 normal dogs. After contraction of the spleen with epinephrine and also after

splenectomy there were no significant differences in either plasma volume or cell volume; however, as mentioned in the discussion on F_{cells}, there was a significant difference in the volume distribution of the cells and plasma. Later, one of the authors (Baker, 1963) reported a mean T-1824 plasma volume of 48.3 ± 7.1 ml. per kg., a mean Cr^{51} erythrocyte volume of 26.4 ± 5.2 ml. per kg. and a mean I^{131} plasma volume of 41.6 ± 4.2 ml. per kg. in 10 normal dogs. Chien (1960) reported average cell volume, plasma volume, and total blood volume values of 32.2, 54.8, and 87.0 ml. per kg., respectively, for sympathectomized-splenectomized dogs. Clark and Woodley (1959) using T-1824 and Cr^{51} reported 81 ml. per kg. in 41 normal dogs. Their own calculation of a mean F_{cells} value of 0.895 led them to believe that the spleens of the dogs were greatly constricted when blood volume measurements were made. No mention of anesthesia is made in the paper so one can conclude that their interpretation is probably correct especially if pentobarbital was not used and the dogs were under some sympathetic stimulation from handling. Data from 21 healthy Beagle dogs show values of 43.0 ± 9.5, 51.4 ± 12.4, and 94.4 ± 16.6 for erythrocyte, plasma, and total blood volumes using Cr^{51} and I^{131} as the test substances (Parkinson and Dougherty, 1958). Deavers *et al.* (1960) presented data on 100 healthy adult mongrel dogs showing

mean Cr^{51} erythrocyte volumes and mean T-1824 plasma volumes of 33.5 ± 0.74 ml. per kg. and 50.2 ± 1.11 ml. per kg. respectively. Analysis of their data based on body weight of the individual dog revealed no significant difference in either plasma volume or cell volume when expressed as a volume per unit of body weight between large dogs (mean body weight of 12.6 kg.) and small dogs (mean body weight of 5.8 kg.). Calculations made by this author from data presented by Reeve et al. (1953b) on nine individual normal dogs give a mean total blood volume of 94.4 ml. per kg. T-1824 plasma volumes and Cr^{51} erythrocyte volumes were measured.

Gregersen and Rawson (1959) state that they abandoned an attempt to compile an extensive table of normal blood volume values for man primarily because it is difficult to evaluate the procedures used and to recalculate or convert the values given to true blood volumes. This author would agree with their statement. A few articles, in which the procedural details are given clearly, are cited here plus a series of three articles on infants which seem to be the only reports in the literature for human infants that compare time-wise with the data presented in this thesis on pigs. A report based on P^{32} and T-1824 studies in eight adult women lists 43.1, 23.4, and 66.5 ml. per kg. for plasma volume, cell volume and total blood volume

respectively (Wadsworth, 1954). Schmidt et al. (1956) reported 40.6, 24.0 and 64.6 ml. per kg. in 70-94 year old adult females and 42.7, 28.5, and 71.3 ml. per kg. in 70-94 year old adult males for plasma volume, cell volume and total blood volume, respectively. They used T-1824 and an F_{cells} value of 0.91. They also recalculated Gibson and Evan's (1937) data on young men and women and by using an F_{cells} value of 0.91 found no difference in the values when expressed identically. Strumia et al. (1958) have listed several parameters related to the measurement of erythropoiesis including plasma volume, cell volume, total blood volume, and F_{cells}, but the only mean value listed is 0.93 ± 0.02 for F_{cells}. Sisson et al. (1959a) in the first of three papers concerning blood volume of infants report average plasma volume, cell volume, and total blood volume values from birth throughout the first year of life. The average blood volume of premature infants has been shown to be slightly higher than that of full term infants (Sisson et al., 1959b). They concluded that original stores of nutrients derived from blood cell destruction in the premature infant are adequate for hemoglobin synthesis until only about 10 weeks after birth, thus explaining in part the development of iron depletion and deficiency at an earlier age in the premature than the full term infant. Sisson and Whalen (1960) concluded, after a study of blood

volumes at birth and three to five hours after birth, that a significant increase of the circulating plasma, erythrocyte, and total blood volumes takes place in the newborn infant during the first few hours of life. The average increments were: plasma volume, 20 per cent; erythrocyte volume, 25.6 per cent; and total blood volume, 22.5 per cent. They postulated that the increase was due to an initial sequestration of blood in the viscera and caudal end of the body and that this blood was then introduced into the general circulation as vascular and pulmonary patterns were stabilized.

Wang (1959), working in Gregersen's laboratory, reported a mean plasma volume of 39.0 ml. per kg. in 50 unanesthetized normal rats. The total blood and red cell volumes calculated by using a F_{cells} factor of 0.74 averaged 59.3 ml. per kg. and 21.4 ml. per kg., respectively. Plasma volumes were determined with T-1824. These values agree with other values on fewer rats presented in the same paper which were determined from the simultaneous measurement of P^{32} cell volume and T-1824 plasma volume. The values presented by Wang for cell volume agree closely with data calculated by this author from average values presented by Doornenbal *et al.* (1962) but not with calculations made for data on plasma volume and total blood volume. Doornenbal *et al.* measured only red cell volume with Cr^{51}

and used no F_{cells} factor for calculation of the other parameters. Application of the figure, 0.74, for F_{cells} to the calculations reveals an approximate total blood volume of 64.0 ml. per kg. for Doornenbal's data. This agrees more closely with that presented by Wang.

Using the T-1824 plasma volume method, Ancill (1956) reported a mean total blood volume of 72 ml. per kg. in the normal guinea pig. No corrections were made for F_{cells} and thus the value reported may be slightly high.

A study involving 42 simultaneous measurements of plasma and cell volumes with T-1824 and P^{32} on 18 normal Rhesus monkeys has been reported by Gregersen et al. (1959). The mean values and standard deviations are as follows: cell volume, 17.7 ± 1.66; plasma volume, 36.4 ± 3.98; and total blood volume, 54.0 ± 4.72 ml. per kg.

Campbell (1959) reported total blood volume values for 12 Leghorn hens but no average values are listed. The methods employed would lead this author to disregard these values. Rychter et al. (1955a) published a micromethod for determining plasma volume with T-1824 in chick embryos from the second day of incubation until hatching. Results of their studies (Rychter et al., 1955b) published later list plasma volumes varying from 8.5 cmm. on the second day of incubation to 1860 cmm. on the 18th day of incubation. Their technics may well lend themselves to prenatal

studies on mammals. Using the Cr^{51} erythrocyte technic, Barnes and Jensen (1959) also reported blood volumes for nine to eighteen day embryonated hen eggs. From the figures presented it would appear that the values obtained for 18 day old chick embryos are similar to those of Rychter et al. (1955b).

Blood volume values for the rabbit have been reported by Burke et al. (1953) and Courtice (1943) using T-1824 plasma volume technics but since the work of Zizza and Reeve (1958) shows that the T-1824 dilution space is erroneous in the rabbit, the values should probably be disregarded.

The plasma volumes of 30 goats have been measured with T-1824 by Courtice (1943) and reported as averaging 53 ml. per kg. A value for total blood volume is also listed as 70 ml. per kg. but no correction for unequal distribution of cells was made and since the individual animal data are not included, true blood volume cannot be calculated. Klement and co-workers (1955) using T-1824 and Cr^{51} measured both cell volume and plasma volume in 20 goats. They reported mean values of 14.7, 55.9, and 70.5 ml. per kg. for erythrocyte volume, plasma volume and total blood volume, respectively.

A classical study by Reynolds (1953) in which she validated the T-1824 method for use in the cow lists a

mean plasma volume for 10 non-pregnant, non-lactating cows as 38.8 ± 1.4 ml. per kg. Quite in contrast to the detailed attention to methods adhered to by Reynolds is the work of Stahl and Dale (1958) on 17 dairy calves. Statements such as "The Cr^{51} method has the advantage of measuring blood volume without hematocrit determinations" and "dye (T-1824) for the standard curve was diluted in water" show a disregard for basic understanding of the technics being used. The words of Gregersen and Rawson (1959, p. 314) seem worthy of repetition:

> Some investigators seem to believe that they are directly determining whole blood volume by determining the concentration of the test substance in samples of whole blood. This is clearly a misunderstanding of the nature of the problem... It should be stated categorically that of all the test substances..., no single one alone measures total blood volume. Each measures either plasma volume or cell volume. In every instance, total blood volume is a derived calculation from the plasma volume or cell volume and the proportion of cells in the blood sample.

E. Erythrocyte Life Span

The methods used and the relationship of methods to interpretation of results are quite similar in erythrocyte life span studies and in blood volume studies. Berlin et al. (1959) and Mollison (1961) have reviewed the various technics and the interpretation of results from them.

To this author's knowledge only five reports regarding the life span of erythrocytes have appeared in the literature as of this date. Bush *et al.* (1955), using C^{14} labeled glycine, reported a mean survival time of 62 days in five growing pigs. Jensen *et al.* (1956) reported an "apparent" life span of 63 days in 18 normal growing pigs. Their studies were conducted with Fe^{59} labeled cells. Bush *et al.* (1956b), using Cr^{51}, determined the mean erythrocyte half-life to be 17 days in four normal growing pigs while Hansard and Kincaid (1956) found the "average" survival time for mature swine to be 71 days. These four studies deduced red cell life spans by using transfusions of red cells from one individual to another within the same species (homologous transfusions). Talbot and Swenson (1963c), reporting a portion of the data from this thesis, have published mean erythrocyte half-lives of 28.0 ± 4.0 days. These values were obtained from Cr^{51} labeled red cells that were removed, labeled and then reinjected back into the donor (autologous transfusions). A discussion of the relationship between the various results reported is presented in the Results and Discussion section of this thesis.

III. METHODS AND MATERIALS

A. Experimental Animals and Design

A total of 176 pigs were used to conduct the studies. Pregnant crossbred sows were obtained from the Veterinary Medical Research Institute herd and from farm herds in the Ames area, allowed to farrow in farrowing crates, and housed on concrete floors for the first six weeks of the pig's life. A 16.5 per cent protein ration (Table 1), fed to all dams before farrowing and during lactation, met the requirements stated by the National Research Council (1959). This ration, which contained no added iron, was the only food other than the dam's milk available to the pigs.

Between 12 and 24 hours after birth, the pigs in each litter were weighed, sexed and selected at random within sex for treatment with iron-dextran. Pigs used as controls were given no iron supplementation; treated pigs were given two ml. of iron dextran,[1] supplying 150 mg. of elemental iron. The iron dextran was administered intramuscularly in the left rear leg between 48 and 72 hours after birth. The litters were divided into two groups; those litters whose

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[1]Ferrextran. Fort Dodge Laboratories, Fort Dodge, Iowa.

Table 1. Ration fed to dams before farrowing and during lactation

Ingredient	Per cent of ration[a]
Yellow corn	72.0
Meat and bone scraps	10.0
Soybean oil meal	7.5
Dehydrated alfalfa meal	10.0
Iodized salt	0.5
Vitamin D supplement	400 I.U.

[a]Except vitamin D supplement which is expressed as international units per pound of ration.

pigs would have determinations made on them at birth, 2, 4, and 6 weeks of age and those whose pigs would have determinations made at 1, 3 and 5 weeks of age. Birth values were determined between 12 and 24 hours after birth. The following determinations were made on each pig at each sampling period: body weight, plasma volume, erythrocyte volume, erythrocyte number, leukocyte number, packed cell volume (PCV), hemoglobin and differential leukocyte count. The following values were calculated from the above data: total blood volume, plasma volume per kilogram of body weight, total blood volume per kilogram of body weight,

F_{cells} factor, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

B. Experimental Technics

1. Plasma volume

The method used to determine plasma volume was a modification of the T-1824 dye dilution method described by Gregersen (1944). This method had not been, to the investigator's knowledge, validated for use in the pig. Since the T-1824 method had been shown to overestimate considerably the plasma volume when used in rabbits (Zizza and Reeve, 1958), the initial part of this investigation involved determining whether or not the T-1824 method was valid for use in swine.

T-1824 dye was found to be an adequate test substance for use in swine (see Results and Discussion section) and the following technic was used for all plasma volume determinations. The T-1824 dye[1] was prepared in a one per cent solution from the same lot for the entire study. A Beckman

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[1]Eastman Organic Chemicals, Distillation Products Industries, Rochester 3, New York.

Model B spectrophotometer[1] with matched cuvettes was used for all spectrophotometric determinations of dye concentration. The T-1824 dye was injected into an ear vein at an approximate dosage of one mg. per kg. of body weight. The exact dosage was determined by using a calibrated syringe. Exact amounts of dye concentrations in the syringe were predetermined by dilution procedures and spectrophotometric comparison with a standard T-1824 solution. After allowing 15 minutes for complete mixing of the dye in the circulatory system, a five ml. sample of blood was withdrawn from the anterior vena cava and placed in a siliconized centrifuge tube. The blood was allowed to clot, centrifuged for 15 minutes at 2800 R.P.M. and the serum harvested for T-1824 dye extraction.

The dye was extracted from the serum by the method of Campbell et al. (1958). This method was developed for use with human serum samples but upon repetition of Campbell's work using swine serum (see Results and Discussion section) the technic was found to be equally as accurate as with human serum.

After extraction of the dye the concentration in mg. per ml. of serum was determined spectrophotometrically. This concentration was corrected mathematically to

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[1]Beckman Instruments, Inc., Fullerton, California.

compensate for an average loss rate of dye from the circulatory system of 22.1 per cent per hour as described in the Results and Discussion section. The formula used to correct the dye concentration was as follows:

$$2.303 \log \frac{A_o}{A} = kt$$

where: t = time elapsed between injection of dye and withdrawal of sample

k = loss rate of dye from blood stream

A = concentration of dye at time t

A_o = concentration of dye at time of injection if it were completely mixed in the bloodstream.

