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Effects of iron on erythrocyte sedimentation rate, serum iron, and plasma proteins of pigs from birth to six weeks of age.

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EFFECTS OF IRON ON ERYTHROCYTE SEDIMENTATION RATE, SERUM IRON, AND PLASMA PROTEINS OF PIGS FROM BIRTH TO SIX WEEKS OF AGE

by

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I. INTRODUCTION

The rate of sedimentation of red blood cells from whole blood has long been thought to be related to disease. This relationship may in part account for the ancient humoral theory of disease. Although the mechanism is still poorly understood, the erythrocyte sedimentation rate is a useful laboratory screening aid as well as a harbinger of change in chronic disease. This rate has been attributed to various factors, most often to the concentration of certain plasma proteins; however, to date it has not been determined that one specific factor characteristically alters the sedimentation rate in all cases.

Iron-deficiency anemia is associated with an increased erythrocyte sedimentation rate; among animals, this has been observed especially well in the porcine species. The "physiological anemia" of baby pigs has been documented to be at least partially due to a deficiency of iron; rapid sedimentation rates have also been observed in these pigs. Even when iron is supplied to the animal, lower than normal hematologic values are observed while the rapid sedimentation rate is corrected. Since a low serum or plasma iron level is also a consistent feature of iron-deficiency anemia, it seems possible that the serum iron level is a determining factor in the erythrocyte sedimentation rate. No literature discussing this possibility has been found. There are other instances in

which both an increased rate of sedimentation and hypoferremia are simultaneously observed, such as human pregnancy, many infectious diseases, and certain malignancies.

The objective of this study was to compare the serum iron levels and the erythrocyte sedimentation rates, as well as other hematologic parameters, of iron-deficient pigs with pigs receiving exogenous iron. These comparisons were planned to achieve the following goals:

1. To determine by appropriate data analysis the correlation, if any, of serum iron levels and sedimentation rates in baby pigs from birth to six weeks of age.
2. To determine whether adrenocorticotropic hormone injected intramuscularly results in decreased serum iron levels in baby pigs, as they do in certain other species, and whether a corresponding alteration occurs in the sedimentation rate.
3. To verify the meager data on serum iron levels in normal and iron-deficient swine.
4. To determine any differences in the percentage of plasma protein components of the two groups and to verify the reported changes in these percentages with time.

II. REVIEW OF LITERATURE

A. Erythrocyte Sedimentation Rate

1. General considerations

The credit for having first appreciated the true cause-and-effect relationship between erythrocyte sedimentation velocity and disease is generally given to an eighteenth century physician William Hewson (1). However, it was not until the twentieth century that any application was made of the relationship when Robin Fahraeus (2), in attempting to develop a simple test for pregnancy, determined much of the evidence presently available on the erythrocyte sedimentation rate (ESR). He and others (3) considered this phenomenon as a blood suspension system which could be described by a hydrodynamic equation, Stoke's law:

$$v = {}^{2}/_{9}\ g\ \frac{S-S_1\ r^2}{n}\ .$$

Here v is the velocity of the particle (RBC) after the opposing downward gravitational force reaches equilibrium with the upward force or resistance exerted by the suspending medium, g is gravitational acceleration, n is the viscosity of the suspending medium (plasma), $S-S_1$ is the difference in the specific gravities of the cell and the plasma, and r is the radius of the red blood cell. The formula actually applies for the sedimentation of particles in colloidal dispersion; it is derived by equating the frictional force of the particles

to the effective downward force. For the valid application of this law, it can be seen that several basic assumptions must be made, at least two of which can only be approximated in the ESR test; first, particles should be small and spherical and erythrocytes do not truly fit this category. Secondly, the equation assumes that the suspending fluid is of infinite size and this certainly is not true because of the geometry of the sedimentation tube and the packed cell volume of the blood.

Studies by Cutler (4) have shown that erythrocyte sedimentation occurs in 3 phases. The first period is the aggregation and acceleration of the particles which average 5 to 15 minutes in duration for man. Phase 2 is the phase of constant velocity; phase 3 is the period of packing and marked deceleration of particles.

An extensive study by Nichols (5) revealed that changes in the ESR can be attributed to many variables, the most important being alterations in either the suspending medium or in the erythrocytes. The changes in the plasma most prominently related to an increased ESR are variances in plasma protein concentration, especially increased levels of fibrinogen and $alpha_2$-globulin, according to Linko et al. (6). Hardwicke and Squires (3) in substitution studies using each of the plasma proteins found that identical increases in concentrations of fibrinogen, $alpha_2$-globulin, and gamma-globulin

resulted in elevations of the ESR in the ratio of 10:5:2. They related these findings to the Stoke's law equation and found that the alterations noted in the sedimentation rates correlated with the variations in the viscosity of the plasma. Shape of the red cell is an important factor also; Abel and Beier (7) concluded that the reduced sedimentation rate in sickle cell anemia related to the altered ability of the abnormally shaped cells to aggregate. In addition, Ruhenstrath-Bauer (8) has reported the formation of a thick coating, presumably of protein, on the erythrocyte surface preceding agglutination. Such an alteration of the red cell surface has been offered as a possible explanation for changes in the ability of some cells to form aggregates.

A number of technical factors are also of importance in the ESR test; among these are the method used and the interpretation of the results. For example, in the Wintrobe-Landsberg method (9), a single observation is made at the end of one hour and normal values are considered to be 0-9 mm. per hour for human males; but for the Cutler method, 5 minute observations are made during the period of constant fall; and the normal value is considered to be less than one mm. settling in 5 minutes. Ham and Curtis (10) noted an 85 per cent increase in the ESR as the temperature rose from 20^0 to 38^0 C. Ponder (11) found that minor inclination of the tube from the vertical position caused a remarkable increase in rate; an

angle of as little as 3 degrees increased the rate by 30 per cent. Other sources of error in determination of the ESR include dirty or moist glassware.

Shanbron and Berger (12) found that a low molecular weight dextran, Rheomacrodex, produced significant lowering of the ESR both *in vitro* and *in vivo*. Their control observations indicated that this inhibiting effect was not a result of dilution of plasma proteins; the lowering of the ESR was apparently explained by the ability of the dextran to inhibit rouleaux formation.

2. *Relationship to iron-deficiency anemia and other diseases*

Iron-deficiency is the most common cause of anemia in human infancy and childhood (13); it usually develops from an inadequate diet, especially an excessive amount of milk, an iron-poor food. Iron-deficiency anemia and an increased ESR are often associated; and because it is known that sedimentation rates of cells diluted with plasma from the same individual is greater than that of undiluted cells (14), corrections for the anemia using the packed cell volume (PCV) are often made. The fact that an increase in the sedimentation rate accompanies a decrease in the packed cell volume has been attributed to a more rapid aggregation phase and a reduction in frictional forces due to fewer numbers of cells. Arbitrary correction factors for the packed cell volume have generally proved of little value, often with overcorrection in obvious

disease states. In addition, Swenson (15) observed that injections of iron dextran alleviated only a portion of the post-natal anemia of pigs, but corrected the rapid ESR. These observations lead to the possibility that a factor in addition to the PCV, possibly the plasma iron level, apparently affects the ESR. Poole and Summers (16) interchanged the red blood cells and plasma of normal and anemic human subjects and showed that "anemic cells" sedimented more slowly than normal cells, both in plasma of normal and anemic individuals. Both types of cells sedimented more rapidly in the plasma of anemic patients. They described the plasma of the patients with iron-deficiency anemia as having a "plasma accelerating factor" but made no attempt to demonstrate what the factor might be. They also described the cells of iron-deficiency anemia as possessing a retarding factor and tentatively inferred that this was associated with anisocytosis. However, it should be pointed out that their graphs indicated no difference in rates existed among the groups at normal PCV's; the differences appeared at great dilutions, i.e., at PCV's of 20% and less. The authors failed to comment on this point.

The ESR is increased in diseases in which there is inflammatory reaction, tissue degeneration, or necrosis (14). Its clinical application lies in the fact that it is often more sensitive than body temperature, white cell count, or

subjective symptoms and thus is of value in guiding therapy or accessing convalescence. The increased ESR is associated with the aggregation of cells into abnormally large rouleaux. Although variations in the size, shape, and perhaps, number of the red cells may influence their capacity to form rouleaux, the essential cause of the excessive aggregation of cells in disease apparently lies in the plasma (3). Ruhenstrath-Bauer (8) states that an increase in fibrinogen or gamma globulin is only coincidental to a high sedimentation rate and not its cause. Although the opposing view is generally held, other evidence also indicates that these proteins do not necessarily affect the ESR; Abel and Beier (7) in their studies on hemoglobinopathies such as homozygous C disease found that even though decreased ESR's were typical, there was not always a decrease in fibrogen and globulin levels.

B. Serum Iron and Iron-binding Capacity

Barkan and Walker (17) demonstrated that iron in the serum was nondialyzable at the pH of blood, but after acidification it appeared in the ultrafiltrate. The existence of a specific iron-binding protein was proved by Schade and Caroline (18) when they investigated various protein fractions obtained by ethanol fractionation of the plasma and found that a color change indicating the presence of iron occurred only with a particular fraction, then called fraction IV: 3,4, and

later named transferrin. Its molecular weight is about 90,000 and at pH 7.5-8.8 transferrin moves with the group of beta-globulins. Determinations of the isoelectric point of transferrin have given varying results because the mobility of the protein is markedly influenced by the kind and concentration of the inorganic ions in the buffer used. However, the isoelectric point of human transferrin is thought to be 5.9 (19).

Some authors have had the opinion that transferrin is not the only plasma protein of importance for the transport of iron. However, Wallenius (20) investigated the distribution of iron between the different serum proteins by means of combined paper electrophoresis and autoradiography; the activity was concentrated in the beta-globulin. He found no support for the assumption that more than one component is responsible for iron transport. Ferritin, the iron-storage protein, is not normally found in plasma; it has been found in plasma in cases of irreversible shock (21).

The same amount of iron is bound by transferrin when either ferrous or ferric iron is added to plasma, and the changes in absorption spectra are identical (22). Each molecule of transferrin combines with two iron atoms; added ferrous iron is rapidly oxidized to ferric iron and the iron is bound in the ferric state. Transferrin constitutes about 3 per cent of the total protein of normal human serum or about 20 per cent of its beta-globulin fraction (22).

Erythrocytes contain no iron-free transferrin, but the possibility that they contain small amounts of iron-transferrin cannot presently be excluded. No one has investigated whether the leukocytes have any function in iron transport in spite of the fact that they have a relatively high iron content (23).

The exchange of iron between plasma and red blood cells seems to be negligible when compared with the rapid plasma iron turnover. Walsh et al. (24) demonstrated with radioactive iron that reticulocytes but not mature erythrocytes can assimilate iron from plasma. Transferrin-bound iron also occurs in the lymph but its concentration is about one-third the concentration found in plasma.

In normal human adults plasma iron shows pronounced diurnal variation with higher morning than evening values. The diurnal rhythm is found reversed in normal subjects working nights and sleeping during the day. There is no diurnal variation in iron-binding capacity (25). There is also a statistically significant sex difference in the serum iron level but not in the total iron-binding capacity. In children there is a hypoferremia from about 8 months of age to 2 years. The values increase slowly up to puberty; the sex difference first appears after puberty (26).

The reaction between iron and transferrin is dependent upon pH (22). If iron is added to a solution of transferrin at neutral or slightly alkaline pH, the added iron is bound

to transferrin, as measured by a typical color change. Upon acidification of the solution, the color disappears at pH 4 and the iron can be dialyzed away. This pH dependency is the basis for most of the iron determination methods currently in use; this includes the bathophenanthroline method, which has been successfully used with various modifications by Kingsley and Getchell, (27), Peters et al. (28), and Beale et al. (29).

Normally, 50 to 80 per cent of the transferrin is free from iron. Teleologically speaking, this excess of unbound transferrin may be considered a safety device capable of binding extra iron that might be put into the blood stream suddenly (30).