The corrected serum dye concentration was then used in the following formula to calculate plasma volume.

$$\text{Plasma Volume} = \frac{\text{amount of dye injected}}{\text{concentration of dye in serum}}$$

2. Erythrocyte volume

A modification of the method of Sterling and Gray (1950) was used for measuring circulating erythrocyte volume. Fifty ml. of blood were obtained aseptically from a donor pig and immediately mixed with 10 ml. of a sterile

anticoagulant solution.[1] Approximately 200 micro-curies of sterile $Na_2Cr^{51}O_4$[2] were added to the blood and the mixture incubated for 30 minutes at 37°C. After incubation two mg. of ascorbic acid were added for every one ml. of blood to reduce the unbound chromium from the anionic hexavalent form to the cationic trivalent form which does not penetrate the red cells. This procedure eliminates the possibility of labeling any erythrocytes in vivo. The blood was then centrifuged, the plasma discarded and the cells washed two times in sterile 0.9 per cent NaCl solution. The washings were discarded and the cells resuspended in 0.9 per cent NaCl solution. A counting standard was prepared by diluting one ml. of the labeled blood to 250 ml. with 0.9 per cent NaCl. One ml. of this dilution was pipetted into a counting tube for radioactive determination. A packed cell volume determination was also made by the microhematocrit method on the labeled blood.

The labeled blood was administered to each pig by injection into an ear vein through the same needle that the T-1824 dye had been administered. Approximately two micro-curies of Cr^{51} per kilogram of body weight were given. The

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[1]Special Formula ACD solution obtained from Abbott Laboratories, Oak Ridge, Tennessee.

[2]Rachromate obtained from Abbott Laboratories, Oak Ridge, Tennessee.

exact amount of blood given was determined volumetrically by using a pre-calibrated syringe.

After allowing 15 minutes for mixture of the labeled cells throughout the circulatory system, a three ml. blood sample was obtained simultaneously with the sample obtained for T-1824 dye analysis. This sample was placed in an oxalated blood tube. Two ml. of the oxalated sample were transferred to a counting tube for radioactive analysis and hemolyzed by adding two ml. of distilled water. The remaining blood was used for other hematological determinations, which included a packed cell volume (PCV) determination.

Determination of the radioactivity present was made by counting the samples in a well-type, thallium activated sodium iodide crystal scintillation counter[1] utilizing a radiation analyzer[1] to reduce the influence of natural radiation. The activity present in each sample was counted to a pre-set count of 10,000 in order to reduce radiation statistical probability error and the activity then recorded as counts per minute per ml. of cells by the appropriate calculation from the packed cell volume. The circulating erythrocyte volume was calculated as follows:

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[1]Nuclear-Chicago Corporation, 333 E. Howard Avenue, Des Plaines, Illinois.

$$\frac{\text{(counts / min. / ml. standard) (250) (ml. injected)}}{\text{counts / min. / ml. cells}}$$

= Circulating erythrocyte volume

3. Erythrocyte life span

Erythrocyte survival time was estimated by labeling the red cells with Cr^{51}. Blood was withdrawn from a donor pig or from the pig to be studied and placed in special formula ACD solution. Two microcuries of radioactive sodium chromate were added for every one ml. of blood and the mixture incubated at 37° C with intermittent agitation. After 30 minutes of incubation two mg. of ascorbic acid were added for every one ml. of blood present. Fifteen ml. of the Cr^{51} labeled blood were injected into an ear vein.

Oxalated blood samples were obtained 15 minutes after the injection. When a normal survival curve was expected, additional samples were taken once daily for the first week, on alternate days for the next 12 days, and every fourth day thereafter until 40 days had elapsed after the injection. When markedly decreased survival curves were anticipated, additional samples were taken at 15 minute intervals for four hours after the injection. The packed cell volume was determined on each blood sample by the

microhematocrit method. One ml. of blood was pipetted into a tube for radioactive counting and hemolyzed with two ml. of distilled water. All samples except the 15 minute sample, which was counted for activity immediately for determination of red cell volume, were refrigerated for counting later. All samples from the same animal were counted on the same day to eliminate correction for radioactive decay and counting apparatus variation. The samples were counted in the same equipment as described in the erythrocyte volume methods. The activity present was counted to a pre-set count of 10,000 and the activity recorded as counts per minute per ml. of red cells by the appropriate calculation from the PCV. The PCV was corrected by a factor of 0.97 which has been shown to correct for the amount of plasma trapped in the packed cell fraction (Talbot and Swenson, 1963a). In order not to bias the survival curve with any early loss of chromium which is probably due to leakage of loosely-bound chromium to intact red cells (Mollison, 1961), the sample obtained at 24 hours after injection was considered to contain 100 per cent of the activity and this day was taken to be day 0 for those animals with normal survival curves. The activity in samples from pigs with cell survivals of less than 24 hours was extrapolated back to time of injection and this value was considered to be 100 per cent of the activity. The activity in all later

samples was calculated as per cent of the activity present at time 0.

Since the animals were growing during the experiment, the concentration of the isotope was diluted by the expanding blood volume as well as by death of individual labeled erythrocytes. Red cell volume at the beginning of the study was calculated from the amount of activity injected and the amount in the 15 minute sample. At the end of the study, plasma volume was estimated using the T-1824 technic and the red cell volume calculated from the total blood volume and the PCV. Total blood volume was calculated from the following formula:

$$\text{Blood Volume} = \frac{\text{Plasma Volume X 100}}{100 - (\text{corrected PCV})\ (0.72)}$$

The corrected PCV was multiplied by a factor of 0.72 which is the F_{cells} factor shown later in this thesis to correct the blood volume for overestimation when the plasma-PCV method is used.

To correct for the changing cell volume during the study period, the determined proportion of the Cr^{51} labeled cells remaining in the circulation was corrected by the following formula:

$$RBC_c^{ACT.} = \frac{(RBC_t^{ACT.})\ (CV_t)}{CV_o}$$

where: $RBC_c^{ACT.}$ is the proportion of Cr^{51} remaining after correction for an increase in cell volume.

$RBC_t^{ACT.}$ is the proportion of Cr^{51} remaining at time t.

CV_t is the calculated cell volume at time t. A linear increase was assumed between the two blood volume determinations.

CV_o is cell volume at day 0.

The corrected amounts of Cr^{51} remaining were expressed as the logarithm of the per cent remaining and analyzed by regression technics (Snedecor, 1956) as well as plotted on graph paper against time for each animal. From the regression calculations the half life (50 per cent survival time) was calculated. The means were tested for significant difference by the Student t-test (Snedecor, 1956).

4. Other hematological parameters

Erythrocyte and leukocyte enumerations were determined in duplicate, utilizing National Bureau of Standards certified pipettes, counting chambers and cover glasses.

Acceptable counts differed no more than twice the square root of the mean of the highest and lowest counts obtained in each counting square of the hemocytometer chamber. The diluting fluids used were 0.90 per cent NaCl solution for erythrocytes and 0.1N HCl for leukocytes.

The PCV was determined by the microhematocrit capillary tube method. Capillary tubes were filled with fresh oxalated blood at the time of venepuncture and centrifuged[1] for five minutes. Readings were made in a microhematocrit reader.[1]

Hemoglobin concentrations were determined by the acid hematin method of Cohen and Smith (1919), utilizing 20 cmm. of whole blood and five ml. of 0.1N HCl. After allowing 30 minutes for color development, samples were read in a spectrophotometer[2] against a 0.1N HCl blank. Standards were prepared for calibration of the spectrophotometer from a blood sample of known hemoglobin content determined by the iron method of Wong (1928).

The MCV, MCH and MCHC were calculated by the formulas described by Wintrobe (1961).

The F_{cells} factor was calculated from the following

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[1]Clay Adams, Inc., New York, New York.

[2]Coleman Jr. spectrophotometer, Coleman Instruments, Inc., Maywood, Illinois.

formula:

$$F_{cells} = \frac{(CV)\ (100)\ /\ (CV + PV)}{VPCV}$$

where: CV is erythrocyte volume.

PV is plasma volume.

VPCV is venous packed cell volume.

IV. RESULTS AND DISCUSSION

A. Plasma Volume

After defining the original objectives of the project and reviewing the literature concerning the objectives and methods concerned, it was found that none of the methods for measuring plasma volume had been validated for use in swine. Of the various methods available the T-1824 dye dilution technic was decided upon as being the method of choice if it could be validated as actually measuring the true circulating plasma volume.

1. Validation of a method

The T-1824 dye dilution technic has been found to be highly useful in measuring the circulating plasma volume in man (Gregersen, 1944), dog (Allen and Semple, 1951), rat (Wang, 1959), monkey (Gregersen *et al.*, 1959) and the cow (Reynolds, 1953). Its validity in the rabbit (Zizza and Reeve, 1958), however, has been questioned. The reasons are not clear but it has been shown by Allen *et al.* (1953a) that in the rabbit T-1824 is less firmly bound to plasma protein than in dog or man. This fact, or the existence of some free dye in the circulation during the

mixing period, or both, may cause overestimation due to unequal distribution and explain the more rapid loss rate seen in rabbits than in dogs and man. A rapid loss rate in itself is not, however, of prime importance since Gregersen and Rawson (1959) in a compilation of data showed that hemoglobin, which has a rapid loss rate in the dog, gives the same volume distribution as test substances that disappear more slowly.

Since no information was available on the validity of the T-1824 technic in the pig, experiments were carried out to determine whether the method was suitable for plasma volume determination in swine.

a. Light absorption of normal porcine serum One of the common methods of determining the concentration of T-1824 dye in serum is to read the absorbance directly. With the information from the work of Stone and Adams (1950) and our own observations of the varying opacities of porcine serums, serum specimens were obtained from 11 of the pigs before any dye had been administered to them. The light absorption was measured spectrophotometrically in these samples at 10 millimicron intervals from 350 through 750 millimicrons. Figures 1 and 2 show the absorption curves obtained from five of the serum samples. The absorption peaks at approximately 420 and 460 millimicrons are attributed to the presence of bilirubin and the peaks