Although anemia is the finding that most frequently alerts the diagnostician of the presence of iron-deficiency, the plasma iron level and the iron-binding capacity provide a very good index of the adequacy with which iron is being supplied to the marrow. A hypoferremia plus an increased binding capacity has proved valuable in the demonstration of iron-deficiency with minimal or insignificant changes in the peripheral blood (31). The importance of a low plasma iron level in the absence of anemia is not presently understood. Smith (31) states that in experimental animals a number of enzymes, such as cytochrome C, become very depleted in the earliest phases of iron deficiency. Thus, anemia may not be the first

symptom of iron deficiency; and the extent of morbidity from tissue deprivation of iron is not known. Smith suggests that perhaps supplementation of infant foods with sufficient iron to raise the serum iron levels might possibly be accompanied by an increase in growth rate or a reduction in morbidity from infections.

The ratio of serum iron to latent iron-binding capacity varies greatly with various disease states (22). In human iron-deficiency anemia and late human pregnancy, the ratio between serum iron and latent binding capacity is considerably below normal, i.e., the serum iron level is low and the binding capacity is increased. In chronic infections, uremia, leukemias, and hepatic cirrhosis, a hypoferremia is present, but the binding capacity is also below normal. In addition, certain malignancies, especially those associated with widespread metastases, present this picture of low serum iron and reduced binding capacity (32). Some diseases are characterized by higher than normal ratios due to a hyperferremia; these include the hemolytic, pernicious, and aplastic anemias and thalassemia major. Laurell (23) has stated that the ratio is higher than normal when depot iron increases and lower than normal when the depot iron decreases. As far as it is known, no one has explained the differences in total binding capacity.

Factors other than disease are known to affect the serum iron level. Laurell (23) states that hyperferremia was

regularly produced in normal subjects ninety minutes after peroral or intravenous administration of 20-50 mg. nicotinic acid. The transferrin level remained unchanged. Slight serum iron decreases often occur some hours after lumbar puncture, encephalography or electroshock in human patients (33). It is theorized by the investigators that such treatment of a patient may constitute a stress which in some manner effects a lowered serum iron or that the variation may be secondary to functional changes in the marrow or reticulo-endothelial system.

Besides this influence of the central nervous system on the serum iron level, hormones are also known to affect it. In rabbits, injections of testosterone and progesterone are without effect, yet estrone administration results in a rapid decrease of the iron level (34). Cartwright et al. (35) demonstrated that a number of agents produced acute transient hypoferremia in dogs. These included sterile turpentine abscesses, histamine, epinephrine, fracture, anaphylactic shock, adrenocortical extract, and adrenocorticotropic hormone. They found that a 25 mg. injection of ACTH, given either intramuscularly or intravenously, resulted in a maximal drop in the plasma iron level of all dogs between 6 and 8 hours. The mean per cent decrease was 47 $\pm$ 7. The iron returned to its preinjection level or a slightly higher level after 24 hours. No hypoferremia occurred in the adrenalectomized control dogs.

They speculated that the acute hypoferremia following stress may have been due to stimulation of the reticuloendothelial cells by the cortical hormones which causes them to take up iron. This mechanism may possibly explain the hypoferremia accompanying infection, although there is no direct evidence of this.

In view of the hypotheses of this thesis, and since both hypoferremia and an accelerated ESR are typical of infections as well as iron-deficiency anemia, it seems beneficial to briefly discuss the anemia of infection. Wintrobe et al. (36), by the use of intravenously injected radioactive iron, found that the utilization of iron for hemoglobin synthesis is impaired during infection. The resulting anemia does not appear immediately, for only when the "old" red blood cells must be replaced does the defect become noticeable. On the other hand, hypoferremia appears early during infection; furthermore, Kuhns et al. (37) found that the intravenous administration of large amounts of iron in man failed to relieve the anemia of infection and the hypoferremia persisted. In studying the fate of injected Fe^{59}, Greenburg et al. (38) concluded that the major diversion of plasma iron during infection is to the liver and that this diversion results in the hypoferremia. The mechanism producing this effect was not elucidated but may possibly be interpreted as being due to the stress reaction discussed above. The possibility that

disturbances in protein metabolism, which often accompanies infection, may in some manner affect the plasma iron level ought also to be considered. Chakrabarty and Banerjee (39) observed that hypoferremia and a decreased total iron-binding capacity were present in patients suffering from cholera, tetanus, or pyogenic meningitis; the percentage of serum albumin was diminished and the globulins elevated in these infections, indicating deranged protein metabolism. However, they state that no relationship between these factors was evident.

Other workers think there is a definite relationship between protein metabolism and serum iron levels. Cartwright and Wintrobe (40) found a significant lowering of the serum iron in swine on a protein-deficient diet. This was apparently due to a greatly reduced amount of iron-binding protein in the deficient pigs; they discovered that there was a proportional reduction in the iron-binding capacity with a reduction in the total serum proteins. Severe iron-deficiency was also produced in some of the pigs in this study, and it resulted in an even more pronounced hypoferremia; the investigators did not conduct total plasma protein studies on these animals, however. No literature has been found on whether the total amounts or percentage of protein components of plasma are altered in iron-deficiency. In the Cartwright and Wintrobe study on swine, the hypoferremia of iron-deficiency averaged

31 micrograms per cent of iron as compared to 115 for the protein-deficient group and 169 per cent for the normal group.

C. Plasma Proteins

Sera from a large number of animal species have been found to possess characteristic and reproducible electrophoretic patterns. There are major changes in the serum patterns of most species with age and development. Cochrane et al. (41) found an extra distinct band in the sera of day old and suckling pigs up to 3 weeks of age. This component was considered to be an alpha$_1$-globulin, or fetuin, and was present between the albumin and main alpha-globulin bands. Using paper electrophoresis, Rutqvist (42) and Leece and Matrone (43) demonstrated that alpha-globulin is the major protein component in the sera of newborn piglets before nursing, whereas albumin is relatively low in comparison with adult serum. They found that during the first five weeks the percentage of albumin increased but that of alpha-globulin decreased. They suggest that the α_1-globulin component is probably present after the first three weeks but that its presence is obscured by increased amounts of albumin with a similar migration velocity.

Normal electrophoretically-determined values for mature swine have been reported by several workers and these are in good agreement with each other (44,45). Koenig and Hogness

(46) noticed a significant difference in the percentage of certain components of identical swine plasma analyzed in various buffers.

The relationship of plasma protein components, especially fibrinogen and alpha globulin, and the ESR has been discussed in a previous section. The relationship between plasma proteins and hypoferremia has also been considered. Unfortunately, no literature relating all three variables has been found.

III. MATERIALS AND METHODS

A. Experimental Animals and Design

A total of 4 litters of pigs were used in this study. Sows and litters were housed on concrete floors for the first 6 weeks of the pigs' lives. A ration meeting the requirements of the Committee of Animal Nutrition of the National Research Council (47) was fed to the dams. It was minimal in iron and contained the following:

ground corn	46.0%
ground oats	20.0%
wheat bran	5.0%
44% soybean oil meal	16.4%
dried whey	10.0%
dicalcium phosphate	1.0%
ground lime	0.6%
NaCl	0.5%
vitamin premix	0.5%

The vitamin premix[1] contained the following:

	per pound
vitamin A USP units	200,000
vitamin D USP units	50,000
riboflavin	500 mg.
calcium dipantothenate	2,400 mg.
niacin	2,000 mg.
vitamin B_{12}	2 mg.

[1] Vit-A-Mix, Inc. Shenandoah, Iowa.

Between 48 and 72 hours after birth, the pigs were weighed and animals within a litter were randomly assigned for injection with iron dextran. Half of the pigs of each litter were used as controls, receiving no iron supplementation. Treated pigs were given two ml. of iron dextran intramuscularly; this supplied 150 mg. of elemental iron.[2] The piglets had access to the sow's ration.

Between 12 and 24 hours after birth and then at 1,2,3,4,5, and 6 weeks of age, blood samples were taken from the anterior vena cava of each pig. Approximately 6 ml. of blood were withdrawn; 3.5 ml. were used for each serum iron determination, and 2.5 ml. of blood were placed in a test tube containing 3 mg. EDTA for the other determinations. The following determinations were made on each pig at each sampling period: body weight, erythrocyte number, leukocyte number, packed cell volume (PCV), hemoglobin concentration, erythrocyte sedimentation rate (ESR), serum iron content, serum iron-binding capacity, electrophoretic analysis of serum and plasma proteins, and differential leukocyte count. In addition, reticulocyte counts were made on the last three litters of pigs.

An additional litter of eleven pigs was treated and sampled in exactly the same manner as above; however, at 2 weeks of age and weekly thereafter 10 USP units of adrenocorticotropic hormone[3] were administered intramuscularly following

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

[3]Depo-ACTH. The Upjohn Company. Kalamazoo, Michigan.

the drawing of the blood sample. Blood samples were again taken six hours later. All above mentioned determinations were also made on blood samples from this litter.

B. Experimental Techniques

1. Serum iron and iron-binding capacity

Glassware used in the serum iron and latent iron-binding capacity determinations was washed in the normal manner, rinsed with sulfuric acid-permanganate solution, rinsed three times in distilled water and finally rinsed with ion-free water. The deionized water was obtained by running distilled water through an exchange column containing Amberlite MB-1 resin.[4]

Blood for these determinations was allowed to clot and then centrifuged for 20 minutes.[5] Serum was harvested with a transfer pipette and was again centrifuged for 20 minutes.

The method of Beale, Bostrum, and Taylor was used for the serum iron determination (29,48). The necessary reagents were prepared in the following ways:

1. Glycine Buffer - 12.5 ml. of 0.2 M glycine and 2.6 ml. M HCl. were diluted to 50 ml. with ion-free water; the solution was adjusted to pH 1.9 and 10 mg. of ascorbic acid were added as a preservative.

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[4]Fisher-Scientific Co. Fair Lawn, New Jersey.

[5]International Equipment Co. Boston, Massachusetts.

2. Bathophenanthroline Reagent - 323.7 mg. of the sodium salt of bathophenanthroline sulfonate were dissolved in water, adjusted to pH 6.7 and diluted to 50 ml. After filtering, a few drops of $CHCl_3$ were added and the reagent was then kept refrigerated until used.
3. A standard iron solution containing 25 µg. Fe per ml. was made using ferrous ammonium hexahydrate.

The procedure was as follows: To 2 ml. of the glycine buffer, 0.5 ml. serum was added; for the blank, 0.5 ml. ion-free water was used rather than serum. After 2 minutes, the serum sample was read against the blank at 534 mµ on a Beckman B spectrophotometer.[6] To both the sample and the blank tubes, 0.08 ml. bathophenathroline reagent was added and the reading was taken after 5 minutes. Finally, 0.04 ml. standard Fe reagent (1 µg Fe) was added to the serum sample only and read against the blank after 1 minute. Readings were made in per cent transmittance and later converted by means of a standard table to optical density.

Calculations:

Increment in linear absorbance due to liberated serum iron = Reading A_2 - Reading A_1.

Increment due to addition of standard iron = Reading A_3 - Reading A_2.

Amount of iron in µg. in 1 ml. serum = $\frac{A_2 - A_1}{A_3 - A_2} \times 2$.

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

Therefore, serum iron content in μg %

$$= \frac{A_2 - A_1}{A_3 - A_2} \times 200.$$

The bathophenanthroline and standard iron reagents for the latent binding capacity technique were the same as above. A buffer was made by mixing 1.21 g. trisaminomethane, 0.98 g. maleic anhydride and 9.3 ml. M sodium hydroxide and diluting to 200 ml. with ion-free water. The solution was adjusted to pH 6.85 and preserved with a few drops of chloroform. Two ml. tris-maleate buffer were added to 0.58 ml. water for the blank and to 0.5 ml. serum for the unknown. To the serum sample only, 0.08 ml. standard iron solution was added and after 2 minutes it was read against the blank at 534 mμ (Reading A_1). To the blank and the sample, 0.08 ml. bathophenanthroline reagent was added; and after 10 minutes, a second reading was made (Reading A_2). Finally, 0.04 ml. standard iron reagent was added to the serum sample and read 1 minute later (Reading A_3).

Calculation:

Calculated absorbance from added iron if none were bound to transferrin = 2 (Reading A_3 - Reading A_2).

Measured absorbance of residual iron complex = Reading A_2 - Reading A_1.

Absorbance of complex which would have been produced by the amount of added iron bound by transferrin = 2 $(A_3 - A_2)$ - $(A_2 - A_1)$.

Iron taken up by transferrin in 1 ml. serum

$$= \frac{2(A_3 - A_2) - (A_2 - A_1)}{A_3 - A_2} \times 2 \text{ µg.}$$

Latent iron-binding capacity in µg %

$$= \frac{2(A_3 - A_2) - (A_2 - A_1)}{A_3 - A_2} \times 200.$$

Total iron-binding capacity = serum iron content + latent iron-binding capacity.

$$\text{Per cent saturation} = \frac{\text{serum iron content}}{\text{total iron-binding capacity}} \times 100 \text{ \%.}$$

Allowances could have been made for the dilution factors to compensate for the volume changes in both this and the preceding calculation, but according to Beale et al. (29), the error is very small (usually less than 2 µg %) and can be ignored.

2. Plasma and serum proteins

Electrophoresis of both plasma and serum proteins was conducted according to the recommendations of the Gelman Instrument Company (49). Cellulose acetate strips, 1" x 6-3/4", were used for the determinations. Approximately a 3 microliter sample was applied to each strip with a Gelman sample applicator.[7] Strips were placed in a Gelman electrophoresis chamber (Model #51100)[7] containing approximately 500 ml. high resolution buffer of pH 8.8; each strip was tensioned and held taut by magnetized clips. Six to eight strips were "run" simultaneously for 90 minutes at 210 volts, which were provided

[7]Gelman Instrument Co. Ann Arbor, Michigan.

by a Heathkit power supply.[8] The strips were removed and placed in Ponceau S stain[9] for at least 5 minutes. Protein bands stained pinkish-red with this stain. Excess stain was removed by several washings in 5% acetic acid; the strips were then allowed to air-dry.

"Clearing" resulted in translucent strips; the procedure consisted of dehydrating a strip in absolute methanol and then placing it for 20-30 seconds in 10-15% acetic acid in absolute methanol. Each strip was placed on a glass slide and allowed to dry. It was then covered with another glass slide and placed in the Gelman scanner (Model #39301).[7] Optical density units were indicated on the meter and these were plotted on standard graph paper. Next, the curves were integrated with a planimeter. In instances where the curve of a protein band did not return to the base line before the next curve began, extrapolations to the base line were made. The percentage of each component was then calculated; components measured were albumin, α-globulin, β-globulin, and γ-globulin. Since fibrinogen cannot be separated as a distinct band with this technique and because it should be between the β and γ-globulins, these 2 percentages were summed for the statistical analyses.

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[8]Heath Company. Benton Harbor, Michigan.

[9]Hartman-Leddon Company. Philadelphia, Pennsylvania.

3. Other hematologic determinations

Hemoglobin concentrations were determined by the cyanmethemoglobin technique.[10] For each blood sample, 5 ml. of reagent and 0.5 ml. blood were mixed; the contents were transferred to a cuvette and read against a reagent blank at 540 mμ in a Beckman B spectrophotometer.[6] Readings were made in per cent transmittance and later converted to optical density. The optical density readings were then multiplied by a factor determined from a known cyanmethemoglobin standard to obtain the hemoglobin concentration in grams per cent. All samples were run in duplicate.

The Wintrobe method was applied for the erythrocyte sedimentation rate. The tubes were placed vertically in a sedimentation apparatus[11] having adjustable screws for leveling the rack. The amount of red cell settling was noted at the end of one hour. In cases where there was a clear plasma layer atop a red hazy layer (the diphasic area) and then the blood cell layer below this, the reading was taken at the junction of the diphasic layer and the blood cells.

Erythrocyte and leukocyte numbers were determined in duplicate using National Bureau of Standards certified pipettes, counting chambers, and cover glasses. The diluting fluids were 0.90% NaCl solution for red blood cells and 0.1

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[10] Hycel, Inc. Houston, Texas.

[11] Aloe Scientific Division. St. Louis, Missouri.

N HCl for leukocytes. Leukocyte counts were corrected for nucleated red blood cells. Differential leukocyte and nucleated red cell counts were made on blood films stained with Fields' stain.

Reticulocyte numbers were determined by diluting 2 to 3 drops of blood with an equal volume of brilliant cresyl blue dye in a small test tube. After five minutes, a small drop was placed on a glass slide and a smear was made. The number of reticulocytes per 1000 red blood cells was recorded.

Packed cell volumes were determined in duplicate by the microhematocrit method. Filled capillary tubes were centrifuged[12] for five minutes and read in a microhematocrit reader.[12]

Mean corpuscular hemoglobin concentration was calculated by the formula: M.C.H.C. in per cent

$$= \frac{\text{hemoglobin in gms. \% x 100}}{\text{PCV per 100 ml. blood}} .$$

Mean corpuscular hemoglobin in micromicrograms was computed as follows:

$$\text{M.C.H.} = \frac{\text{hemoglobin in gms. \% x 100}}{\text{RBC count in millions per cu. mm.}} .$$

Mean corpuscular volume in cubic microns was calculated as follows:

$$\text{M.C.V.} = \frac{\text{PCV per 1000 ml. blood}}{\text{RBC count in millions per cu. mm.}} .$$

For plasma exchange studies, fresh blood containing EDTA

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

was centrifuged[13] for 15 minutes. The plasma from each sample was pipetted off the cells; 0.90% NaCl solution was added to the cells to "wash" them, and they were recentrifuged. The saline solution was withdrawn and plasma from an anemic animal was added to cells from an iron-injected animal and vice versa. Packed cell volumes (PCV) and sedimentation rates (ESR) were then determined.

The dilution studies were made by placing fresh blood from an animal injected with iron into 2 test tubes containing EDTA. One tube was centrifuged as described above. The plasma withdrawn from it was used for diluting the blood in the other tube. A curve of ESR vs. PCV for an individual animal was then plotted.

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[13]International Equipment Co. Boston, Massachusetts.

IV. RESULTS AND DISCUSSION

A. Erythrocyte Sedimentation Rate

1. Changes with age and during anemia

The analysis of variance (Table 2a) was set up in such a way that the time factor could be tested as a linear, quadratic, cubic or quintic effect. For this reason, four degrees of freedom are listed for time rather than six, as might be expected. However, it is evident from Table 1 that the most drastic change in the erythrocyte sedimentation rate (ESR) occurred from birth to 1 week. Since the birth data reflect maternal rather than treatment effects, it seemed advisable to also do the analysis of variance on only the data from weeks 1 through 6 (Table 2b). In this analysis of variance, no restrictions are placed on the time variable, so that 5 degrees of freedom are present. Similar analyses were made on the other two major parameters under study here, the packed cell volume and the serum iron content (Tables 4b and 10b).

Comparison of Tables 2a and 2b reveals that time is a significant variable only in the ANOV in which the birth data are included. However, there are rather large weekly changes in the mean ESR's of the control group (Table 1) even though this is not reflected in the analysis of variance.

There exists a significant difference in the erythrocyte sedimentation rate (ESR) between the iron-injected and control groups, as well as a significant difference due to time. It

Table 1. Effects of iron dextran and age on the erythrocyte sedimentation rate of pigs

Age	Controls			Iron Dextran		
	Mean (mm./hr.)	s[a]	n[b]	Mean (mm./hr.)	s	n
Birth	23.5	25.1	13	30.3	29.6	12
1 week	13.7	13.1	21	4.0	4.6	15
2 weeks	18.6	15.4	21	2.6	2.9	21
3 weeks	16.9	12.1	21	3.3	3.2	20
4 weeks	16.8	13.7	20	5.8	4.5	20
5 weeks	17.4	26.0	20	7.9	16.9	20
6 weeks	9.0	15.3	18	6.3	6.5	20

[a]Standard deviation of the mean.

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

Table 2a. Analysis of variance of the erythrocyte sedimentation rate of pigs (including birth values)

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	5,192.1	5,192.1	10.77***
Error (a)	40	19,288.3	482.2	
Time	4	6,619.0	1,654.7	9.14***
Time x Treatment	4	2,713.7	678.4	3.75***
Error (b)	212	38,350.8	180.9	

***P<.005

Table 2b. Analysis of variance of the erythrocyte sedimentation rate (excluding birth values)

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	6,170.21	6,170.21	12.75***
Error (a)	40	19,648.92	491.22	
Time	5	345.23	69.05	0.65
Time x Treatment	5	724.12	144.82	1.36
Error (b)	185	19,613.42	106.02	

***P<.005

will be noticed that the mean rates at birth are much higher than at any other time. The relatively large difference in the two birth means is an illustration of the introduction of random error into the experiment; all animals were randomly assigned to one group or the other, yet the mean ESR's are very dissimilar.

It will be noticed also that the standard deviations of the mean are large, especially in the control group. Great variations in the rates were encountered each testing period; however, duplicate samples consistently gave the same results, so the large differences among individual samples were not due to technique. Litter differences were not tested, but Coulter (50) in his swine studies found significant litter differences (1% level) at ages 1 and 3 weeks. Schalm (51) states that the ESR of pigs is highly variable, and his book presents a table showing a weekly change of 10 mm./hr. in the average ESR of one litter of pigs.

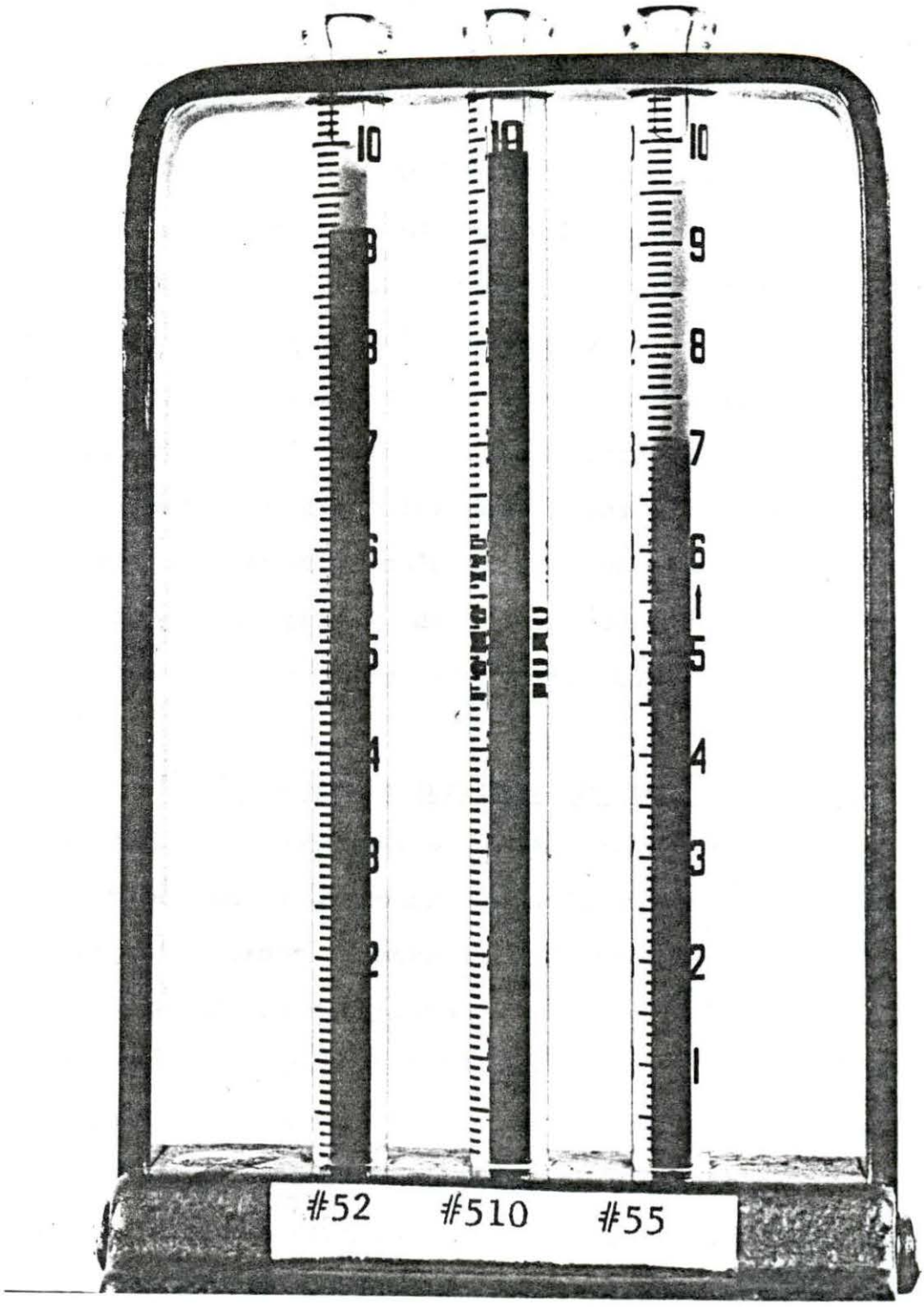
The peculiar manner in which the erythrocytes of young pigs sediment is illustrated in Figure 1. Pigs 52 and 510 were injected with 150 mg. of elemental iron as iron dextran. Tube #55 is characteristic of the appearance of many blood samples from iron-deficient pigs, although the iron-injected pigs which had high sedimentation rates typically displayed this same appearance. Schalm (51) reports that a diphasic sedimentation phenomenon is observed mainly in suckling pigs

during the period of intense erythrogenesis. Sometimes long strands of cells can be seen trailing down the hazy diphasic area. Microscopic examination of material from this layer revealed mostly reticulocytes and occasionally nucleated red cells. The explanation for this type of sedimentation is generally thought to be that the slightly immature cells do not form rouleaux well (51).