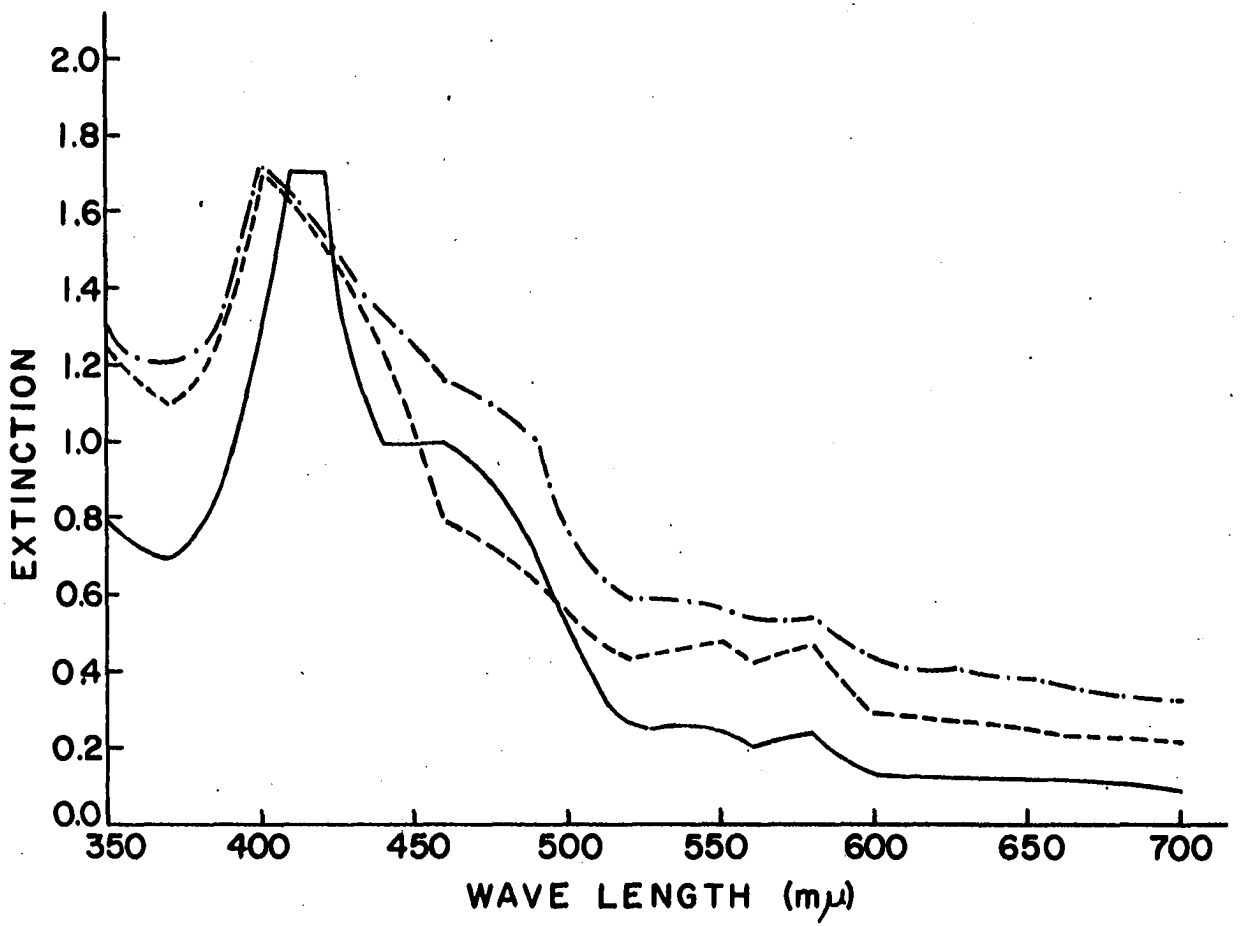


Figure 1. Absorption spectrum curves of serum from three pigs

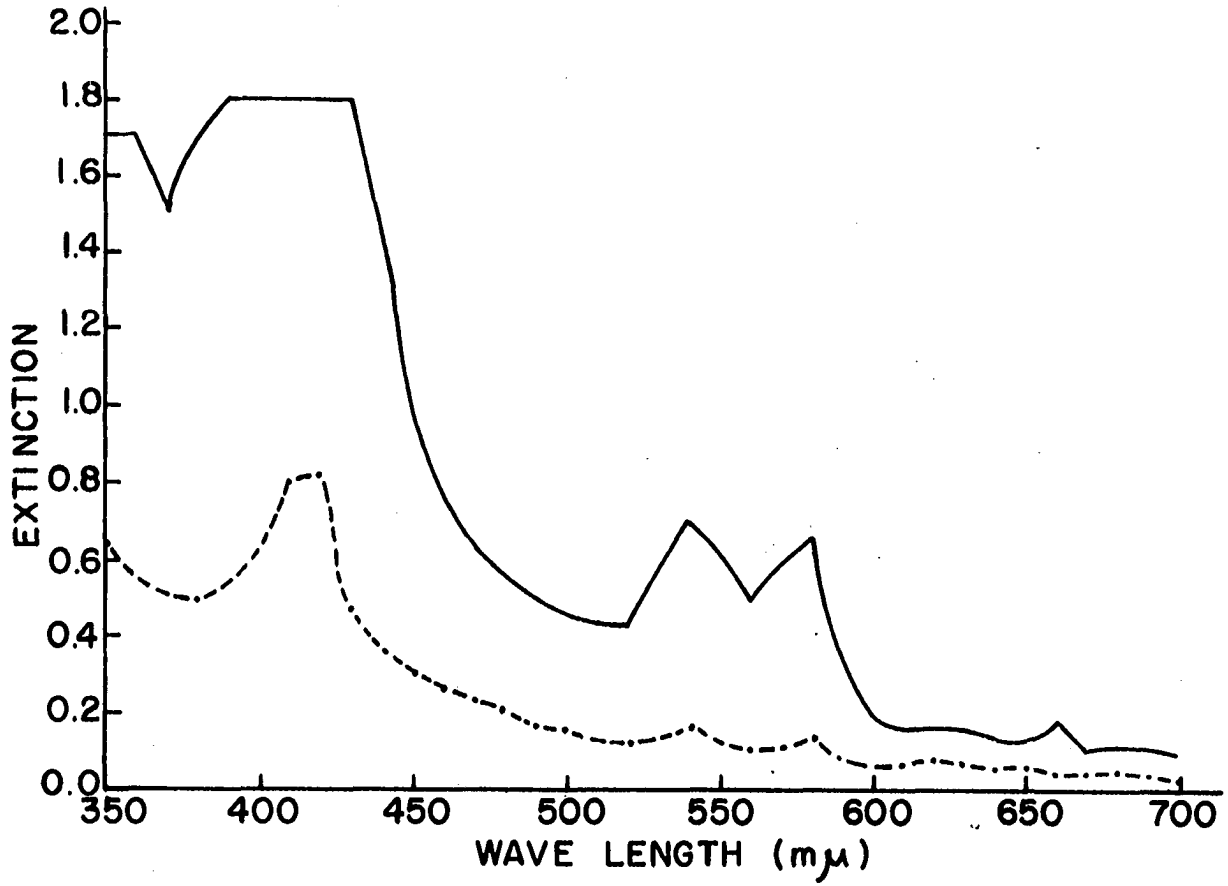


Figure 2. Absorption spectrum curves of serum from two pigs

around 540 and 580 probably are due to free hemoglobin even though none was visible to the naked eye. The feature of the curves of importance to the dye dilution technic is the variation of absorbance among experimental animals in the region of 620 millimicrons. This is the region of peak absorbance for T-1824 dye. Stone and Adams (1950) have stated that the aberrancy in absorption spectra curves may be due to lipemia. Regardless of the cause, the aberrancy itself would seem to invalidate the method of reading T-1824 dye concentration in the spectrophotometer directly from porcine serum samples.

b. Extraction of T-1824 dye from porcine serum The T-1824 dye extraction technic developed by Allen (1953) and modified by Campbell et al. (1958) has proved to be satisfactory under a variety of conditions; therefore, it seemed logical to investigate its use for extracting the dye from porcine serum. If the method were reproducible, it not only would offer the advantage of being free from variations due to inherent plasma color and lipemia but also would eliminate any errors due to hemolysis. The method was tried with varying known amounts of T-1824 dye added in vitro to serum samples from 18 pigs (Table 2). The recovery of the dye varied between 94 and 98 per cent with a mean of 96 per cent, which was similar to the results reported by Campbell et al. (1958) for human plasma. The

Table 2. Per cent recovery of T-1824 dye from porcine serum by an extraction technic

Sample number	Per cent recovery
1	97
2	94
3	97
4	97
5	94
6	97
7	97
8	97
9	98
10	94
11	96
12	97
13	97
14	97
15	96
16	97
17	95
18	98
mean	96.2

technic was, therefore, deemed valid for use with porcine serums.

c. Behavior of T-1824 dye in the porcine circulatory system All test substances used for determining plasma

volume have a measurable loss rate. In those species that T-1824 dye excretion has been investigated, graphic analysis of the time-concentration curve on a semi-log plot with back extrapolation of the disappearance curve is now the accepted method of arriving at a value for the amount of dye that would have been present had there been no loss between the time of injection and the time of sampling (Gregersen and Rawson, 1959). The validity of graphic analysis depends upon the assumption that the loss rate during the mixing period is correctly estimated from the slope of the disappearance curve. In order to validate this assumption, two different plasma volume determinations were performed in each of six of the experimental pigs. One determination was made with an amount of dye that would result in approximately 0.01 mg. of dye per ml. of plasma. This determination was immediately followed by an injection of dye that would result in a plasma dye concentration of approximately 0.2 mg. per ml. of plasma. The actual amounts of dye injected were determined by weighing to the third decimal place. The two determinations on all pigs each gave the same plasma volume within experimental error. Table 3 summarizes the data obtained. It is highly improbable that the same volume distribution would be obtained with both large and small amounts of injected dye if the loss rate during the mixing period were different

Table 3. Consecutive plasma volume determinations using varying concentrations of T-1824 dye

Pig no.	Plasma volume A[a] (ml.)	Plasma volume B[b] (ml.)	Difference A-B (ml.)	Percentage error
1	816	817	-1	0.1
2	857	820	37	4.3
3	882	871	11	1.2
4	884	934	-50	5.5
5	2,133	2,036	97	4.5
6	1,092	1,035	57	5.3

[a]Determination made with about 1 mg. of dye per kilogram of body weight.

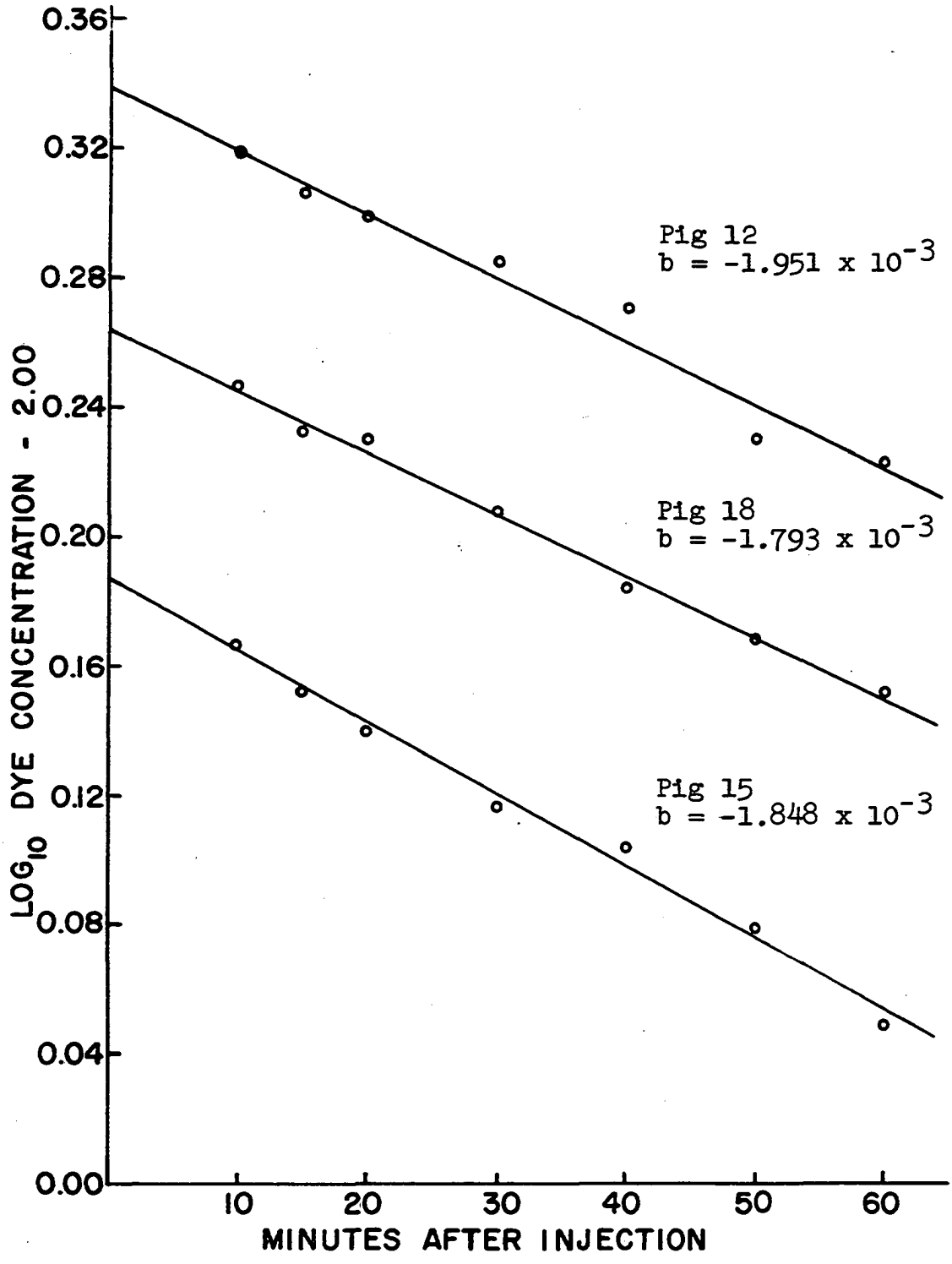
[b]Determination made with about 10 mg. of dye per kilogram of body weight.

than the loss rate thereafter (Gregersen and Rawson, 1959). Therefore, for the pig, graphic analysis with back extrapolation is a valid method of obtaining the initial dye concentration.

In order to obtain information concerning mixing time and loss rate of the dye in the porcine circulatory system, the change in dye concentration with respect to time was determined in 14 pigs. The pigs were anesthetized with sodium pentobarbital, the heart was catheterized through the external jugular vein and a pre-injection blood sample

obtained. Approximately one mg. of T-1824 dye per kg. of body weight (the exact amount was determined by using a calibrated syringe) was injected through the catheter and rinsed with five ml. of heparin solution. Five ml. blood samples were then withdrawn through the catheter at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50 and 60 minutes after the injection. The catheter was kept full of heparin solution between sampling times. A portion of blood was always withdrawn before and reinjected after the sample was taken in order to prevent contamination of the sample with any blood or heparin solution that was static within the catheter. The blood samples were allowed to clot, centrifuged for 15 minutes, and the serum harvested for extraction of the dye. The concentration of dye in each sample was determined after extraction by spectrophotometric comparison with a known standard solution of the dye and the results were plotted on semilog paper against time. Figure 3 shows the results from three of the experimental animals and Figure 4 shows the results from one of the animals as well as the regression data calculated for the individual dye excretion curve. There is a straight line relationship after the first ten minutes. The mixing phase occurs during the first ten minutes. Table 4 summarizes the calculations made for each decay curve to evaluate the relationship between dye concentration and time. The regression

Figure 3. T-1824 dye decay curves from circulatory systems of three pigs (b = regression coefficient in mg. T-1824/ml./min.)


0.36
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
LOG10 DYE CONCENTRATION - 2.00
10
20
30
40
50
60
MINUTES AFTER INJECTION
Pig 12
b = -1.951 x 10-3
Pig 18
b = -1.793 x 10-3
Pig 15
b = -1.848 x 10-3

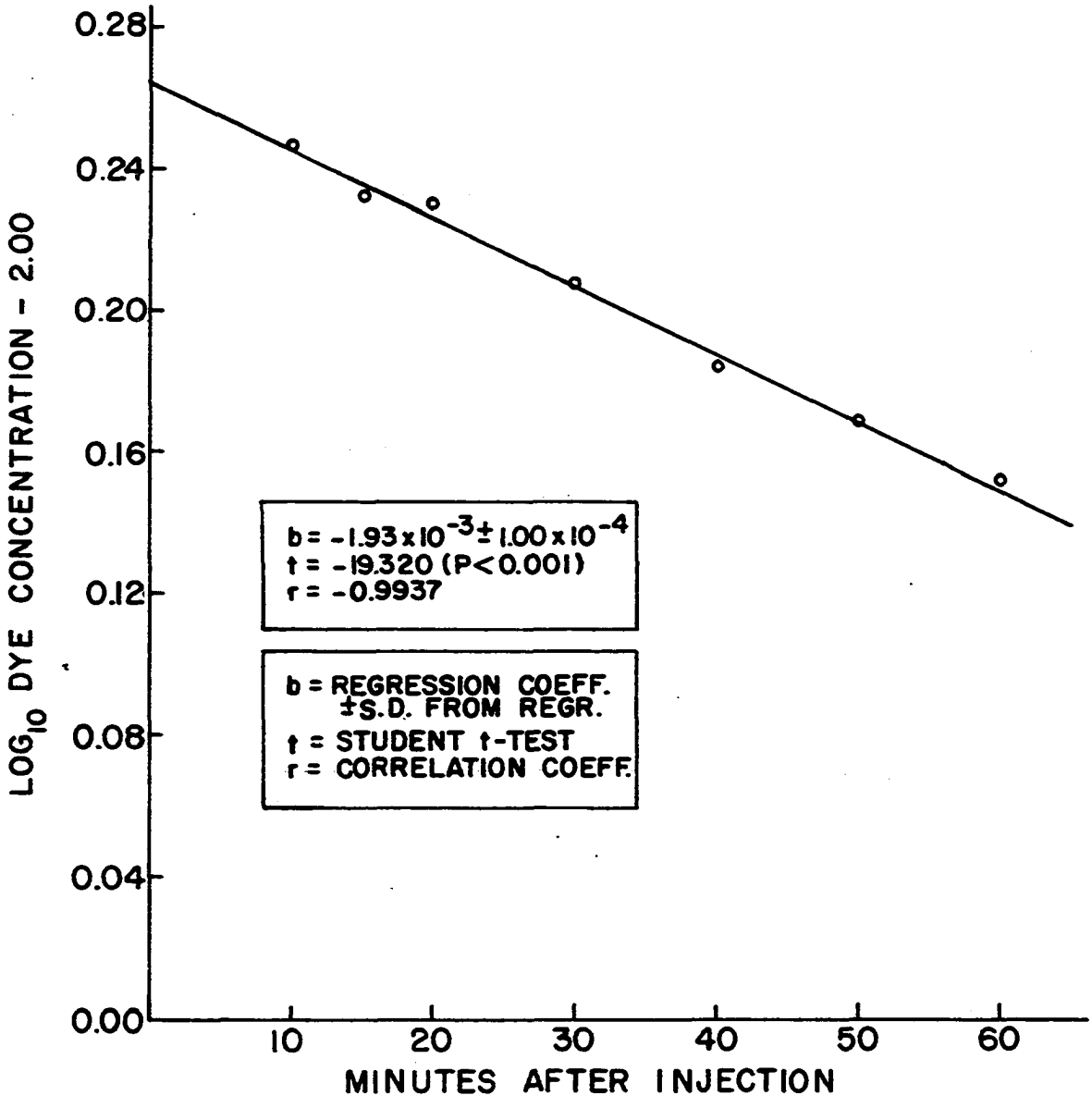


Figure 4. T-1824 dye decay curve and calculated regression data from circulatory system of one pig

Table 4. Disappearance of T-1824 dye from the porcine circulatory system[a]

Pig no.	b ($\times 10^{-3}$)	SD ($\times 10^{-4}$)	t	r	Loss rate (%/hr.)
6	-1.806	2.91	-6.210**	-0.9404	22.4
7	-1.113	1.98	-5.621**	-0.9415	15.4
8	-2.123	2.34	-10.073***	-0.9934	25.1
9	-1.345	2.34	-5.748**	-0.9202	17.3
10	-1.740	3.87	-5.496**	-0.8968	21.1
11	-1.110	1.65	-6.727**	-0.9678	14.4
12	-1.951	1.51	-12.920***	-0.9864	23.4
13	-1.494	1.60	-10.338**	-0.9860	21.2
14	-2.010	1.66	-12.108***	-0.9831	35.8
15	-1.848	3.89	-4.751**	-0.9231	22.3
16	-2.229	0.93	-23.968***	-0.9968	26.6
17	-1.505	2.80	-5.375**	-0.9239	18.9
18	-1.793	1.61	-12.137***	-0.9799	22.1
19	-1.932	1.00	-19.320***	-0.9937	23.4

[a]b = regression coefficient; SD = standard deviation from regression; t = Student's "t" test value; r = correlation coefficient; average r = -0.9734; Chi square = 16.583 ($P > 0.24$); and mean loss rate = 22.1 (SE_{mean} = 1.42).

**$P < 0.01$.

***$P < 0.001$.

coefficient (b) with its standard deviation from regression (SD) represents the slope of the decay curve. Each regression coefficient was tested with the Student-t test (Snedecor, 1956). The probability that the experimentally obtained values do not represent true values that lie on the regression line is less than 0.001 for six of the individual experimental animals and less than 0.01 but greater than 0.001 for the other eight. The regression coefficients were all analyzed by analysis of covariance (Snedecor, 1956) which showed that none of the slopes of the curves were significantly different from each other and are thus sampling variations of a common decay curve.

The correlation coefficient (r) of dye concentration with respect to time varied from -0.8968 to -0.9968 with a mean of -0.9734. The null hypothesis that all 14 of the correlation coefficients came from the same population was tested by the Chi Square test (Snedecor, 1956) and could not be rejected.

The preceding analyses demonstrate that the loss of T-1824 from the blood vascular system of the porcine species is constant during the first hour after injection and that the loss rate can be described by the following first order rate reaction decay equation:

$$2.303 \log \frac{A_o}{A} = kt$$

where: t is the time elapsed between injection of the dye and withdrawal of the sample.

k is the loss rate of the dye from the blood stream.

A is the concentration of the dye at time t.

A_o is the concentration of the dye at the time of injection if it were completely mixed in the blood stream.

The mean loss rate during the first hour after injection for the 14 animals was 22.1 per cent of that injected. This is rather high compared with the rates reported for several other species. This loss of dye from the blood vascular stream is considered by many people to represent a turnover of plasma albumin (Reynolds, 1953) and it seems reasonable to postulate, as Reynolds (1953) has done for the bovine, that a rapid lymph turnover may be a characteristic of swine.

Table 5 shows the values for plasma volume and estimated total blood volume obtained from each of the pigs used to study the behavior of T-1824 dye in the circulatory system. The plasma volume and estimated total blood volume, when expressed as ml. per kg. of body weight, vary inversely with the weight of the animal. This portion of the experiment was not designed specifically to ascertain average

Table 5. Plasma volume and estimated total blood volume of pigs

Pig no.	Weight (kg.)	Venous PCV[a] (%)	Plasma volume (ml.)	Plasma volume (ml./kg.)	Estimated total blood volume[b] (ml.)	Estimated total blood volume[b] (ml./kg.)
6	25.2	29	1,096	43.5	1,543	61.2
7	28.0	32	1,436	51.3	2,112	75.4
8	31.2	29	1,623	52.0	2,286	73.3
9	28.9	37	1,518	52.5	2,410	83.4
10	22.9	32	1,314	57.4	1,932	84.4
11	26.3	26	1,503	57.1	2,031	77.2
12	20.7	29	1,238	59.8	1,744	84.2
13	7.3	18	673	92.2	821	112.5
14	19.5	34	1,070	54.9	1,621	83.1
15	10.1	27	676	66.9	926	91.7
16	15.0	27	974	64.9	1,334	88.9
17	19.4	27	1,195	61.6	1,637	84.4
18	19.5	24	1,234	63.3	1,624	83.3
19	20.9	33	1,141	54.6	1,703	81.5

[a]Corrected by a factor of 0.97 for plasma trapped among the cells.

[b]Calculated from: $\frac{\text{Plasma volume}}{\text{100-corrected PCV}} \times 100$

porcine blood volume values but the values obtained do compare favorably with those reported by Hansard et al. (1951) and Bush et al. (1955) who used P^{32} and Cr^{51}, respectively, to determine erythrocyte volume and estimated total blood

volume in swine. Emphasis is placed on the fact that the method used (T-1824-PCV method) overestimates total blood volume in swine considerably, as is discussed in the section on F_{cells} because of the fact that the venous packed cell volume measurement is not a true representation of the volume distribution of cells in the entire circulatory system.

d. Packed cell volume correction factor One of the sources of error in any blood volume computation which is estimated by measuring only the plasma volume and the packed cell volume (PCV) or only the cell volume and the PCV involves the PCV determination. The PCV reading obtained is not identical with the volume percentage of cells in a blood sample. In order to obtain the true volume percentage of cells in a blood sample one must correct the PCV for the plasma that is trapped within the packed cell fraction. The correction depends upon the centrifugal force and the time of spinning. Using the microhematocrit method, it was determined, by means of the T-1824 dilution technic in vitro on twenty-four 50 ml. samples of porcine blood that the packed cell column contained one to six per cent plasma with a mean of 3.13 per cent (Table 6). A correction factor of 0.97 has therefore been used to correct PCV determinations used in blood volume computations.

Table 6. Packed cell volume correction factor for micro-hematocrit method

Sample no.	PCV (%)	Plasma volume (ml.)	Cell per cent	Difference (%)
1	16	44	12	4
2	16	44	12	4
3	17	46	12	5
4	16	43	14	2
5	30	36	28	2
6	24	40	20	4
7	29	38	24	5
8	29	36	28	1
9	29	36	28	1
10	32	35	30	2
11	38	32	36	2
12	36	33	34	2
13	38	34	32	6
14	36	33	34	2
15	34	34	32	2
16	27	37	26	1
17	32	36	28	4
18	36	34	32	4
19	29	37	26	3
20	24	40	20	4
21	21	42	16	5
22	23	41	18	5
23	24	40	20	4
24	27	37	26	1
			Mean	3.13
			Standard deviation	1.64

2. Changes with age and during anemia

The data obtained for plasma volume in pigs from birth through six weeks of life are presented in Tables 7 and 8 and Figures 5 and 6. Table 7 and Figure 5 show the total plasma volume at weekly intervals for anemic control pigs as well as iron dextran treated pigs. Table 8 and Figure 6 show plasma volume expressed as ml. per kilogram of body weight in the same animals. The values presented in both tables are means plus or minus one standard deviation. The number of animals used to derive the mean is given in parenthesis following the standard deviation.

Table 7. Effect of iron dextran and age on total plasma volume of pigs

Age	Controls			Iron dextran		
	Mean (ml.)	SD[a]	n[b]	Mean (ml.)	SD	n
Birth	89.9	13.3	(21)	88.0	12.7	(19)
1 week	170.5	27.9	(27)	154.9	31.2	(25)
2 weeks	247.7	21.7	(27)	222.3	40.7	(24)
3 weeks	327.2	66.9	(24)	318.0	59.6	(24)
4 weeks	439.1	120.0	(23)	395.0	103.6	(25)
5 weeks	468.4	106.0	(20)	519.4	98.2	(20)
6 weeks	569.0	134.0	(23)	553.8	141.0	(21)

[a]Standard deviation of the mean.

[b]Number of animals upon which mean is based.

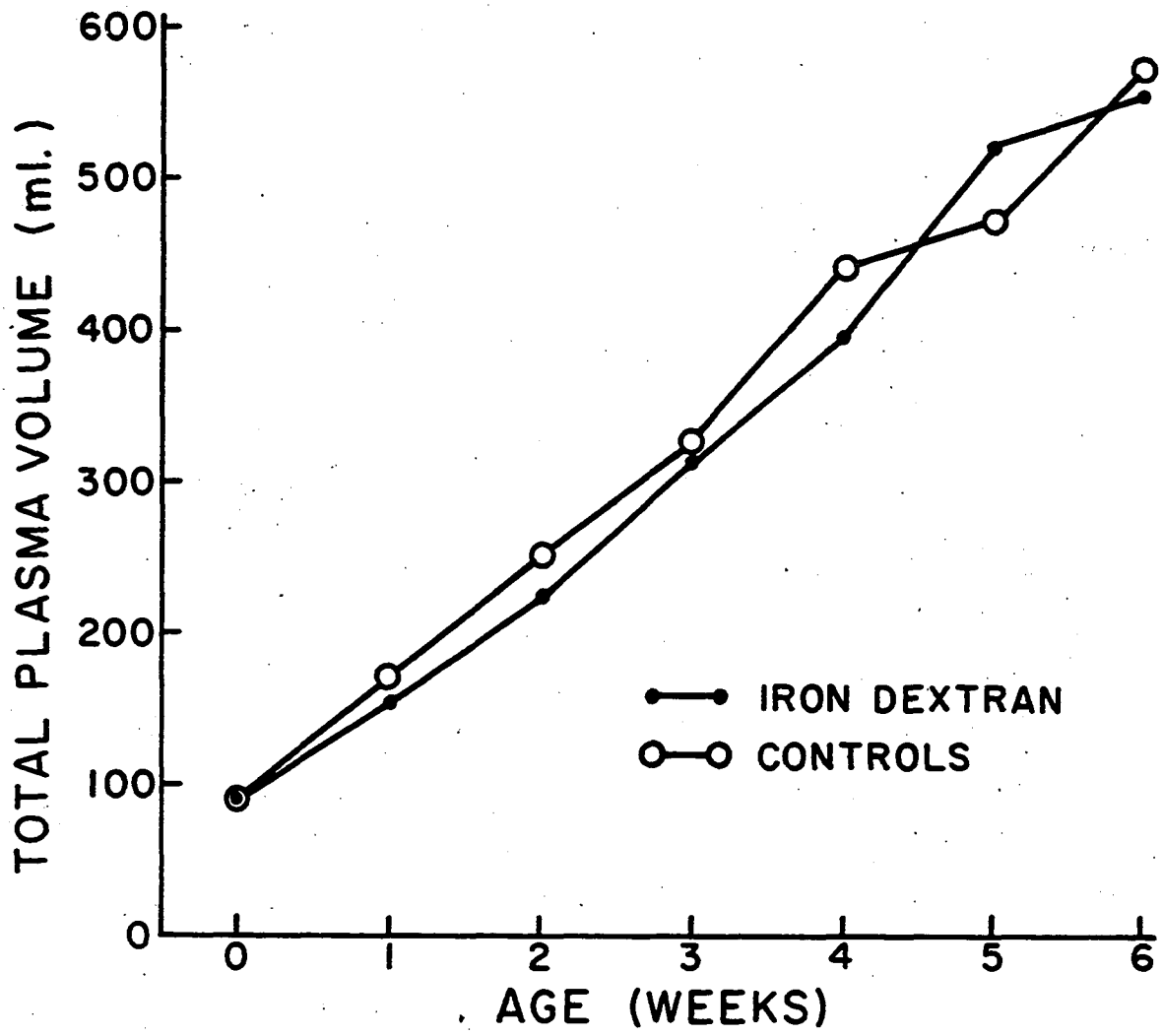


Figure 5. Effect of iron dextran and age on total plasma volume of pigs

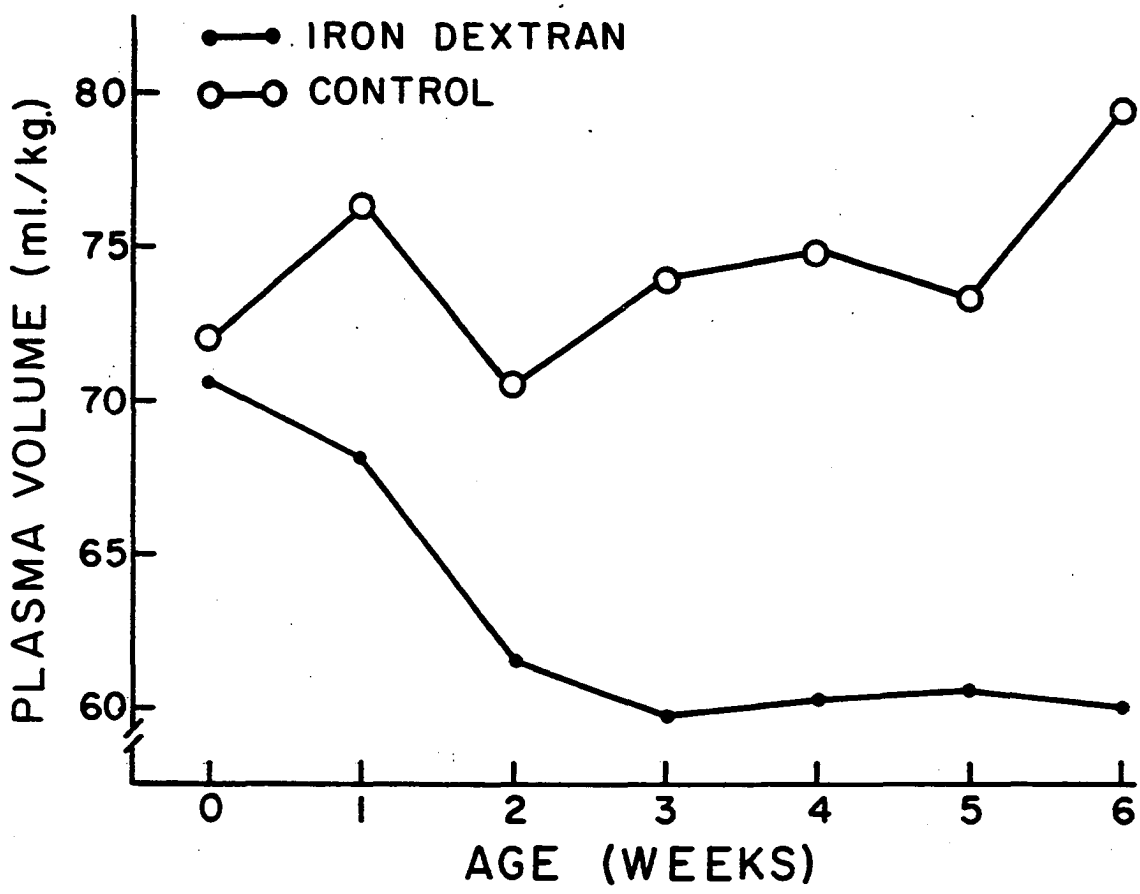


Figure 6. Effect of iron dextran and age on plasma volume per kilogram of body weight of pigs