2. Effect of serum iron level

Table 3 shows that the mean serum iron content of the iron deficient group is much less than the iron injected group. It parallels the decrease in the packed cell volume (Table 9) and erythrocyte number (Table 13). The effect of serum iron on the ESR was demonstrated in this experiment by testing the hypothesis that the regression coefficient of ESR on serum iron content was equal to zero (52). The value for testing the hypothesis, $\beta_{ESR.SI} = 0$, was $t=-0.54$, which is not significant and permits the hypothesis to be accepted. In the regression analysis excluding birth data, the t value was -0.71, which leads to the same conclusion. As has been stated previously (3,5,8,9,10), the sedimentation rate is known to vary with the packed cell volume; therefore the hypothesis that the regression coefficient of ESR on PCV in this experiment was equal to zero was also tested. For this hypothesis, $\beta_{ESR.PCV} = 0$, the value $t = -9.25$ ($P<.001$) is very significant and leads to the rejection of the hypothesis and

Figure 1. Appearance of the sedimentation of blood from two iron-injected pigs (#52 and #510) and an iron-deficient pig (#55)


10
10
10
9
8
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4
3
2
1
#52
#510
#55

to the conclusion that the ESR is highly dependent on the packed cell volume. The value of t for the analysis excluding the birth data was -9.49, which leads to the same conclusion. A comparison of the weekly means of the serum iron values and the PCV shows that they follow a similar trend, so that the t values might be expected to be also similar. Although the mean serum iron was significantly lower in the control group, the ranges of the iron values were very large and the lower serum iron values were not consistently associated with the higher sedimentation rates, while the packed cell volumes were. Since it is the plasma which delivers the iron to the erythropoietic tissue, the level of plasma iron reflects the degree of erythrogenesis; and the weekly values of the packed cell volume and serum iron level would be expected to follow a similar trend.

3. Dilution curves and exchange studies

It was not known whether a chart such as that made by Wintrobe (14), which attempts to correct the sedimentation rate for the decreased PCV of anemia, could be applied for swine sedimentation rates. Nevertheless, the ESR's of all samples from the first four litters were corrected by this chart. Of 201 observed rates, all except 14 corrected to 0 mm./hr. and only 5 of these 14 corrected to ESR's greater than 3 mm./hr. Again, it appeared from these data that the decreased PCV accounted for the more rapid ESR of the anemic

pigs, if the Wintrobe human chart is valid for swine blood. To determine whether the ESR's of normal swine blood paralleled those of humans, blood from 3 iron-injected pigs was diluted to various packed cell volumes, each with its own plasma; and the ESR's were determined. Figure 2 shows these curves. Although the curves are not smooth exponential curves as those of the Wintrobe chart, they do demonstrate that the human chart seems to be valid for correcting swine rates. The Wintrobe chart may overcorrect the swine values since at a PCV of 10, it corrects ESR's from 10 to 50 mm./hr. to 0 mm./hr. A larger number of samples would establish whether an array of such dilution curves exists for swine also.

Exchanges of plasma from the cells of anemic pigs to cells from iron-injected pigs and vice versa were also made. At first, all packed cell volumes were adjusted to levels more or less "normal" for 4-6 weeks old pigs. For 18 such exchanges (36 determinations) in which the final PCV's ranged from 33-37%, no real differences were observed between the ESR's of the anemic cells in normal plasma and those of the normal cells in anemic plasma. In no instance was the ESR greater than 3 mm./hr. So, it seemed obvious that if any plasma factor of the anemic pigs were affecting the sedimentation rate, its effect was of no consequence at normal packed cell volumes. Therefore, dilutions were made on four such ex-

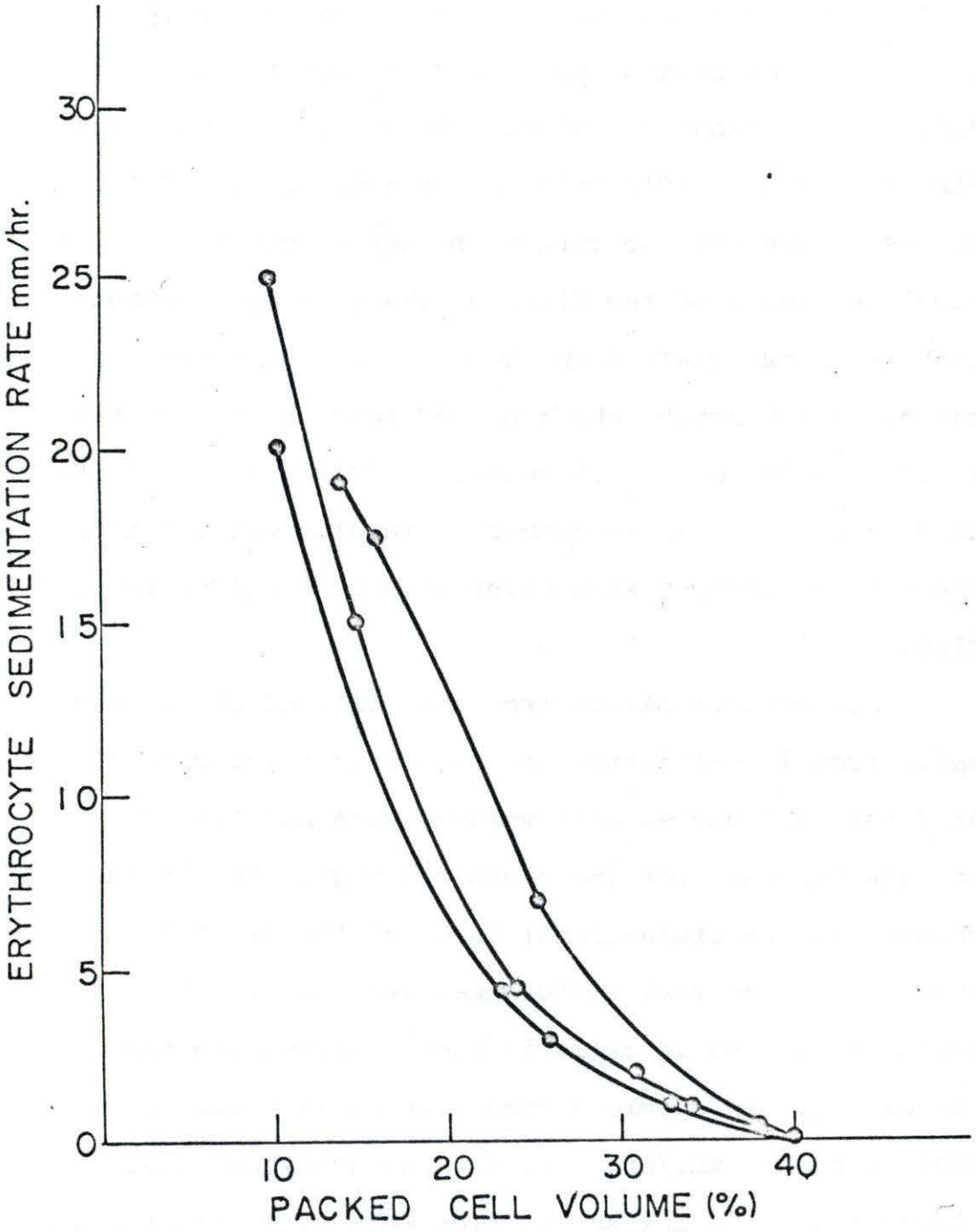

Figure 2.. PCV-ESR curves for 3 iron-injected pigs

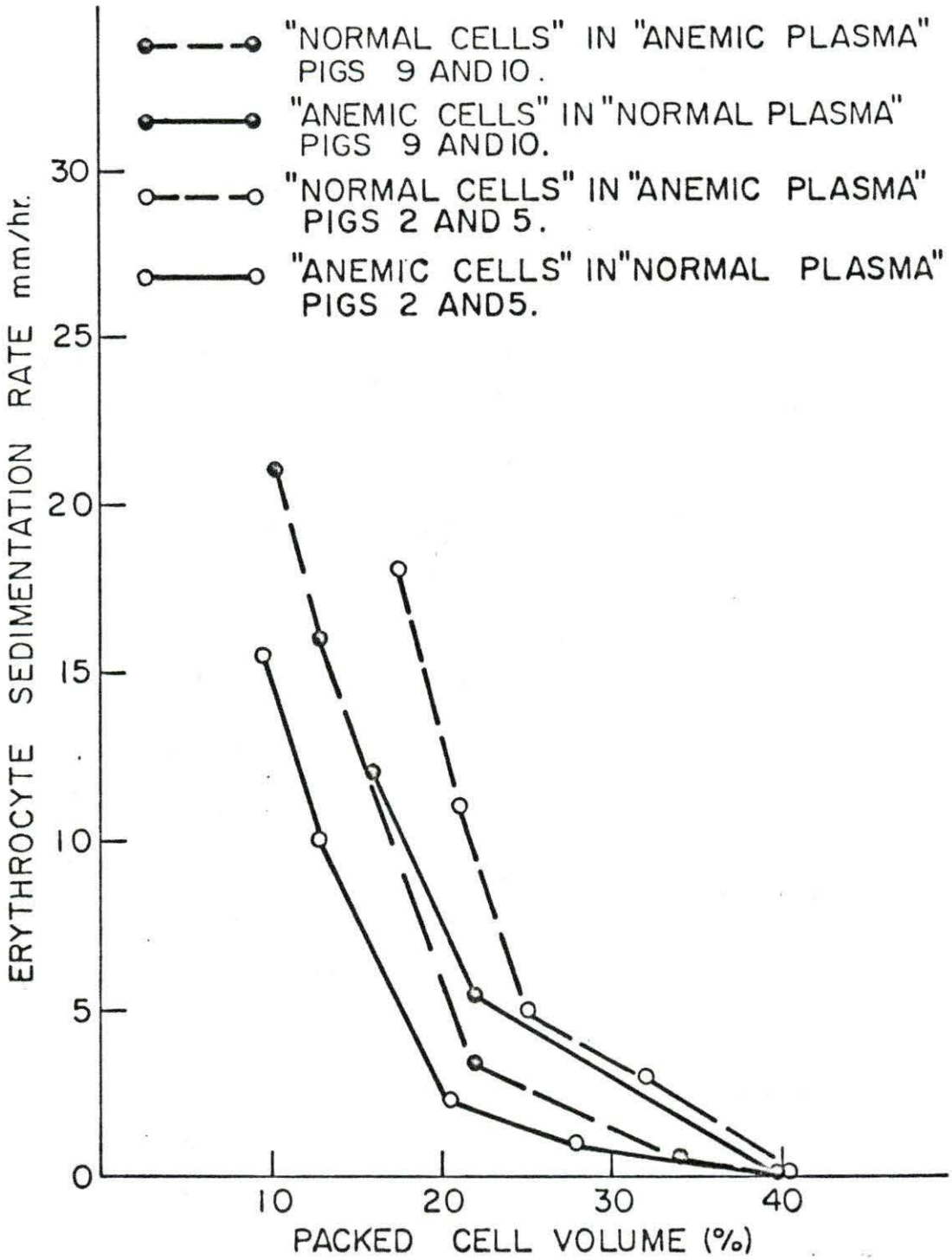

Figure 3. PCV-ESR curves for exchanged plasma and erythrocytes of 4 pigs (control animals #2 and #9; iron-injected animals #10 and #5)