Table 8. Effect of iron dextran and age on plasma volume per kilogram of body weight of pigs

Age	Controls			Iron dextran		
	Mean (ml.)	SD[a]	n[b]	Mean (ml.)	SD	n
Birth	71.8	10.6	(21)	70.3	6.7	(19)
1 week	76.4	12.0	(27)	68.1	13.8	(25)
2 weeks	70.5	9.6	(26)	61.6	6.4	(23)
3 weeks	74.3	10.3	(24)	59.4	8.7	(24)
4 weeks	74.8	11.7	(23)	60.4	7.7	(25)
5 weeks	73.2	8.4	(20)	60.8	6.5	(20)
6 weeks	79.3	9.5	(23)	59.8	6.5	(21)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

When the data are expressed as total plasma volume without regard for weight of the animal, it is apparent from Figure 5 that there is no appreciable difference between the iron dextran treated and the control pigs. This is verified by observing the statistical analysis of the data (Table 9), which shows no significant differences between treatments. The analysis of variance did show a significant difference due to age of the pig. This is to be expected since there is approximately a 900 per cent increase in plasma over the six week study period.

A more meaningful way of expressing the data is to

Table 9. Analysis of variance of total plasma volume of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	731,386.79	121,964.46	202.08***
Sex	1	1,172.23	1,172.23	1.94
Treatment	1	523.67	523.67	0.87
Time X Sex	6	945.32	157.55	0.26
Time X Treatment	6	5,312.11	885.35	1.47
Sex X Treatment	1	483.65	483.65	0.80
Time X Sex X Treat.	6	4,394.09	732.35	1.21
Individuals	295	-	603.52	-

***P < 0.005.

relate the plasma volume to the physical size of the pig. This was done by expressing the data as plasma volume per kilogram of body weight (Table 8 and Figure 6). The data show that there is a higher plasma volume per unit of body weight in the untreated pigs from one through six weeks of age when compared to the iron dextran group. The analysis of variance (Table 10) verifies this statement statistically and also shows that there is a significant difference due to age. The relationship of these differences to the changes in the other blood volume parameters is discussed under total blood volume changes.

Table 10. Analysis of variance of plasma volume per kilogram of body weight of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	131.8009	21.9668	2.63*
Sex	1	4.8722	4.8722	0.58
Treatment	1	911.7732	911.7732	109.20***
Time X Sex	6	40.5134	6.7532	0.81
Time X Treatment	6	199.9838	33.3306	3.99***
Sex X Treatment	1	2.4249	2.4249	0.29
Time X Sex X Treat.	6	24.8716	4.1453	0.50
Individuals	293	-	8.3492	-

*P < 0.05.

***P < 0.005.

B. Erythrocyte Volume

The first objective of this study was to ascertain whether or not the baby pig is capable of producing erythrocytes and hemoglobin in proportion to the increase in total body mass during the first six weeks of life. The body weight gains are shown in Figure 7 and Table 11. The changes in erythrocyte volume are shown in Tables 12 and 13 as well as graphically in Figures 8 and 9. The data for total red cell volume, as evidenced by the analysis of

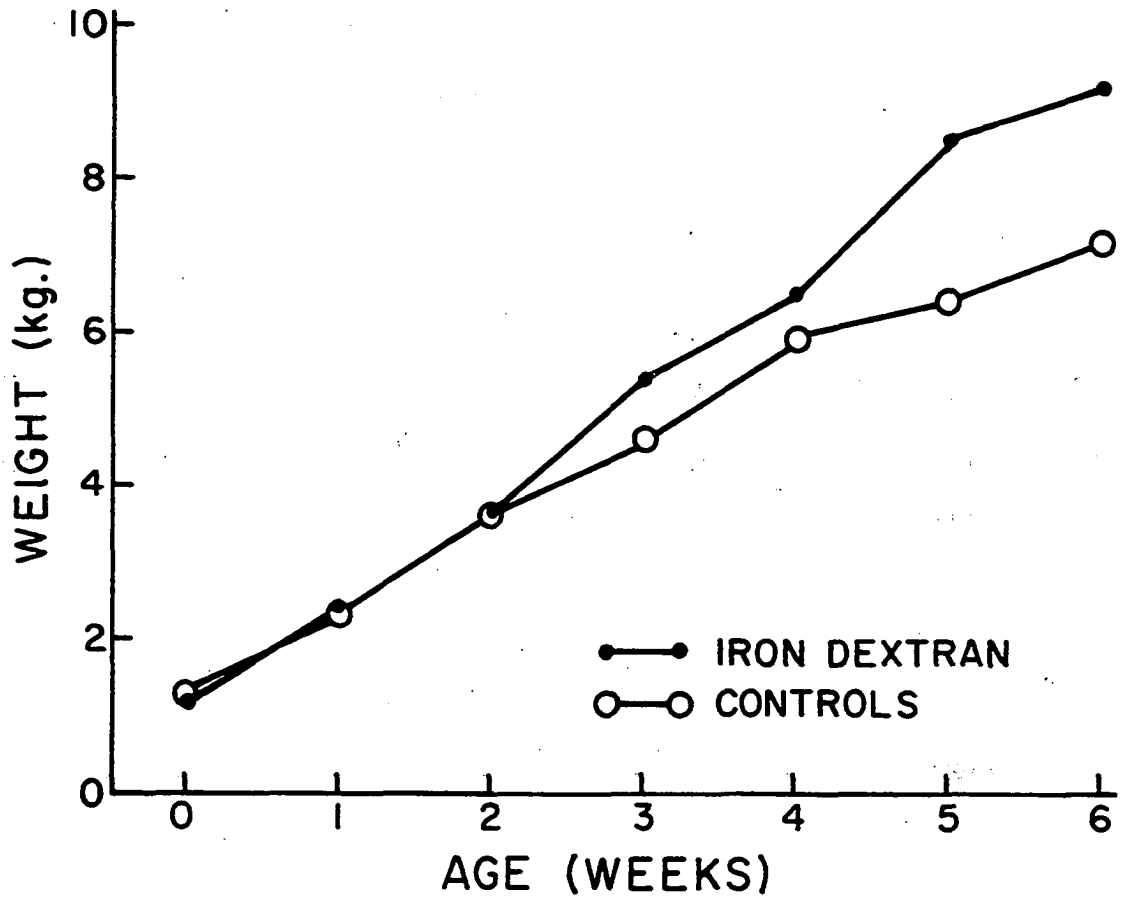


Figure 7. Effect of iron dextran and age on total body weight of pigs

Table 11. Effect of iron dextran and age on body weight of pigs

Age	Controls			Iron dextran		
	Mean (kg.)	SD[a]	n[b]	Mean (kg.)	SD	n
Birth	1.29	0.19	(21)	1.25	0.17	(19)
1 week	2.29	0.55	(27)	2.36	0.64	(25)
2 weeks	3.58	0.84	(27)	3.63	0.85	(23)
3 weeks	4.56	1.10	(25)	5.40	1.00	(24)
4 weeks	5.91	1.70	(28)	6.52	1.60	(26)
5 weeks	6.45	1.30	(20)	8.54	1.40	(20)
6 weeks	7.22	2.00	(25)	9.16	2.10	(23)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

variance (Table 14), show a significant increase in red cell volume due to both increasing age as well as treatment with iron dextran. Those animals that received iron dextran more than doubled their total red cell mass during the first week of life and almost doubled it again (84 per cent increase) during the second week of life. This is in contrast to the control animals who showed only a 25 per cent increase during the first week of life and a similar 25 per cent increase the second week. When body weight and erythrocyte volume are correlated so that the erythrocyte volume is expressed as erythrocyte volume per kilogram

Table 12. Effect of iron dextran and age on total red cell volume of pigs

Age	Controls			Iron dextran		
	Mean (ml.)	SD[a]	n[b]	Mean (ml.)	SD	n
Birth	29.4	9.8	(18)	25.3	7.6	(16)
1 week	36.8	10.7	(25)	55.4	18.6	(22)
2 weeks	45.9	13.0	(25)	91.2	23.5	(21)
3 weeks	61.5	20.4	(19)	145.2	22.8	(18)
4 weeks	84.8	35.4	(22)	172.2	54.2	(22)
5 weeks	86.4	28.2	(18)	180.8	26.7	(17)
6 weeks	102.4	41.2	(21)	194.6	45.3	(17)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

Table 13. Effect of iron dextran and age on red cell volume per kilogram of body weight of pigs

Age	Controls			Iron dextran		
	Mean (ml.)	SD[a]	n[b]	Mean (ml.)	SD	n
Birth	22.6	5.9	(18)	20.2	2.7	(16)
1 week	16.5	4.6	(25)	22.9	3.5	(22)
2 weeks	13.1	1.9	(24)	25.4	2.1	(20)
3 weeks	14.4	4.6	(19)	28.0	3.2	(18)
4 weeks	13.4	3.9	(22)	25.2	3.3	(22)
5 weeks	13.8	3.9	(18)	21.1	2.9	(17)
6 weeks	13.3	3.9	(21)	20.9	3.8	(17)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