changed samples. Figure 3 illustrates the results. Pig #10 was an iron-injected pig which originally had a PCV (at age 5 weeks) of 34.3 and an ESR of 1.0; pig #9 was a control animal with an original PCV of 22.0 and an ESR of 10. It can be seen that there is very little difference in the sedimentation rates of the exchanged samples of these two pigs. Had a large number of dilutions and ESR determinations been made, so that curves rather than straight lines could have been plotted, the differences in these two may have appeared even less. The exchanged samples from the other two pigs gave larger differences in the rates, but no statistical calculations were attempted. Pig #2 was an iron-injected pig and thus was considered to have the "normal" cells and plasma, but it actually had an original PCV of 26.8 and an ESR of 6.0, while pig #5 was uninjected and had an original PCV of 27.3 and an ESR of 3.0. No explanation for the fairly large difference in the curves can be offered. A large number of such exchanges and dilutions would perhaps allow definite conclusions to be made on this point. It should be pointed out that while Poole and Summers (16) reported that normal cells in anemic plasma of humans sedimented more rapidly than normal cells in normal plasma and that anemic cells in normal plasma sedimented more slowly than anemic cells in anemic plasma, at packed cell volumes of 10 and 15, the mean rates of normal cells in anemic plasma were <u>not</u> out of the range they reported for normal blood di-

luted with its own plasma to 10 and 15% PCV's.

B. Serum Iron, Iron-binding Capacity and Per Cent Saturation

Table 3. Effects of iron dextran and age on the serum iron content of pigs

Age	Controls			Iron Dextran		
	Mean (mcgm./100 ml.)	s[a]	n[b]	Mean (mcgm./100 ml.)	s	n
Birth	101.8	8.2	7	50.7	9.8	8
1 week	71.6	27.3	16	131.2	41.7	10
2 weeks	59.9	14.9	17	145.2	34.3	16
3 weeks	70.9	29.1	17	119.5	35.9	15
4 weeks	69.3	21.6	14	111.3	28.3	15
5 weeks	63.0	20.6	14	116.2	30.5	15
6 weeks	74.9	22.3	12	104.5	41.8	15

[a]Standard deviation of the mean.

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

Table 4a. Analysis of variance of the serum iron content of pigs (including birth values)

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	109,577.48	109,577.48	59.3***
Error (a)	32	59,119.52	1,847.49	
Time	4	9,294.72	2,323.68	2.02
Time x Treatment	4	50,086.23	12,521.55	10.88***
Error (b)	168	193,373.90	1,151.04	

***P<.005

Table 4b. Analysis of variance of the serum iron content of pigs (excluding birth values)

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	142,510.97	142,510.97	76.5***
Error (a)	32	59,888.33	1,871.51	
Time	5	7,054.24	1,410.85	1.27
Time x Treatment	5	14,923.77	2,984.75	2.68*
Error (b)	149	165,620.84	1,111.55	

*** P<.005

*P<.05

The iron-treated pigs show a statistically significant higher serum iron level than the control animals. No serum iron values for both iron-deficient and iron-injected young pigs were found in published literature for comparison, but the means for these iron-deficient pigs are somewhat higher than those reported for weanling pigs by Cartwright and Wintrobe (40). They stated that chronic iron-deficiency anemia in swine resulted in a marked hypoferremia with an average serum iron content of 31 ± 7.2 mcgm. %. On the other hand, their reported mean for the normal group, 169 ± 38.8 mcgm. % is much higher than the means presented here. Since the rate of erythropoiesis is a principal factor determining serum iron turnover, the age difference may explain some of the variance; some may also be due to the techniques used. Dvorak (53) reported that in baby pigs with an average age of 46 days and a hemoglobin level of 7.5 grams %, there was an average serum

iron level of 109.0 mcgm. %.

As can be inferred from the large standard deviations, much variation of the serum iron level occurred within the treatment groups. A large range of apparently normal serum iron values for humans, from 90-142 mcgm. %, has been reported by various authors (22). Laurell (22) also states that several workers have found great variations in repeated determinations of serum iron in the same individual at intervals of days, weeks, and months.

Table 5. Effects of iron dextran and age on the total serum iron-binding capacity of pigs

Age	Controls			Iron Dextran		
	Mean (mcgm./100 ml.)	s[a]	n[b]	Mean (mcgm./100 ml.)	s	n
Birth	427.2	10.7	7	406.2	12.8	8
1 week	383.9	62.1	16	418.2	85.8	10
2 weeks	377.6	46.6	17	452.2	68.8	16
3 weeks	384.2	56.9	17	443.1	45.4	15
4 weeks	376.9	63.7	14	447.4	45.5	15
5 weeks	359.0	70.4	14	454.0	39.4	15
6 weeks	393.9	47.3	12	447.7	52.7	15

[a]Standard deviation of the mean.

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

There exists a significant difference in the total serum iron-binding capacity of the two groups, but contrary to the expected results, the iron-injected group had a higher total iron-binding capacity (TIBC). In normal human adults, the TIBC averages 315 mcgm. %, while the mean TIBC of iron-defi-

Table 6. Analysis of variance of the total serum iron-binding capacity of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	225,223.52	225,223.52	21.96***
Error (a)	32	328,211.10	10,256.59	
Time	4	39,378.93	9,844.74	1.74
Time x Treatment	4	63,003.56	15,750.80	2.78*
Error (b)	168	952,922.88	5,672.16	

***P<.005

*P<.05

cient individuals is slightly over 400 mcgm. % (53,54,22). Cartwright and Wintrobe (40) also found the iron-binding capacity reduced rather than increased in iron-deficient pigs; however, they drew no definite conclusions because of the small number of pigs used. Possibly, a species difference may account for the apparently inconsistent results; the above authors mention that in humans iron-deficiency anemia is accompanied by a marked increase in erythrocyte protoporphyrin and plasma copper, but this does not occur in swine.

The TIBC values reported here are much lower than those presented by Cartwright and Wintrobe (40). They gave values of 845 mcgm. %, 298 mcgm. %, and 540 mcgm. % respectively for normal, protein-deficient, and iron-deficient swine. This discrepancy in the TIBC is probably due to the two different methods employed, although age may also be a contributing factor. There is a lack of reported data for comparison of the TIBC of very young pigs or other young animals. Not even for

humans has a systematic study on the serum iron-binding capacity during infancy and childhood been published. Laurell (56) found that the transferrin level of umbilical blood was significantly lower (TIBC = 226 mcgm. %) than adult serum while Smith et al. (57) found higher TIBC values in children from 2½ to 10 years than in adults.

Table 7. Effects of iron dextran and age on the per cent saturation of transferrin with iron in pigs

Age	Controls			Iron Dextran		
	Mean (%)	s[a]	n[b]	Mean (%)	s	n
Birth	24.2	1.7	7	12.1	2.0	8
1 week	18.7	8.2	16	31.2	8.8	10
2 weeks	16.3	7.2	17	31.8	7.1	16
3 weeks	18.5	6.6	17	26.9	8.2	15
4 weeks	18.2	4.8	14	24.6	6.4	15
5 weeks	17.5	5.8	14	25.3	6.6	15
6 weeks	19.1	5.1	12	23.1	10.3	15

[a]Standard deviation of the mean.

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

Table 8. Analysis of variance of the per cent saturation of transferrin with iron in pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	3,084.08	3,084.08	38.97***
Error (a)	32	2,532.00	79.13	
Time	4	607.69	151.92	2.83*
Time x Treatment	4	2,270.38	567.59	10.56***
Error (b)	168	9,028.32	53.74	

***P<.005

*P<.05

The per cent saturation of transferrin (Table 8) is significantly greater in the iron-injected pigs; the values are in fair agreement with reported values of 31.0 per cent for non-anemic swine (40). Transferrin is said to be about 30-40% saturated with iron in normal human adults and usually less than 20 per cent saturated in individuals with iron-deficiency anemia (57). The time factor is significant at only the 5 per cent level; this appears to be due mainly to the very low percentage saturation at birth of the iron dextran group and the great increase after the iron dextran injection. Also, there is a gradual decrease in the mean per cent saturation after the second week in the iron dextran group which agrees with the serum iron data (Table 3).

Since no similar studies on serum iron levels, iron-binding capacity, and per cent saturation in piglets could be found for comparison, and especially since the data are not in good agreement with the study of Cartwright and Wintrobe on older swine (40), more investigation is needed on these parameters.

C. Other Hematologic Parameters

Tables 9 through 22 list the mean values, the standard deviations, and analyses of variance of the packed cell volume (PCV), erythrocyte count, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular

hemoglobin concentration (MCHC), and leukocyte count. For all these parameters except the leukocyte count, the means for the iron dextran group are significantly higher than the control group at the 0.5 per cent level. Time and treatment verus time interaction significances are also present for the same variables. In some cases the effects of time are more obvious in one group than in the other; for instance, the mean total erythrocyte count increased weekly in the iron dextran group only, but the PCV did not continuously increase with time in this same group. A significant time verus treatment interaction indicates that the rate of change of one group does not parallel that of the other; for example, while the mean PCV was decreasing from birth to three weeks in the control group, it was increasing in the iron dextran group.

Table 9. Effects of iron dextran and age on the packed cell volume of pigs

Age	Controls			Iron Dextran		
	Mean (%)	s[a]	n[b]	Mean (%)	s	n
Birth	29.1	6.3	13	30.7	5.4	12
1 week	24.1	3.4	21	32.1	3.0	15
2 weeks	19.7	4.3	21	34.2	3.3	21
3 weeks	17.6	3.7	21	34.9	2.3	20
4 weeks	18.0	4.4	20	31.3	2.7	20
5 weeks	20.3	8.1	20	29.3	5.1	20
6 weeks	24.5	7.8	18	30.8	4.8	20

[a]Standard deviation of the mean.

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

Table 10a. Analysis of variance of the packed cell volume of pigs (including birth values)

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	7,051.60	7,051.60	118.12***
Error (a)	40	2,389.30	59.73	
Time	4	747.73	186.93	9.90***
Time x Treatment	4	1,443.67	360.91	19.12***
Error (b)	212	4,002.56	18.88	

***P<.005

Table 10b. Analysis of variance of the packed cell volume of pigs (excluding birth values)

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	694.42	694.42	11.95***
Error (a)	40	2,323.88	58.10	
Time	5	540.68	108.14	6.54***
Time x Treatment	5	872.90	174.58	10.59***
Error (b)	185	3,056.75	16.52	

***P<.005

Table 11. Effects of iron dextran and age on the hemoglobin values of pigs

Age	Controls			Iron Dextran		
	Mean (gm./100 ml.)	s[a]	n[b]	Mean (gm./100 ml.)	s	n
Birth	9.4	2.4	13	9.2	2.1	12
1 week	8.1	1.4	21	9.9	2.6	15
2 weeks	5.8	1.6	21	10.8	1.2	21
3 weeks	4.9	1.3	21	11.2	0.7	20
4 weeks	4.9	1.7	20	10.0	0.9	20
5 weeks	5.7	2.5	20	9.1	1.9	20
6 weeks	7.4	2.8	18	9.9	1.6	20

[a]Standard deviation of the mean.

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

Table 12. Analysis of variance of hemoglobin values of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	854.37	854.37	96.97***
Error (a)	40	352.47	8.81	
Time	4	114.31	28.58	14.15***
Time x Treatment	4	190.33	47.58	23.57***
Error (b)	212	428.24	2.02	

***P<.005

Table 13. Effects of iron dextran and age on the total erythrocytes per cubic millimeter of blood of pigs

Age	Controls			Iron Dextran		
	Mean ($x10^6$)	s[a]	n[b]	Mean ($x10^6$)	s	n
Birth	4.12	1.8	13	3.83	0.8	12
1 week	4.06	0.9	21	4.45	0.3	15
2 weeks	3.81	0.6	21	4.69	0.8	21
3 weeks	3.70	0.5	21	4.99	1.0	20
4 weeks	4.04	0.6	20	5.29	0.7	20
5 weeks	4.01	1.1	20	5.36	1.1	20
6 weeks	4.72	1.2	18	5.69	1.1	20

[a]Standard deviation of the mean

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

Table 14. Analysis of variance of total erythrocytes per cubic millimeter of blood of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	64.15	64.15	27.07***
Error (a)	40	94.91	2.37	
Time	4	30.17	7.54	11.35***
Time x Treatment	4	18.63	4.66	7.01***
Error (b)	212	139.92	0.66	

***P<.005

Table 15. Effects of iron dextran and age on the mean corpuscular hemoglobin values (in micromicrograms) of erythrocytes of pigs

	Controls			Iron Dextran		
Age	Mean	s[a]	n[b]	Mean	s	n
Birth	23.0	2.8	13	23.6	4.1	12
1 week	19.9	2.8	21	22.1	3.4	15
2 weeks	15.8	5.3	21	23.6	4.4	21
3 weeks	13.1	1.9	21	23.1	4.5	20
4 weeks	12.3	3.0	20	19.4	3.9	20
5 weeks	13.8	4.0	20	17.5	4.0	20
6 weeks	15.4	4.2	18	16.8	3.0	20

[a]Standard deviation of the mean.

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

Table 16. Analysis of variance of the mean corpuscular hemoglobin of erythrocytes of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	1,513.95	1,513.95	36.57***
Error (a)	40	1,655.21	41.38	
Time	4	1,636.78	409.19	42.05***
Time x Treatment	4	647.85	168.71	17.34***
Error (b)	212	2,062.76	9.73	

***P<.005

Table 17. Effects of iron dextran and age on the mean corpuscular volume of erythrocytes of pigs

Age	Controls			Iron Dextran		
	Mean (cu. microns)	s[a]	n[b]	Mean (cu. microns)	s	n
Birth	72.4	10.8	13	81.0	12.1	12
1 week	63.4	12.7	21	76.3	19.9	15
2 weeks	52.7	17.6	21	74.7	12.2	21
3 weeks	47.4	6.2	21	72.3	14.2	20
4 weeks	43.7	10.5	20	60.7	12.7	20
5 weeks	49.8	12.0	20	56.3	10.8	20
6 weeks	51.2	10.6	18	52.9	11.7	20

[a]Standard deviation of the mean.

[b]Number of animalsupon which mean is based.

Table 18. Analysis of variance of the mean corpuscular volume of erythrocytes of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	11,324.81	11.324.81	26.22***
Error (a)	40	17,273.79	431.84	
Time	4	15,756.72	3,939.18	32.05***
Time x Treatment	4	4,099.91	1,024.98	8.34***
Error (b)	212	26,050.56	122.88	

***P<.005

Table 19. Effects of iron dextran and age on the mean corpuscular hemoglobin concentration of pigs

Age	Controls			Iron Dextran		
	Mean (%)	s[a]	n[b]	Mean (%)	s	n
Birth	30.8	3.8	13	29.5	4.2	12
1 week	32.2	2.3	21	30.8	2.5	15
2 weeks	29.9	5.8	21	31.6	2.5	21
3 weeks	26.9	3.6	21	32.0	1.5	20
4 weeks	27.8	4.3	20	31.9	1.3	20
5 weeks	28.4	4.0	20	31.1	2.2	20
6 weeks	29.8	4.0	18	32.2	3.6	20

[a]Standard deviation of the mean.

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

Table 20. Analysis of variance of the mean corpuscular hemoglobin concentration of erythrocytes of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	247.36	247.36	9.59***
Error (a)	40	1,031.37	25.78	
Time	4	118.40	29.60	2.91*
Time x Treatment	4	258.50	64.63	6.37***
Error (b)	212	2,149.68	10.14	

***P<.005

*P<.05

Table 21. Effects of iron dextran and age on the total leukocytes per cubic millimeter of blood of pigs

Age	Controls			Iron Dextran		
	Mean ($x10^3$)	s[a]	n[b]	Mean ($x10^3$)	s	n
Birth	7.5	3.7	13	8.5	2.9	12
1 week	8.9	2.1	21	10.5	3.1	15
2 weeks	7.5	1.9	21	9.1	3.2	21
3 weeks	7.5	2.4	21	8.3	2.3	20
4 weeks	8.5	2.4	20	9.1	3.8	20
5 weeks	10.5	3.3	20	11.3	2.4	20
6 weeks	12.4	3.8	18	11.6	3.6	20

[a]Standard deviation of the mean.

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

Table 22. Analysis of variance of total leukocytes per cubic millimeter of blood of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	64.07	64.07	4.47*
Error (a)	40	573.72	14.34	
Time	4	479.94	119.99	14.15***
Time x Treatment	4	9.98	2.47	0.29
Error (b)	212	1,797.76	8.48	

*P<.05

***P<.005

The mean packed cell volumes (Table 9) agree in general with those cited by Schalm (51) for the birth through 2 weeks values in iron-deficient pigs and those cited by Miller et al. (59) for iron-injected pigs. The values for both groups are somewhat lower than those reported by Talbot (60), but the trends are the same. These data agree more closely with those reported earlier by Talbot and Swenson (61) for control and iron-dextran groups. In consistencies in various reports are probably due to litter differences; Coulter (50) reported that litter differences exist for the PCV at a significant level (1%) for weeks 1, 2, and 3. The analyses of variance (Tables 10a and 10b) show statistical significances attached to treatment, time, and the treatment-time interaction both with and without the birth values.

Hemoglobin values presented here compare favorably with those reported by other investigators (50, 58, 60, 61). The typical hematologic picture of iron deficiency of piglets is well illustrated by these hemoglobin values; Table 11 shows that the lowest values occur at weeks 3 and 4 in the control group and then the hemoglobin values begin to return to a more normal level.

The erythrocyte counts (Table 13) show the same general trend and the same significances as the hemoglobin and PCV. In the iron dextran group, the highest mean erythrocyte values (at 6 weeks) do not correspond in time to the highest packed

cell volumes (at 3 weeks). This probably relates to the size of the erythrocytes (Table 17).

Values for the MCH (Table 15) for both groups and the MCV for the control group agree well with those previously reported (51, 58, 60), but the MCV means (Table 17) for the iron dextran group are higher than those reported by the same investigators. The low values for the control group indicates the degree of microcytosis. MCHC values reported here (Table 19) are slightly less than those reported by Talbot (60) for both groups. The values are in better agreement with those reported by Schalm (51) and Miller et al. (59).

Although the total leukocyte count is not consistently higher in the iron dextran group (Table 21), the analysis of variance shows a significant difference in the two groups at the 5% level. The mean values agree with those reported for similar ages by Coulter (50), Schalm (51), and Palmer (63). No significant difference in anemic and non-anemic groups was reported by Ullrey et al. (64); this may be due to their failure to correct the WBC counts for nucleated erythrocytes. As shown in Table 23, the number of nucleated RBC's is somewhat higher in the control group and this produces a relatively greater decrease in the corrected leukocyte count of that group compared with the iron dextran group. Wintrobe (65) points out that the fact should not be overlooked that the hematopoietic system functions as a physiologic unit and that

when red cell formation is stimulated, as a rule increased leukopoiesis is also found. He further states that when erythropoieses is impaired owing to iron or vitamin B_{12} deficiency, evidence of disturbed leukocyte production is often found; for instance, frank leukopenia is a common accompaniment of pernicious anemia.

Table 21 shows that there is a significance attached to time in the leukocyte counts. Various workers (51, 58, 62) have presented evidence that the leukocyte count increases in swine to a maximum at three to four months of age. Dukes (66) reports the mean to be about 17,000 per cubic mm. blood in the normal adult pig.

Table 23 presents means and ranges for the reticulocyte number, nucleated erythrocyte, lymphocyte, and neutrophil counts. No statistical analysis of any difference in the groups was made, although rather large differences in the means of the control and iron-injected groups occurred at some time periods. Coulter (50) observed a statistically significant difference between anemic and non-anemic pigs in the nucleated RBC counts at 2 and 3 weeks. The values of Table 23 for the reticulocyte and nucleated RBC counts are in general agreement with those reported by others (51, 58). One outstanding change with age is the differential count. Waddill et al. (68) state that a relative lymphopenia and neutrophilia exist in newborn pigs in comparison with older swine. The

Table 23. Effects of iron dextran and age on reticulocyte, nucleated red cell, neutrophil, and lymphocyte counts of pigs

Age in Weeks		Reticulocytes per 1,000 RBC's		Nucleated RBC's #per 100 WBC's		Lymphocytes		Neutrophils	
		C[a]	I[b]	C	I	C	I	C	I
0	N[c]	10	6	13	12	13	12	13	12
	Range	6-48	18-46	0-8	0-10	7-66	5-70	32-90	28-93
	Mean	36.6	27.7	5.0	5.2	25.1	31.7	70.9	66.4
1	N	5	6	21	23	21	23	21	23
	Range	16-72	24-84	1-53	0-120	16-70	14-70	27-81	30-91
	Mean	44.8	50.7	50.6	42.0	46.6	39.8	48.9	52.5
2	N	10	13	19	18	19	18	19	18
	Range	0-138	12-176	0-82	1-71	32-77	6-75	21-65	29-92
	Mean	78.8	80.8	28.9	22.4	55.8	48.9	40.1	45.5
3	N	11	10	12	14	12	14	12	14
	Range	40-262	20-140	11-89	2-45	26-76	44-70	16-49	22-50
	Mean	118.4	74.6	37.6	19.2	60.7	53.9	33.0	38.9
4	N	11	14	16	17	16	17	16	17
	Range	8-266	20-144	2-41	0-15	12-78	20-84	17-85	11-78
	Mean	123.5	53.0	13.4	8.0	57.6	60.4	38.2	34.5
5	N	11	12	7	9	7	9	7	9
	Range	6-240	12-114	0-52	0-8	40-74	50-75	25-65	23-46
	Mean	93.1	56.0	13.0	3.7	58.4	59.7	36.0	34.6

[a]Controls.

[b]Iron Dextran Group.

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

Table 23 (Continued)

Age in Weeks		Reticulocytes per 1,000 RBC's		Nucleated RBC's #per 100 WBC's		Lymphocytes		Neutrophils	
		C[a]	I	C	I	C	I	C	I
6	N	14	14	13	18	13	18	13	18
	Range	16-182	14-164	0-13	0-32	14-70	12-89	23-80	9-85
	Mean	60.3	44.7	7.2	6.6	52.1	54.7	42.1	40.3

trend of decreasing per cent of neutrophils and increasing per cent of lymphocytes with age is in accord with data supplied by Schalm (51).

D. Plasma Proteins

Means for the percentage of the plasma protein components are shown in Table 24. The β- and γ- globulin fractions were summed together because in most cases it was impossible to separate them well electrophorectically. Other workers (67, 72) have reported encountering this difficulty and have stated that they used subjective judgement in separating the β- and γ- globulin curves. Also, it was impossible to determine the fibrinogen percentage by this electrophoretic method; since the fibrinogen migrates at a velocity between that of the β- and γ- globulins, the values for both the serum and plasma globulins are presented. Waddill et al. (68) found that plasma electrophoresis was unsatisfactory in providing a quantitative measure of fibrinogen content. They estimated from the differences in serum and plasma curves that fibrinogen comprised about 8% of the total plasma protein of the newborn pig. This corresponds to the fibrinogen value observed by Foster et al. (69) with the Tiselius electrophoresis technique.