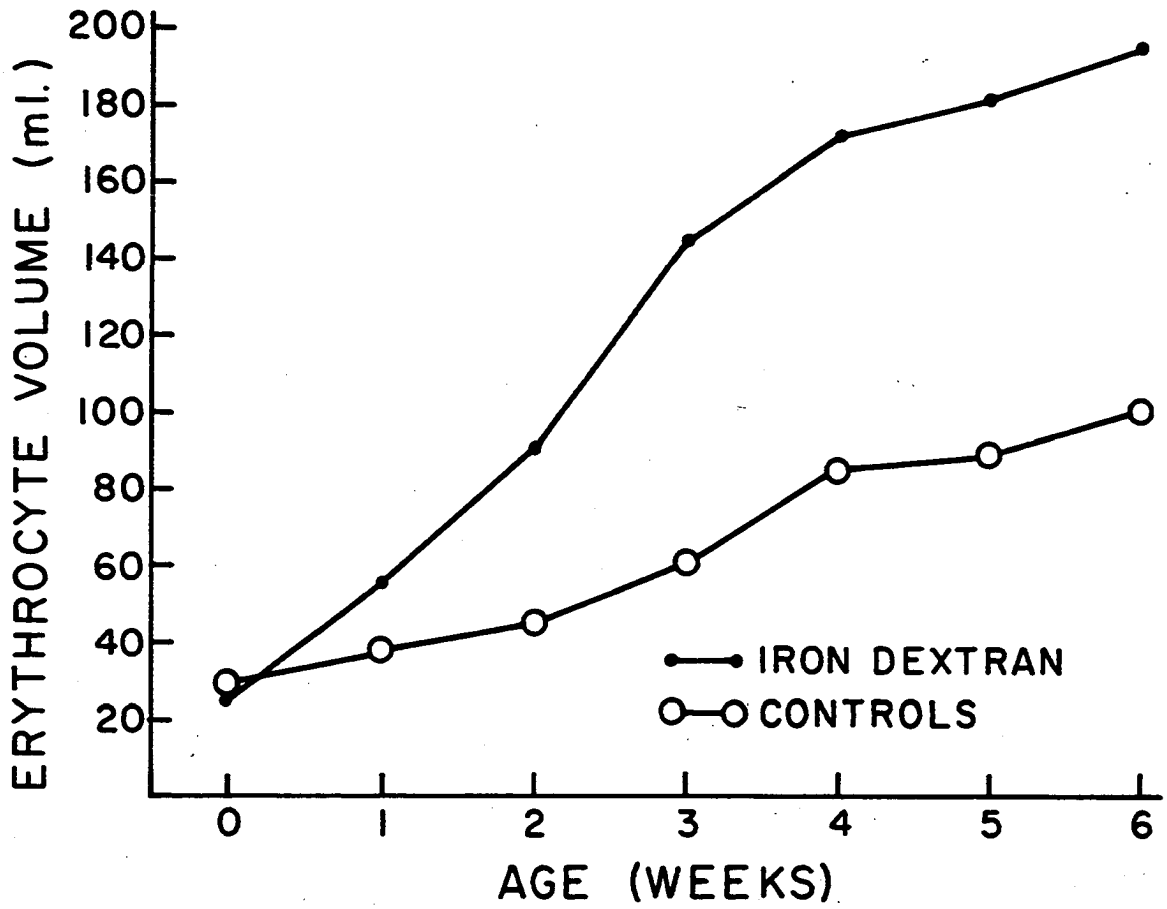


Figure 8. Effect of iron dextran and age on total erythrocyte volume of pigs

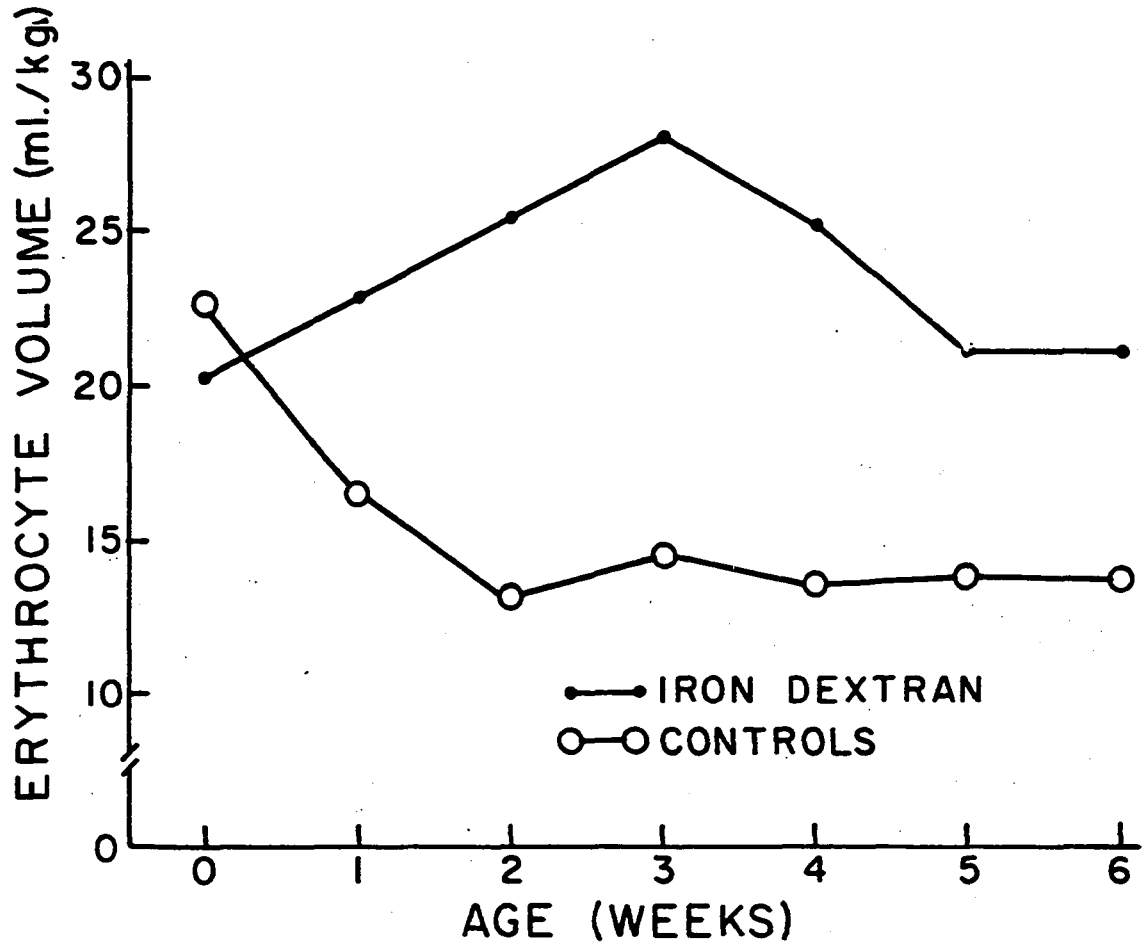


Figure 9. Effect of iron dextran and age on erythrocyte volume per kilogram of body weight of pigs

Table 14. Analysis of variance of total red cell volume of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	52,717.5694	8,786.2616	105.52***
Sex	1	53.5996	53.5996	0.64
Treatment	1	24,916.4024	24,916.4024	299.23***
Time X Sex	6	754.2299	125.7050	1.51
Time X Treatment	6	9,520.6177	1,586.7696	19.06***
Sex X Treatment	1	450.4032	450.4032	5.41*
Time X Sex X Treatment	6	509.7058	84.9510	1.02
Individuals	253	-	83.2677	-

*P < 0.05.

***P < 0.005.

of body weight, as has been done in Table 13 and Figure 9, it becomes apparent that the pig is capable of producing erythrocytes at a rate that is proportional to the increasing body weight. If the line in Figure 9 were exactly parallel with the base line of the graph, then the erythrocyte volume would be increasing in direct proportion to the increase in body weight. The animals that received iron dextran were not only capable of this but were capable of increasing the red cell volume at a more rapid rate than they were increasing total body weight, as evidenced by

the positive slope on the graph. The fact that adequate iron is necessary is verified by the values for the control pigs. Their mean erythrocyte volume dropped during the first two weeks to almost one-half of the birth value and maintained itself there until the end of the study. The analysis of variance (Table 15) shows a significant difference due to age and treatment as would be expected from Figure 9. The significant interaction between age and treatment in the analysis of variance is also demonstrated in Figure 9 by the positive curve for the iron dextran pigs during the first three weeks of life and the negative curve for the control animals during the same period. If there were no interaction between the two variables of age and treatment, the curves would have been parallel. The decrease in cell volume per unit of body weight after three weeks in the iron dextran treated pigs is not completely answered by this study. Previous studies by Hansard et al. (1951), Bush et al. (1955) and Jensen et al. (1956) on older swine have shown that there is a decrease of red cell volume per unit of weight as pigs increase in body size. Whether the decrease seen in this study after three weeks represents a normal physiological control mechanism or is due to some other influencing factor was not defined by this study. A possibility exists that at three weeks of age the pigs had utilized most of the storage iron from

Table 15. Analysis of variance of total red cell volume per kilogram of body weight of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	64.5238	10.7540	7.50***
Sex	1	4.8223	4.8223	3.36
Treatment	1	455.8743	455.8743	317.82***
Time X Sex	6	7.9469	1.3245	0.92
Time X Treatment	6	173.9716	28.9953	20.21***
Sex X Treatment	1	1.5841	1.5841	1.10
Time X Sex X Treatment	6	9.9320	1.6553	1.15
Individuals	251	-	1.4344	-

***P < 0.005.

the injected iron dextran and then did not have sufficient dietary iron to maintain the same production rate of red blood cells as when adequate iron stores were present.

C. Total Blood Volume

The total blood volume parameter is a calculated value. In this study the values were obtained by summation of the red cell volume and the plasma volume at each time of determination. The effect of iron dextran and age on total blood volume is shown in Table 16 and Figure 10.

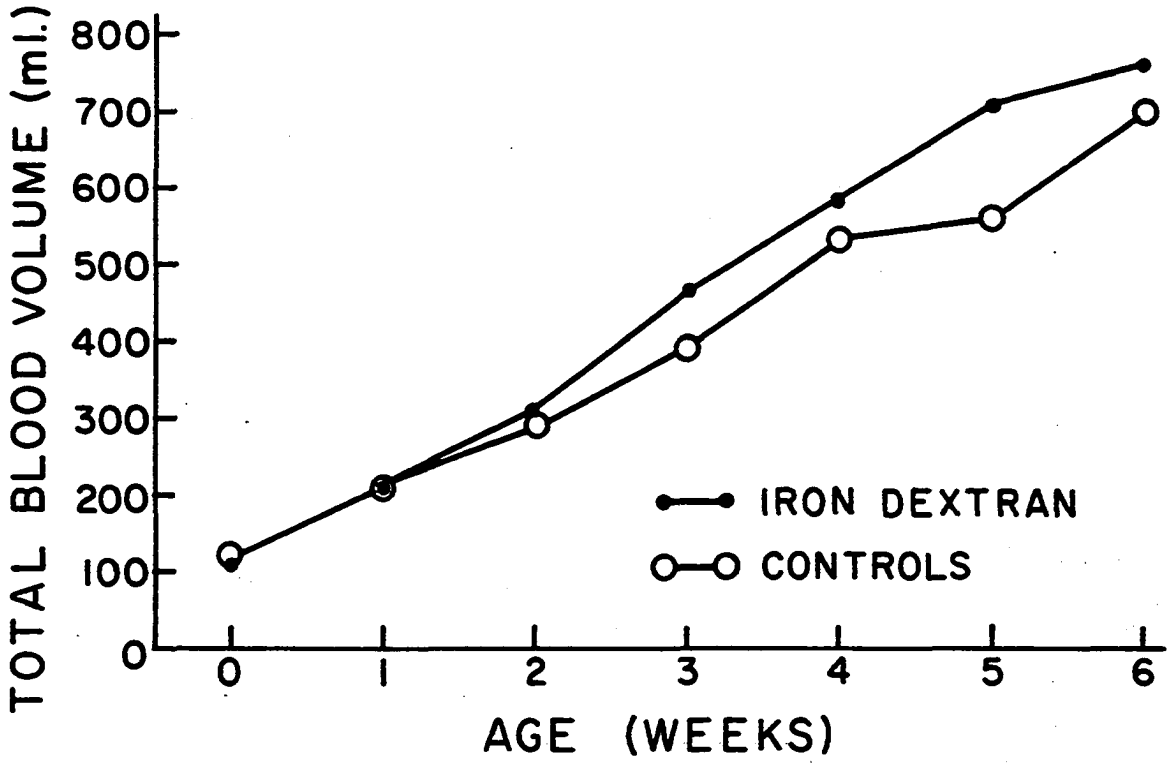


Figure 10. Effect of iron dextran and age on total blood volume of pigs

Table 16. Effect of iron dextran and age on total blood volume of pigs

Age	Controls			Iron dextran		
	Mean (ml.)	SD[a]	n[b]	Mean (ml.)	SD	n
Birth	118.3	15.9	(18)	112.5	17.0	(16)
1 week	206.3	37.5	(25)	211.5	48.0	(22)
2 weeks	293.0	56.3	(25)	314.0	63.1	(21)
3 weeks	392.5	85.0	(19)	469.0	73.1	(18)
4 weeks	534.5	145.5	(20)	585.5	145.0	(22)
5 weeks	557.0	125.3	(18)	710.5	112.2	(17)
6 weeks	697.0	134.1	(21)	758.0	159.3	(17)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

The analysis of variance is given in Table 17. There is a significant difference between the means of the iron dextran treated pigs and the control pigs from the third week until the end of the study but the values are biased by the fact that the control pigs did not weigh as much as the treated pigs. This bias is removed by presenting the data as blood volume per kilogram of body weight in Table 18 and Figure 11. The analysis of variance for these data is given in Table 19. In general, the values for treated and control animals tend to be quite similar. This is in agreement

Table 17. Analysis of variance of total blood volume of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	1,242,183.21	207,830.54	208.19***
Sex	1	2,091.57	2,091.57	2.10
Treatment	1	18,720.57	18,720.57	18.83***
Time X Sex	6	5,096.93	849.49	0.85
Time X Treatment	6	17,517.93	2,919.66	2.94**
Sex X Treatment	1	2,304.15	2,304.15	2.32
Time X Sex X Treatment	6	7,067.36	1,177.89	1.18
Individuals	251	-	994.45	-

**P < 0.01.

***P < 0.005.

Table 18. Effect of iron dextran and age on total blood volume per kilogram of body weight in pigs

Age	Controls			Iron dextran		
	Mean (ml.)	SD[a]	n[b]	Mean (ml.)	SD	n
Birth	93.1	9.9	(18)	90.0	6.1	(16)
1 week	94.1	13.0	(25)	90.7	11.4	(22)
2 weeks	83.8	10.6	(24)	88.2	6.2	(20)
3 weeks	90.5	11.9	(19)	90.3	9.8	(18)
4 weeks	85.7	10.7	(20)	86.6	9.1	(22)
5 weeks	88.1	9.2	(18)	82.4	8.8	(17)
6 weeks	92.6	10.1	(21)	81.2	10.5	(17)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

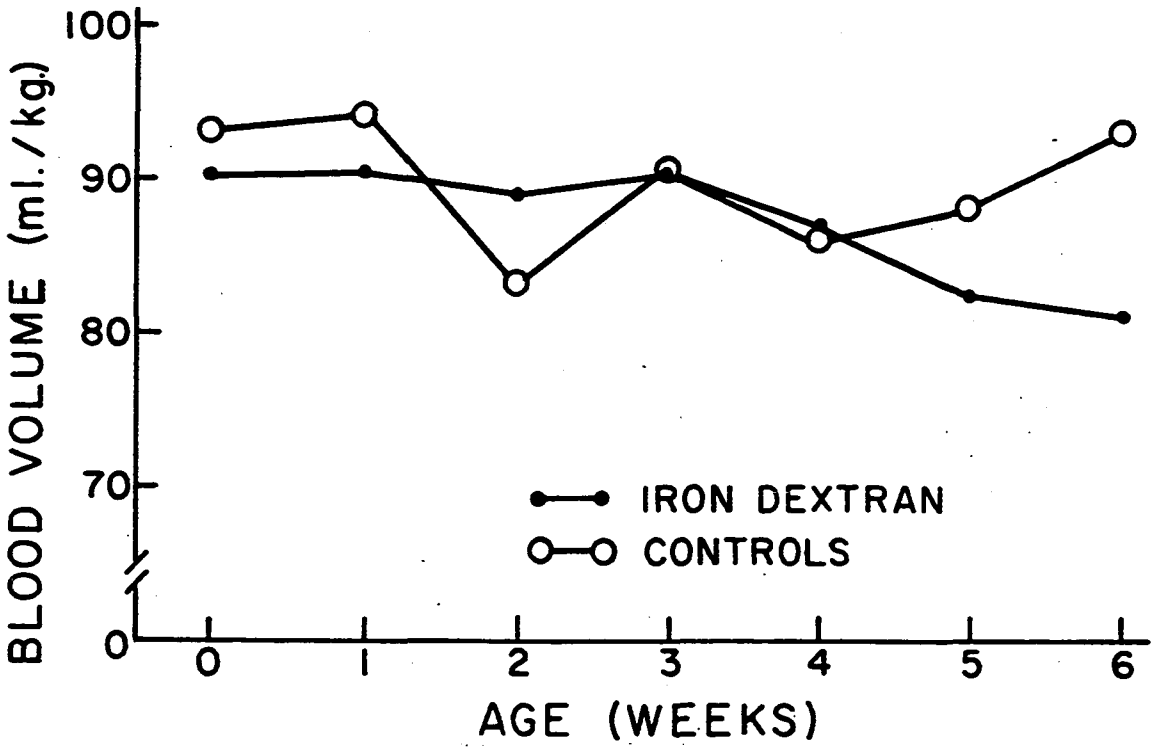


Figure 11. Effect of iron dextran and age on blood volume per kilogram of body weight of pigs

Table 19. Analysis of variance of total blood volume per kilogram of body weight of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	211.8567	35.3095	3.31***
Sex	1	29.1516	29.1516	2.73
Treatment	1	50.4109	50.4109	4.72***
Time X Sex	6	26.6348	4.4391	0.42
Time X Treatment	6	153.6872	25.6145	2.40*
Sex X Treatment	1	4.3766	4.3766	0.41
Time X Sex X Treatment	6	20.2513	3.3752	0.32
Individuals	249	-	10.6793	-

*P < 0.05.

***P < 0.005.

with the theory presented by Reeve et al. (1960) that a homeostatic mechanism of the body tends to keep the total blood volume constant. When the three parameters of red cell volume, plasma volume, and total blood volume are correlated, it is evident that the plasma volume adjusts in order to compensate for varying amounts of erythrocyte volume. While the values for blood volume per kilogram of body weight are quite similar for treated and control pigs, some differences do exist as evidenced by the analysis of variance in Table 19. The treated pigs maintained a

relatively uniform blood volume per unit size for the first three weeks of age and then began to decrease. The control animals showed fluctuating values both above and below the level of the treated pigs' values. At six weeks of age the values for the control pigs had not dropped but were still quite similar to their previous values. This is probably due to the lesser physical size of the control animals. This study was not designed to examine specific control mechanisms that regulate total blood volume. The data obtained though would suggest that whatever the control mechanisms in the pig's body are, they can and do maintain a relatively normal total blood volume even under the stress of anemia. The weekly fluctuations shown by the data could represent overcompensations by a sensitive feedback mechanism which regulates the blood volume.

D. F_{cells} Factor

Most of the published results on blood volumes of animals have been derived from determinations of plasma volume or erythrocyte volume alone. With either of these values and a packed cell volume (PCV) determination, the total blood volume is calculated. Gregersen and Rawson (1959) prefer to call this value "estimated blood volume" and to call the value obtained from determining both cell and

plasma volumes simultaneously, "true or measured blood volume." When the blood volume is estimated from a single volume determination and a PCV, there are two main sources of error. First of all, the PCV value is not identical with the volume percentage of cells in the blood sample being analyzed. Some of the plasma is always trapped in the packed cell fraction and the PCV should be corrected. This correction factor for swine blood has been discussed previously in this thesis. The second source of error in an estimated blood volume value involves the fact that the concentration of erythrocytes is not the same in all parts of the circulatory system. It is a generally accepted physiological fact that the cell/plasma ratio is lower in the capillaries and small vessels than in the larger arteries and veins. Therefore, the average cell percentage is lower than the venous cell percentage in most species of animals. This phenomenon is verified by the fact that plasma volume-PCV estimates of total blood volume are consistently higher than cell volume-PCV estimates. If both the cell volume and plasma volume as well as the PCV are known, the ratio of the average cell percentage to the venous cell percentage can be determined. This ratio gives the factor by which the venous cell percentage must be corrected in order to compute the average cell percentage and has been termed the F_{cells} factor (Gregersen and

Rawson, 1959). In order that previous and future studies of blood volume involving measurement of only one volume parameter may be more correctly calculated, the F_{cells} factor for pigs was calculated in this study. The analysis of variance of the data is presented in Table 20. The data show no appreciable differences due to age of the animal as evidenced by the non-significant F value, and therefore it can be stated that the F_{cells} factor is a constant as far as age of the pig is concerned between birth and six weeks of life. The analysis shows a significant difference in F_{cells} factor due to treatment and sex so the data were arranged without regard for age into treatment groups and sex groups. These data are presented in Table 21. When the data are observed as controls and iron dextran treated with no regard for sex, the iron dextran treated pigs had a mean value of 0.74 ± 0.07 while the anemic control pigs had a mean value of 0.71 ± 0.08. The difference of 0.03 was significant by the LSD test at the 0.005 level. Physiologically this difference could represent a simple protective phenomenon in the body. Since the control pigs were anemic and had considerably fewer red blood cells than the treated pigs, they could tend to keep a higher proportion of the available red cells in the capillaries, where nutrient transfer could take place, than in the larger vessels. Such a distribution would lower the

Table 20. Analysis of variance for F_{cells} factor of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	0.00326093	0.00054349	0.99
Sex	1	0.00268129	0.00268129	4.88*
Treatment	1	0.00704057	0.00704057	12.80***
Time X Sex	6	0.00131121	0.00021854	0.40
Time X Treatment	6	0.00615593	0.00102598	1.87
Sex X Treatment	1	0.00010414	0.00010414	0.19
Time X Sex X Treatment	6	0.00119336	0.00019889	0.36
Individuals	251	-	0.00054990	-

*P < 0.05.

***P < 0.005.

Table 21. F_{cells} factors for pigs from birth through six weeks of age[a]

		n	$\bar{x}$	s	d
Iron Dextran	Males	65	0.75	0.07	
	Females	68	0.73	0.07	0.02
Controls	Males	72	0.71	0.08	
	Females	74	0.70	0.08	0.01
Add Over Treatment	Males	137	0.73	0.07	
	Females	142	0.72	0.07	0.01
Add Over Sex	Iron D.	133	0.74	0.07	
	Controls	146	0.71	0.08	0.03***

[a]n = number of observations, $\bar{x}$ = mean, s = standard deviation, d = difference between means.

***P < 0.005.

F_{cells} value. Another possibility that should be considered is the physiological state of the spleen. The effect of greater storage of red cells in the spleen is a lowered F_{cells} value. Since the anemic control pigs probably had spleens that were functioning as erythropoietic organs, it is possible that they were also functioning as greater storage organs in proportion to the total mass of red cells available than were the spleens of the non-anemic iron dextran treated pigs. Future studies will be necessary to clarify the functional role of the spleen in relation to blood storage. Since the analysis of variance also showed a sex difference, the data were further analyzed with no regard for treatment. The mean for the males was 0.73 ± 0.07 and for the females was 0.72 ± 0.07. When the difference of 0.01 was compared to the calculated LSD, the difference was not significant but approached significance at the 0.05 level. This lack of agreement with the F test in the analysis of variance can be explained by the method used in calculating the analysis of variance. Unweighted means were used for the analysis of variance because of unequal subclass numbers. The F test for these calculations was significant at the 0.05 level which implies that the chances of being correct are 19 times out of 20 and the chances of being wrong are 1 out of 20. To this author's knowledge, no information is available concerning

sex differences in splenic function or volume distribution of erythrocytes. Since the means do approach statistical significance, future studies designed to elucidate possible sexual differences in these functions seem necessary.

Regardless of the above mentioned discrepancies, it seems logical to assume that blood volume estimations utilizing only a plasma volume or a red cell volume determination and a venous packed cell volume on non-anemic pigs between birth and six weeks of age should utilize a factor of 0.74 to correct the packed cell volume for unequal distribution of cells. It also seems logical to postulate that because of the relatively low numerical value of the F_{cells} factor, when compared to other species, the spleen of the pig from birth through six weeks of age assumes a significant physiological function as an erythrocyte storage organ.

E. Erythrocyte Life Span

Survival study of transfused erythrocytes is of general use both as a diagnostic aid and as a research tool in man and several domestic animals. The study of erythrocyte survival in swine was not an original objective of this research. During the process of performing Cr^{51} erythrocyte volume determinations, one litter of seven

pigs was found to have four individuals who appeared to destroy some of the donor's red blood cells when they were injected intravenously. This litter of pigs was not used for the blood volume studies but rather was investigated along with eight other pigs for possible variation in erythrocyte life span. In previous studies discussed in review of the literature, the erythrocyte life span in swine has been deduced from the results of cross-transfusion experiments (homologous transfusions). That is to say, the red cells were obtained from a donor pig and transfused into another pig, the recipient. The use of this method presupposes that the "new environment" for the injected cells will have no influence upon their survival time. Such an assumption seems not justified for various reasons. Hence, an "a priori" better method is estimation of the life span of the individuals own erythrocytes in its own circulation (autologous transfusion). A comparison of these two methods was the objective of this ancillary study and is reported here.

Fifteen pigs varying in age from 10 to 12 weeks at the beginning of the study were used. Four of the pigs were Durocs from two litters, four were Hampshires from two litters and the remaining seven were Landrace-Poland crossbreeds from one litter. The pigs were fed a commercial grower ration and water ad libitum throughout the

study. Erythrocyte survival time was estimated by labeling the red cells with Cr^{51}. Four of the animals received injections of autologous labeled cells while the other 11 received homologous labeled cells from a donor pig.

In Figure 12, the disappearance curve of radiochromium from the blood of one pig of each group is plotted on a semi-logarithmic scale. The data closely approximated a straight line in all of the experimental pigs and were verified by testing the "goodness of fit" of the experimental values to the calculated regression line by use of the Student-t test. In no case were the values significant at the 0.05 or less level and thus can be interpreted as sampling variations from the calculated regression line. The statistical methods used were identical to those used for the T-1824 dye dilution curves.

Differing opinions appear in the literature concerning whether Cr^{51} red cell survival curves are simple exponential or linear with respect to time. Practically speaking, the disappearance of radiochromium from the blood stream of swine represents both cell destruction and chromium elution. Bush *et al.* (1955) interpreted from their C^{14} glycine data that most of the red blood cells from swine are destroyed in a random manner. To this author's knowledge, the rate of chromium elution from swine erythrocytes has not been investigated. If the elution process is

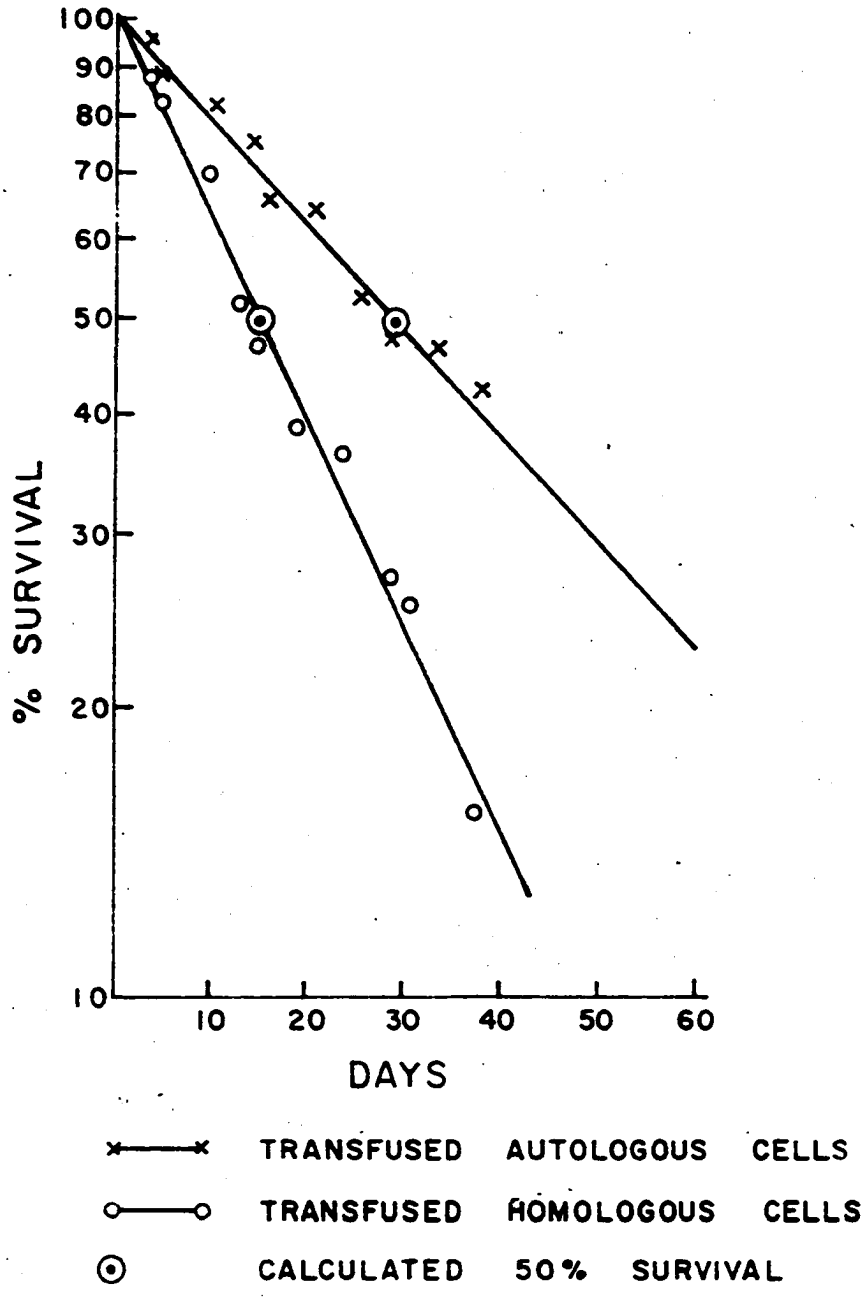


Figure 12. Survival of transfused porcine erythrocytes

exponential in swine, it would theoretically be possible for the aging process to be linear and still have a logarithmic curve when the two are combined. Regardless of whether the cell destruction process is linear or logarithmic, the disappearance of Cr^{51} from the blood stream when not corrected for chromium elution, represents a logarithmic process quite similar to that reported by Bush et al. (1956b).

The results concerning mean half-lives (50 per cent survival) of erythrocytes in swine are presented in Table 22. The mean half-life for the autologous injections of labeled red cells was 28.0 days while that for homologous injections was only 13.8 days. These values are quite different from those reported by Bush et al. (1956b), who found a mean half-life of 17.0 days in four normal growing swine that had received Cr^{51} labeled homologous cells. According to Mollison (1961), differences in time taken for 50 per cent of the chromium to leave the circulation of man after injecting labeled cells are due to a large extent to differences in the rate of elution of chromium, depending upon the medium in which the red cells are suspended at the time of labeling and whether they have been washed before labeling. Since all transfused cells in these studies were treated similarly, differences due to these factors can be ruled out and a true difference said

Table 22. Erythrocyte half-life determined by the Cr^{51} technic

Type of cells injected	No. of pigs	Half-life	
		Mean (days)	SD[a]
Autologous	4	28.0	4.0
Homologous	11	13.8	5.7

[a]Standard deviation of the mean; "t" value for difference between means is 12.56, $P < 0.001$.

to exist between the life spans of autologous and homologous injections of erythrocytes in swine.

The values for autologous injected cells indicate that the life span of swine erythrocytes can be considerably longer than values previously reported and may approximate the 25 to 30 day half-life value reported for man. Since the half lives for both man and swine are quite similar, it seems logical to assume that the true life span of swine erythrocytes may be close to the value of 110 days reported for man rather than the 62 to 63 day values listed by Calhoun and Smith (1958).

F. Other Hematological Parameters

The classical parameters of erythrocyte count, hemoglobin, and packed cell volume were determined on each pig at the same time erythrocyte and plasma volume determinations were being made. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also calculated from the experimental values. These were done not only to verify previous results but also to correlate these data with the blood volumes of anemic and iron dextran treated pigs.