The analyses of variance show no significant difference in any component due to the iron treatment. This is, of course, only for the percentage of components; since total plasma

Table 24. Effects of iron dextran and age on the mean electrophoretic values of blood proteins of pigs

Week	% Albumin				%α-Globulin			
	Control	s[a]	Iron Dextran	s	Control	s	Iron Dextran	s
0	15.9 (19)[b]	4.1	18.4 (16)	5.0	17.9 (19)	7.4	18.6 (16)	8.8
1	34.5 (17)	5.7	25.8 (10)	7.4	19.6 (17)	4.5	28.2 (10)	8.8
2	45.3 (19)	8.7	48.9 (13)	4.9	20.7 (19)	4.4	19.3 (13)	5.1
3	49.9 (11)	10.7	50.4 (12)	12.8	20.6 (11)	5.5	20.7 (12)	8.3
4	46.8 (19)	9.2	51.8 (19)	11.3	20.3 (19)	3.7	23.5 (19)	12.7
5	50.3 (19)	2.8	55.3 (18)	4.8	20.9 (19)	4.4	15.4 (18)	3.9
6	55.6 (10)	8.3	54.0 (19)	9.6	18.6 (10)	4.5	17.6 (19)	6.2

	%(Plasma) β-and γ-Globulins				%(Serum) β-and γ-Globulins			
	Control	s	Iron Dextran	s	Control	s	Iron Dextran	s
0	66.3 (19)	9.7	62.8 (16)	10.1	56.4 (14)	12.3	59.4 (12)	6.4
1	44.7 (17)	6.3	45.9 (10)	6.4	43.5 (11)	6.8	39.6 (12)	8.0
2	34.2 (19)	5.4	33.5 (13)	5.2	30.9 (15)	9.5	31.0 (10)	7.7
3	29.4 (11)	9.4	28.8 (12)	7.7	26.5 (10)	12.1	24.2 (10)	8.6
4	28.9 (19)	9.8	27.0 (19)	4.9	27.6 (12)	7.7	24.5 (11)	10.1
5	28.8 (14)	6.0	29.3 (18)	3.9	29.1 (14)	9.5	22.6 (13)	7.8
6	25.8 (10)	5.4	25.7 (19)	4.5	25.8 (14)	9.1	21.6 (11)	5.3

[a]Standard deviation of the mean.

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

protein content was not determined, whether a treatment difference exists in the actual quantities is unknown. Except for one unreferenced statement by Schalm (70), no reported values could be found comparing the total protein concentration in iron-deficient and iron-injected pigs. Schalm states that as anemia progresses, blood viscosity is reduced because the plasma protein concentration is decreased.

Figure 4 illustrates the typical change in the percentage of the proteins with time. The electrophoretic curves for day 1 and week 6 for one pig are shown. The small extra curve following the albumin curve in the day 1 electrophoretic analysis is the α-globulin component referred to as fetuin by Cochrane et al. (41). It disappears or is masked by other components after the first or second week.

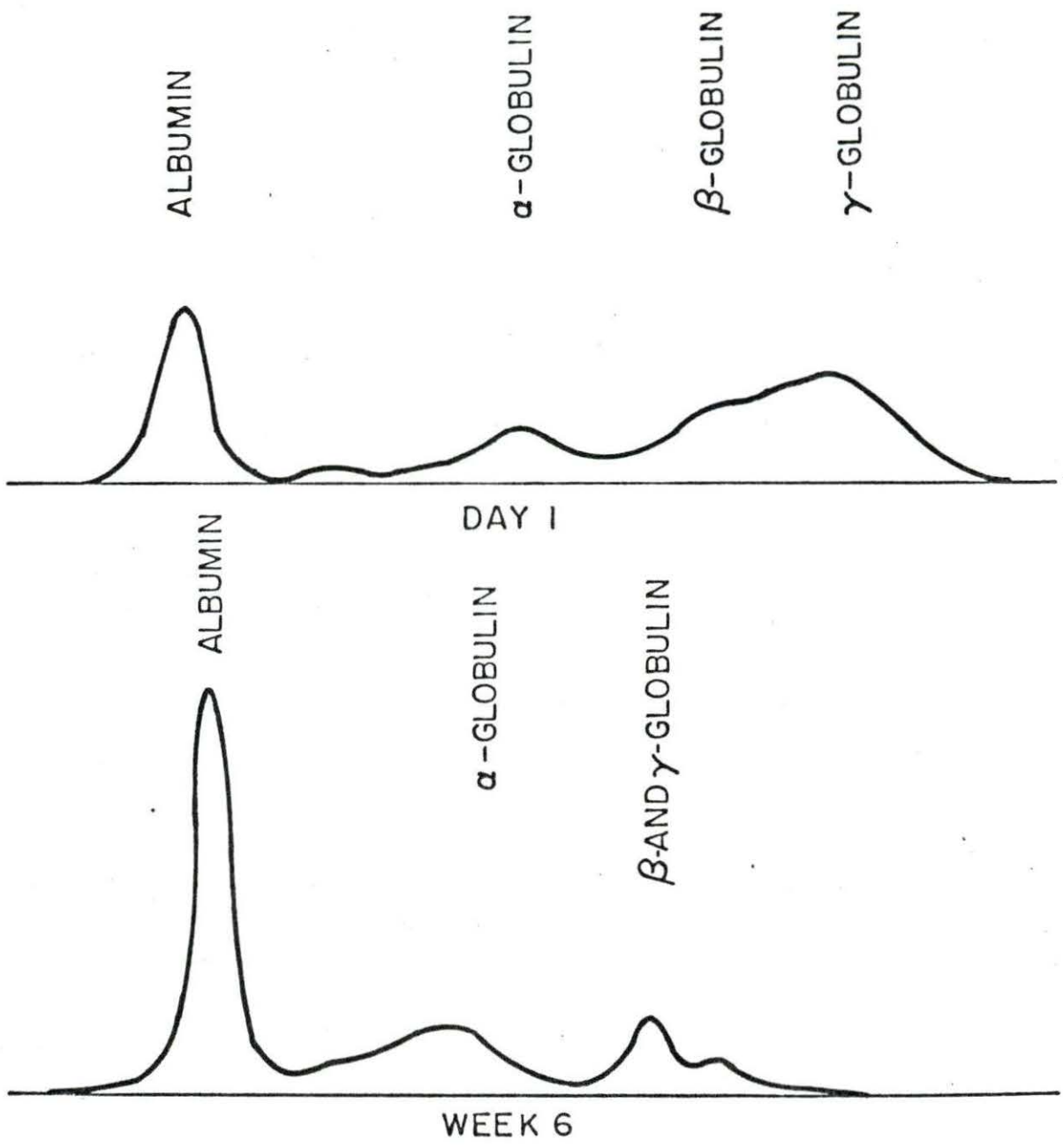

Figure 4. Electrophoresis curves of plasma proteins from pig #55

Table 25. Analysis of variance of the per cent plasma albumin of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	52.8	52.8	0.5
Time	6	44,686.6	7,448.1	74.4***
Error	258	25,819.8	100.1	

***P<.005

Table 26. Analysis of variance of the per cent plasma α-globulin of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	31.2	31.2	1.04
Time	6	1,118.9	186.5	6.19***
Error	258	7,774.9	30.1	

***P<.005

Table 27. Analysis of variance of the per cent plasma β- and γ - globulins of pigs

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	65.3	65.3	1.9
Time	6	7,311.6	1,218.6	36.1***
Error	258	8,686.1	33.7	

***P<.005

Table 28. Analysis of variance of the per cent serum β- and γ - globulins

Source of variation	d.f.	S.S.	M.S.	F
Treatment	1	1,268.8	1,268.8	2.0
Time	6	24,500.5	4,083.4	6.5***
Error	174	10,883.9	625.5	

***P<.005

The means presented in Table 24 on plasma proteins are in good accord with those for similar ages reported by Cochrane et al. (41) and Miller et al. (67). Table 25 shows that there is a statistically significant increase in the percentage of albumin with time. Miller et al. (67) found that the serum albumin reached its peak value at 3 weeks of age and remained fairly constant thereafter as was present in this research.

Table 26 shows that there is a significance attached to time in the analysis of variance of the percentage of α-globulin. Pirtle and Deyoe (71) demonstrated that the α-globulin is the major protein component in the serum of newborn piglets before nursing; Miller et al. (67) found that the α-globulin made up 60.8% of the serum proteins before nursing, but it accounted for only 26.4% of the proteins at 18 hours of age. The reason for this is not an actual decrease in the amount of α-globulin; it is due to an increase in other components, mainly the β- and γ-globulins. They found that the total proteins increased from 2.2 grams per cent at birth to 5.2 grams per cent 18 hours later and that the β- and γ-globulins fractions increased from 22.4% to 67.7% during this period. These same investigators found that while the β-globulin percentage remained fairly constant, the γ-globulin decreased until the fourth week. The β- and γ-globulins constituted 28.2% of the serum proteins at 6 weeks

of age. Tables 27 and 28 reveal that time causes a significant change in the percent of globulins also. Since the iron dextran treatment did not reveal significant differences by the analyses of variances of either the plasma or serum globulins, it is inferred that the treatment did not affect the fibrinogen content. No conclusion can be drawn from these data as to whether time affected the fibrinogen level; however, Deutsch and Goodloe (45) report that chemical analysis of adult swine plasma indicated that approximately 8% of the plasma protein is fibrinogen while their electrophoretic analysis revealed it to be 13.9%; as previously stated, other workers (68,69) estimated the fibrinogen content in piglets to be about 8%.

It can be seen from Table 1 that the erythrocyte sedimentation rates for week 0 are much greater than those of other weeks even when the packed cell volumes are comparable. Since the blood samples were taken between 12 and 24 hours after birth, possibly the total plasma proteins had not reached their maximum values and this may account for the higher sedimentation rates. Since there is not significant difference between treatment groups for any of the plasma proteins, these could not account for the significant difference in the ESR between the two groups. However, it is possible that the changing ratios among the plasma proteins with time may account for some of the significance attributed to

time in the ESR analysis of variance. This, as well as the above suggestion, is merely speculative and has no real statistical basis.

E. Effects of ACTH on Serum Iron, Erythrocyte Sedimentation Rate, and Other Hematologic Factors

Tables 29-34 reveal the action of adrenocorticotropic hormone on the serum iron content, per cent saturation, total iron-binding capacity, PCV, ESR, and total leukocyte count of baby pigs. Student's t-distribution was used as the test of significance in evaluating the differences in each of the above parameters before and 6 hours after the injection of 10 U.S.P. units of ACTH; Table 35 shows the results. The serum iron content was significantly reduced after the ACTH treatments. Table 29 shows that the mean decrease varied considerably, but inconsistently, from week to week and between the iron dextran and control groups; however, the overall mean decrease for all paired observations was 40.6 mcgm. %. For comparison purposes, Cartwright et al. (35) found that 25 mg. ACTH injected into each of 7 normal dogs produced a mean decrease in 6-8 hours in the plasma iron of 82 mcgm. % with a range of 23-157 mcgm. % decrease. The plasma iron level of the dogs returned to its normal or a slightly higher level after 24 hours. These investigators did not determine

the iron-binding capacity nor the per cent saturation in this experiment. They found that no significant decrease occurred after ACTH injections into adrenalectomized dogs.

There are also significant decreases in the per cent saturation of transferrin and the total iron-binding capacity. Although both are changed significantly, it can be seen from Tables 30 and 31 that the relative changes are much greater in the per cent saturation than in binding capacity. It is unknown for certain whether iron leaves the blood stream as an iron-transferrin complex or in ionized form; but Laurell (56) states that most data support the latter. In either case, it is difficult to explain the results here since both the factors decrease significantly. However, since the PCV also decreased significantly, possibly the ACTH injection resulted in a greater plasma volume and this could account for a relative decrease in the total iron-binding capacity and the packed cell volume. The sedimentation rate increased significantly after the ACTH injection, but this is probably due to the decrease in the packed cell volume. If a slightly greater plasma volume resulted from the ACTH injection, it could possibly account for the slight, but significant, decrease in total binding capacity and packed cell volume and thus also for the slight increase in the ESR; however it undoubtedly does not account for the relatively large decreases in the serum iron content and the per cent satura-

tion. Further experiments along this line are needed before more definite conclusions can be reached.

The most plausible explanation for these results seems to be that the ACTH stimulated the secretion of aldosterone which in turn caused sodium retention and, therefore, water retention. Goodman and Gilman (72) state that the manner in which ACTH is involved in the physiological regulation of aldosterone secretion is not clear, but that it is evident that ACTH acts directly on the adrenal cortex to influence the rates of secretion of cortisol, corticosterone, and aldosterone. They further state that hypophysectomy of experimental animals results in an immediate and lasting reduction of cortical secretion and a significantly reduced rate of aldosterone secretion, at least initially. Furthermore, Slater et al. (73) found that definitely increased aldosterone secretion is observed in hypophysectomized-nephrectomized dogs at high rates of infusion of ACTH.