Tables 23 through 34 list the mean values, the standard deviations and the analyses of variances for the above parameters. For any given age except birth, the mean of the iron dextran group is significantly higher ($P < 0.001$) than the corresponding control group for all parameters listed with the exception of the MCHC. The difference between the means was tested by use of the LSD test using a standard error of the mean calculated from the variance for individuals (error term) in an analysis of variance (Snedecor, 1956).

The total erythrocyte count (Table 23) not only demonstrated the marked difference between control and iron dextran treated groups, but also showed a significant ($P < 0.005$) progressive increase in numbers of red cells

Table 23. Effect of iron dextran and age on total erythrocyte count (per cubic mm. of blood) of pigs

Age	Controls			Iron dextran		
	Mean ($X 10^6$)	SD[a]	n[b]	Mean ($X 10^6$)	SD	n
Birth	5.130	1.03	(21)	4.865	1.07	(19)
1 week	3.985	0.98	(22)	4.648	0.95	(20)
2 weeks	4.460	0.70	(28)	5.829	1.00	(23)
3 weeks	4.778	1.23	(25)	6.326	0.97	(24)
4 weeks	4.534	1.28	(28)	6.514	0.85	(26)
5 weeks	5.873	1.62	(20)	6.857	0.57	(20)
6 weeks	5.275	1.76	(24)	7.250	0.72	(21)

[a]Standard deviation of the mean.

[b]Number of observations upon which the mean is based.

Table 24. Analysis of variance of total erythrocyte count of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	12.5559	2.0926	18.36***
Sex	1	0.0079	0.0079	0.07
Treatment	1	9.5940	9.5940	84.16***
Time X Sex	6	1.2776	0.2129	1.87
Time X Treatment	6	3.7979	0.6330	5.55***
Sex X Treatment	1	0.0364	0.0364	0.32
Time X Sex X Treat.	6	0.1475	0.0246	0.22
Individuals	294	-	0.1140	

***P < 0.005.

Table 25. Effect of iron dextran and age on hemoglobin values (grams per 100 ml. of blood) of pigs

Age	Controls			Iron dextran		
	Mean	SD[a]	n[b]	Mean	SD	n
Birth	11.8	2.2	(21)	11.0	2.3	(19)
1 week	8.3	1.4	(22)	11.1	2.7	(20)
2 weeks	7.4	1.1	(28)	13.4	1.3	(23)
3 weeks	7.4	1.7	(25)	14.2	1.5	(24)
4 weeks	6.4	1.7	(28)	13.4	1.7	(26)
5 weeks	7.1	1.9	(20)	12.4	1.4	(20)
6 weeks	6.3	3.1	(24)	12.3	2.9	(21)

[a]Standard deviation of the mean.

[b]Number of observations upon which the mean is based.

Table 26. Analysis of variance of hemoglobin of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	11.7689	1.9615	7.48***
Sex	1	2.5804	2.5804	9.83**
Treatment	1	156.8928	156.8928	597.91***
Time X Sex	6	2.9712	0.4952	1.89
Time X Treatment	6	48.3662	8.0610	30.72***
Sex X Treatment	1	0.0015	0.0015	0.01
Time X Sex X Treat.	6	1.1394	0.1899	0.72
Individuals	307	-	0.2624	-

**P < 0.01.

***P < 0.005.

Table 27. Effect of iron dextran and age on packed cell volume of pigs

Age	Controls			Iron dextran		
	Mean (%)	SD[a]	n[b]	Mean (%)	SD	n
Birth	33.0	6.7	(21)	30.6	6.7	(19)
1 week	25.5	3.8	(22)	34.6	5.8	(20)
2 weeks	22.1	3.6	(28)	39.2	3.1	(23)
3 weeks	23.6	5.9	(25)	41.1	3.2	(24)
4 weeks	20.8	4.7	(28)	39.1	4.1	(26)
5 weeks	22.4	5.8	(20)	35.2	3.2	(20)
6 weeks	20.4	8.0	(24)	35.2	4.1	(21)

[a]Standard deviation of the mean.

[b]Number of observations upon which the mean is based.

Table 28. Analysis of variance of packed cell volume of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	59.1020	9.8503	4.65***
Sex	1	16.2641	16.2641	7.68**
Treatment	1	1089.5040	1089.5040	514.38***
Time X Sex	6	21.9424	3.6571	1.73
Time X Treatment	6	313.7970	52.2995	24.69***
Sex X Treatment	1	0.3434	0.3434	0.16
Time X Sex X Treat.	6	12.4568	2.0761	0.98
Individuals	307	-	2.1181	

**P < 0.01.

***P < 0.005.

Table 29. Effect of iron dextran and age on mean corpuscular volume of pigs

Age	Controls			Iron dextran		
	Mean (μ^3)	SD[a]	n[b]	Mean (μ^3)	SD	n
Birth	64.2	8.1	(21)	63.1	7.6	(19)
1 week	66.0	11.9	(22)	78.4	13.1	(20)
2 weeks	50.3	5.9	(28)	69.2	10.0	(23)
3 weeks	50.3	9.7	(25)	66.2	9.3	(24)
4 weeks	47.3	6.8	(28)	61.3	10.9	(26)
5 weeks	38.6	5.5	(20)	51.4	5.1	(20)
6 weeks	40.2	6.5	(24)	49.7	6.5	(21)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

Table 30. Analysis of variance of mean corpuscular volume of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	2328.9820	388.1637	61.63***
Sex	1	36.2521	36.2521	5.76*
Treatment	1	966.2025	966.2025	153.41***
Time X Sex	6	59.1432	9.8572	1.57
Time X Treatment	6	245.7769	40.9628	6.50***
Sex X Treatment	1	0.1263	0.1263	0.02
Time X Sex X Treat.	6	21.9003	3.6500	0.58
Individuals	293	-	6.2980	

*P < 0.05.

***P < 0.005.

Table 31. Effect of iron dextran and age on mean corpuscular hemoglobin of pigs

Age	Controls			Iron dextran		
	Mean (μμgm.)	SD[a]	n[b]	Mean (μμgm.)	SD	n
Birth	23.2	2.6	(21)	22.7	2.7	(19)
1 week	21.2	2.9	(22)	25.4	3.6	(20)
2 weeks	16.7	1.7	(28)	23.5	3.3	(23)
3 weeks	15.8	3.1	(25)	22.8	3.3	(24)
4 weeks	14.4	3.0	(28)	21.1	4.4	(26)
5 weeks	12.3	1.9	(20)	18.0	2.2	(20)
6 weeks	12.9	2.7	(24)	17.4	3.0	(21)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

Table 32. Analysis of variance of mean corpuscular hemoglobin of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	264.4093	44.0682	56.69***
Sex	1	5.2117	5.2117	6.70**
Treatment	1	169.9372	169.9372	218.62***
Time X Sex	6	7.5726	1.2621	1.62
Time X Treatment	6	42.0163	7.0027	9.01***
Sex X Treatment	1	0.0513	0.0513	0.07
Time X Sex X Treat.	6	3.3658	0.5610	0.72
Individuals	293	-	0.7773	

**P < 0.01.

***P < 0.005.

Table 33. Effect of iron dextran and age on mean corpuscular hemoglobin concentration of pigs

Age	Controls			Iron dextran		
	Mean (%)	SD[a]	n[b]	Mean (%)	SD	n
Birth	36.1	4.8	(21)	36.0	2.7	(19)
1 week	31.7	2.0	(22)	31.7	1.8	(20)
2 weeks	33.3	2.5	(28)	34.1	1.7	(23)
3 weeks	31.3	2.3	(25)	34.5	1.9	(24)
4 weeks	30.2	3.4	(28)	34.3	1.8	(26)
5 weeks	31.8	1.7	(20)	35.0	1.9	(20)
6 weeks	31.9	3.8	(24)	34.9	2.5	(21)

[a]Standard deviation of the mean.

[b]Number of animals upon which the mean is based.

Table 34. Analysis of variance of mean corpuscular hemoglobin concentration of pigs

Source of variation	d.f.	S.S.	M.S.	F
Time	6	45.6674	7.6112	14.57***
Sex	1	0.3657	0.3657	0.70
Treatment	1	28.8463	28.8463	55.23***
Time X Sex	6	2.3793	0.3966	0.76
Time X Treatment	6	18.1258	3.0210	5.78***
Sex X Treatment	1	0.1008	0.1008	0.19
Time X Sex X Treat.	6	2.4171	0.4028	0.77
Individuals	306	-	0.5223	

***P < 0.005.

per unit of blood with increasing age in the non-anemic treated pigs. The inability of the control pigs to increase the red cell count at the same rate as the treated pigs is demonstrated by a significant ($P < 0.005$) treatment versus time interaction in an analysis of variance (Table 24). There were no sex differences in the erythrocyte counts.

The data for hemoglobin (Tables 25 and 26) demonstrated the same differences as the erythrocyte counts, also with a statistical probability of being correct in making the assumption 995 times out of 1000. The age differences in the treated pigs were biphasic, in that the mean hemoglobin values increased from 11.0 gm. per 100 ml. of blood at birth to 14.2 at three weeks of age and then progressively decreased to 12.3 at six weeks of age. These results are similar to values reported previously (Talbot and Swenson, 1963b). The hemoglobin values also showed a significant ($P < 0.01$) difference due to sex. The differences are small and inconsistent when observed by age groups and since the analysis of variance shows no significant interaction between sex and treatment, no interpretation of the difference is made.

The PCV values (Tables 27 and 28) show the same general trend as hemoglobin and the same significances. The peak PCV value is also at three weeks of age. When

this general trend of the hemoglobin and PCV values is compared to the erythrocyte volume per kg. of body weight (Figure 9) and the total blood volume per kg. of body weight (Figure 11), the explanation for the peak values at three weeks becomes evident. The total blood volume per unit of body weight is gradually decreasing from birth through six weeks of age. The red cell volume per unit of body weight, however, is increasing for the first three weeks and then begins to decrease. Since it has been shown that the F_{cells} factor is not age dependent in these pigs, the PCV and/or the hemoglobin seem to be direct correlates of the red cell volume per unit of body weight. Obviously, for a perfect correlation, the parameters of total blood volume per kg. of body weight, F_{cells}, MCV, and MCH would have to remain as constants. These factors were constant enough in this study to make the previous statement logical.

The results obtained from calculating the MCV (Tables 29 and 30) and MCH (Tables 31 and 32) were similar to those previously reported by Talbot and Swenson (1963b). In this research baby pigs were born with a relatively large mean erythrocyte volume of approximately 63 cubic microns. During the first week of life, when iron was available for production of erythrocytes, the mean volume increased to approximately 78 cubic microns. From this time on there

was a progressive decrease in the mean volume of the erythrocytes until at six weeks of age they presented mean values of about 50 cubic microns. The initial increase in mean values probably represented increased numbers of young red cells being poured into the rapidly expanding total blood volume. Since young red cells are larger than older ones, an imbalance in the average age of cell would tend to elevate the MCV. The control animals failed to show a significant increase in MCV the first week of life and then progressively decreased during the rest of the study, thus demonstrating that the anemia seen in iron deficiency is microcytic in character.

The results obtained from the MCHC calculations (Table 33) are also similar to data previously reported (Talbot and Swenson, 1963b). The analysis of the data (Table 34) showed no sex differences but did show differences ($P < 0.005$) for age and treatment. The differences due to age are not consistent from week to week and merely represent the lack of perfect correlation between the MCV and the MCH. For example, at one week of age the cells of the treated pigs were quite large and even though this was the time of greatest corpuscular hemoglobin, the MCHC was at its lowest because of the large size of the container (the erythrocyte) that was holding the hemoglobin. In general, even though there were treatment differences

($P < 0.005$) at three, four, five, and six weeks of age, the concept proposed by Talbot and Swenson (1963b) is not altered. This concept, that iron deficiency anemia in young pigs is not hypochromic, is based on a MCHC value of 30 being the lower limit of normal. Since the control pigs did not fall below 30 but did show significantly depressed MCV values, the nomenclature of normochromic microcytic anemia would still seem to be the most descriptive terminology for iron deficiency anemia of baby pigs.

V. SUMMARY

Comparisons of the plasma, erythrocyte, and total blood volumes as well as the classical hematological parameters of erythrocyte count, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were made between iron deficient pigs and pigs that received an intramuscular injection of iron dextran. Additional data were calculated to determine the relationship of the venous packed cell volume to the average circulatory system packed cell volume. The survival time of Cr^{51} labeled erythrocytes and validation of a method for determining porcine plasma volume were ancillary studies.

The plasma volume of iron dextran treated pigs was shown to decrease from 70.3 to 59.8 ml. per kg. of body weight from birth through six weeks of age. The anemic control pigs showed mean values that fluctuated between 70.5 and 79.3 during the same period.

The measurements of red cell volume proved the hypothesis that young pigs are capable of producing erythrocytes at a rate proportional to their increasing body weight if adequate iron is available. The control pigs of this study showed a gradual depression of red cell volume per kilogram of body weight from 22.6 ml. at birth to 13.3 ml. at six

weeks of age. The iron dextran treated pigs increased their red cell volume per kilogram of body weight from 20.2 ml. at birth to 28.0 ml. at three weeks of age. Their mean values then gradually decreased to 20.9 ml. at six weeks of age.

The total blood volume per kilogram of body weight of the iron dextran treated pigs decreased from 90 ml. per kg. at birth to 81.2 at six weeks of age. The control pigs showed fluctuating values both above and below the treated pigs' values. At six weeks of age the values for the control animals had not dropped but were still quite similar to their previous values.

The F_{cells} factors calculated for pigs were shown not to be age dependent. Treatment differences were significant. The iron dextran pigs presented a mean F_{cells} factor of 0.74 ± 0.07 while the anemic control pigs had a mean value of 0.71 ± 0.08. It was postulated that the spleen of the pig from birth through six weeks of age assumes a significant physiological function as an erythrocyte storage organ, because of the relatively low numerical value of the F_{cells} factor.

The erythrocyte survival time of autologous transfused cells was shown to approximate the 110 day value reported for man. Comparison of transfused autologous erythrocytes with transfused homologous erythrocytes showed 50 per cent

survival times of 28.0 and 13.8 days, respectively.

The T-1824 dye dilution technic was shown to be valid for measuring plasma volume in the pig.

Data obtained for erythrocyte count, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration showed significantly higher values for the iron dextran treated pigs than the control pigs. The mean corpuscular hemoglobin concentration of the anemic controls, however, did not fall below 30 per cent. These data verify previous studies in which post-natal iron deficiency anemia of pigs less than six weeks of age was shown to be microcytic and normochromic rather than hypochromic.

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