The leukocyte count increased significantly after the ACTH injection. It has long been recognized clinically that leukocytosis accompanies a wide variety of insults to an animal. Selye (74) has collected much evidence implicating the adrenal cortex as the agency through which many of the nonspecific reactions to stress are mediated. Physiologic mechanisms brought into play by stress appear to include adrenocortical stimulation and to result in blood changes

resembling those obtained by administration of ACTH. Dougherty and White (75) reported that adrenal stimulation by ACTH in mice or rats causes an increase in circulating neutrophils and a decrease in circulating lymphocytes. Hills et al. (76) confirmed this in humans as well as finding the eosinophil number decreased and the total number of leukocytes increased. They found that the total leukocyte count per cubic mm. of blood increased to a maximum within 4 hours after a 25 mg. injection of ACTH in humans; the average increase for 8 normal subjects was 3.9×10^3.

In this experiment, the overall average increase was 2.0×10^3 leukocytes per cubic mm. blood. Cartwright et al. (35) reported that administration of ACTH to dogs resulted in a rise in the total leukocyte and polymorphonuclear cell counts, which was maximal at 6 hours following the injection. They also noted significant decreases in the eosinophils and lymphocytes and observed that there was a correlation between the degree of lymphopenia produced and the degree of hypoferremia which developed following the ACTH injection.

Table 29. Effects of ACTH, iron dextran, and age on the serum iron content of pigs

Age in Weeks	Group	Mean Serum Iron (mcgm.%) Before ACTH	Mean Serum Iron (mcgm.%) After ACTH[a]	Mean Decrease in serum iron level (mcgm.%)	s[b]	n[c]
2	Controls	42.6	18.4	24.2	13.4	5
	Iron Dextran	150.7	77.5	73.3	52.6	6
3	Controls	79.1	34.8	44.4	26.6	5
	Iron Dextran	80.1	54.0	26.1	15.3	4
4	Controls	83.9	57.0	26.9	14.4	4
	Iron Dextran	101.3	40.9	60.6	36.6	5
5	Controls	44.9	39.5	5.4	7.2	4
	Iron Dextran	105.5	45.6	60.0	53.9	5
6	Controls	89.5	41.0	48.5	10.8	4
	Iron Dextran	64.3	41.4	22.9	41.4	6

[a]6 hours after 10 U.S.P. units of adrenocorticotropic hormone injection.

[b]Standard deviation of the mean decrease with ACTH.

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

Table 30. Effects of ACTH, iron dextran, and age on the per cent saturation of transferrin with iron in pigs

Age in Weeks	Group	Mean % Saturation Before ACTH	Mean % Saturation After ACTH[a]	Mean Change in % Saturation	s[b]	n[c]
2	Controls	12.8	5.5	-7.3	4.3	5
	Iron Dextran	36.2	21.4	-14.8	9.4	6
3	Controls	17.4	8.2	-9.2	4.8	5
	Iron Dextran	20.6	12.6	-8.0	4.5	5
4	Controls	19.9	13.9	-5.8	4.6	4
	Iron Dextran	21.5	10.2	-11.3	6.9	5

[a]6 hours after 10 U.S.P. units of ACTH injection.

[b]Standard deviation of the mean decrease with ACTH.

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

Table 30 (Continued)

Age in Weeks	Group	Mean % Saturation Before ACTH	Mean % Saturation After ACTH[a]	Mean Change in % Saturation	s[b]	n[c]
5	Controls	12.8	11.0	-1.6	3.0	3
	Iron Dextrans	23.4	10.3	-13.2	8.9	5
6	Controls	23.0	12.6	-10.4	2.9	4
	Iron Dextrans	15.1	9.8	-5.3	9.6	6

Table 31. Effects of ACTH, iron dextran, and age on the total iron-binding capacity of serum of pigs

Age in Weeks	Group	Mean Binding Capacity (mcgm.%) Before ACTH	Mean Binding Capacity (mcgm.%) After ACTH[a]	Mean Change in binding capacity	s[b]	n[c]
2	Controls	337.5	324.0	-13.5	46.0	5
	Iron Dextran	409.6	352.6	-56.9	53.7	6
3	Controls	440.7	406.8	-33.8	19.9	5
	Iron Dextran	419.5	416.9	-2.6	27.5	5
4	Controls	426.8	407.7	-19.1	49.0	4
	Iron Dextran	462.2	397.4	-64.7	38.4	5
5	Controls	369.0	434.2	+65.2	95.0	3
	Iron Dextran	441.9	432.8	-8.9	53.8	5
6	Controls	397.6	369.7	-27.9	44.7	4
	Iron Dextran	418.9	403.1	-15.9	20.6	6

[a] 6 hours after 10 U.S.P. units of ACTH injection.

[b] Standard deviation of the mean change with ACTH.

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

Table 32. Effects of ACTH, iron dextran, and age on the sedimentation rate of erythrocytes of pigs

Age in Weeks	Group	Mean ESR (mm/hr.) Before ACTH	After ACTH[a]	Mean Change in ESR (mm/hr.)	s[b]	n[c]
2	Controls	8.2	8.9	+0.7	3.1	5
	Iron Dextran	5.2	5.9	+0.7	2.0	6
3	Controls	13.1	13.8	+0.7	2.4	5
	Iron Dextrans	6.1	6.3	+0.2	3.5	6
4	Controls	7.5	7.6	+0.1	0.9	5
	Iron Dextrans	8.3	12.6	+4.3	3.0	6
5	Controls	3.9	5.1	+1.2	1.1	5
	Iron Dextrans	5.1	9.5	+4.4	3.4	6
6	Controls	2.8	2.9	+0.1	0.7	5
	Iron Dextrans	6.6	6.3	-0.3	0.6	6

[a]6 hours after 10 U.S.P. units of ACTH injection.

[b]Standard deviation of the mean change with ACTH.

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

Table 33. Effects of ACTH, iron dextran, and age on the packed cell volumes of pigs

Age in Weeks	Group	Packed Cell Vol. (%) Before ACTH	After ACTH[a]	Mean Change in PCV (%)	s[b]	n[c]
2	Controls	24.6	25.0	+0.4	3.8	5
	Iron Dextran	31.7	29.9	-1.8	4.7	6
3	Controls	21.1	19.0	-2.1	3.1	5
	Iron Dextran	34.8	33.5	-1.3	1.3	6
4	Controls	23.7	22.2	-1.4	1.2	5
	Iron Dextran	32.5	31.1	-1.4	0.4	6

[a]6 hours after 10 U.S.P. units of ACTH injection.

[b]Standard deviation of the mean change with ACTH.

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

Table 33 (Continued)

Age in Weeks	Group	Packed Cell Vol.(%) Before ACTH	After ACTH[a]	Mean Change in PCV (%)	s[b]	n[c]
5	Controls	28.0	26.0	-1.9	1.6	5
	Iron Dextran	32.2	30.2	-2.0	1.3	6
6	Controls	30.2	28.9	-1.4	0.9	5
	Iron Dextran	32.6	30.9	-1.7	0.6	6

Table 34. Effects of ACTH, iron dextran, and age on the total leukocyte count per cubic mm. of blood of pigs

Age in Weeks	Group	Leukocyte Count ($x10^3$) Before ACTH	After ACTH[a]	Mean Change in leukocyte count ($x10^3$)	s[b]	n[c]
2	Controls	7.3	9.2	+1.9	0.8	5
	Iron Dextran	9.4	10.7	+1.3	2.7	6
3	Controls	5.9	7.8	+2.1	2.4	5
	Iron Dextran	6.2	6.3	+0.1	0.8	6
4	Controls	8.5	9.9	+1.5	3.1	5
	Iron Dextran	6.3	6.2	-0.1	2.0	6
5	Controls	10.5	14.3	+3.8	1.2	5
	Iron Dextran	9.3	8.5	-0.8	2.2	6
6	Controls	14.4	21.2	+6.8	1.7	4
	Iron Dextran	12.3	17.5	+5.2	2.3	6

[a] 6 hours after 10 U.S.P. units of ACTH injection.

[b] Standard deviation of the mean change with ACTH.

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

Table 35. T values for testing the hypotheses that means of specified factors were equal before and after ACTH injections

Factor	$\bar{d}$[a]	$s_{\bar{d}}$[b]	t value	n[c]
Serum Iron (mcgm. %)	-40.6	5.6	7.31	48
% Saturation of Transferrin	-9.0	1.1	8.54***	48
Total Iron-Binding Capacity (mcgm. %)	-21.8	7.4	2.93**	48
ESR ($\frac{mm.}{hr.}$)	+1.3	0.4	3.23***	55
PCV (%)	-1.5	0.3	4.84***	55
Total Leukocyte Count ($\frac{x10^3}{cu.mm.}$)	+2.0	0.4	5.00***	55
Albumin (%)	+1.7	2.2	0.80	32
α-globulins (%)	+1.6	1.7	1.37	32
β- and γ-globulins (%)	-3.9	1.7	2.29*	32

[a]Average difference between all before and after observations.

[b]Standard error of the mean difference.

[c]Total number of paired observation.

***P<.005

**P<.01

*P<.05

As can be seen from Table 35 there was no significant change in the percentages of albumin and α-globulin. The change in the β- and γ-globulin was significant only at the 5% level; because of the non-significance of the other two plasma components, no conclusion was drawn as to a possible explanation for the change in the β- and γ-globulins.

V. SUMMARY

Comparisons of the serum iron levels, total iron-binding capacity, per cent saturation of transferrin, erythrocyte sedimentation rate, plasma protein percentage, and packed cell volume, as well as the usual hematologic parameters of erythrocyte count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte number, leukocyte differential count, and leukocyte count were made between iron-deficient pigs and pigs which received an intramuscular injection of iron dextran supplying 150 mg. elemental iron. Data were also calculated to determine the relationship of the serum iron content to the erythrocyte sedimentation rate. An ancillary study involved the use of intramuscularly injected adrenocorticotropic hormone on one litter of pigs and noting its effect on the serum iron level, total iron-binding capacity, per cent saturation of transferrin with iron, and leukocyte count.

The erythrocyte sedimentation rate was significantly higher in the iron-deficient pigs, and this was calculated by testing the regression coefficients to be due to the lower values of the packed cell volumes of these pigs rather than to the lower serum iron values. However, the weekly changes in the mean plasma iron values paralleled in general the weekly trend of the packed cell volume. Apparently, the degree of anemia is a reflection of the plasma iron content

and this anemia is associated with the erythrocyte sedimentation rate. Evidently the plasma iron neither physically nor chemically influences the sedimentation of erythrocytes, but because it supplies the iron for erythrogenesis, it affects the packed cell volume. This packed cell volume in turn has an effect on the sedimentation rate. Furthermore, in some instances the packed cell volume was low (27%) and a normal erythrocyte sedimentation rate was present; this indicates that factors other than the packed cell volume are important in determining the sedimentation rate and that the Wintrobe-Landsberg chart may not accurately predict the packed cell volume for a given rate and vice versa. Plasma-red cell exchange studies revealed that at normal packed cell volumes there was no difference in the sedimentation rates of "normal erythrocytes" in "anemic plasma" and erythrocytes from anemic pigs in "normal plasma." Results from dilution of exchanged cells and plasma were inconclusive.

The iron-deficient pigs showed a significantly lower serum iron level, per cent saturation of transferrin, and total iron-binding capacity. The latter finding is the reverse of that usually observed in iron-deficiency anemia in humans; this may be a species difference.

Data obtained for the erythrocyte count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and leukocyte count re-

vealed significantly higher values for the iron dextran treated pigs than for the control pigs. As has been reported previously by other workers, the differential leukocyte count revealed that with increasing age, there was a relative decrease in neutrophils and a relative increase in lymphocytes.

There was no statistically significant difference in the percentages of plasma protein components between the two groups. The main findings were a decrease in the percentage of β- and γ-globulins with time and an increase in the percentage of albumin.

The intramuscular injections of adrenocorticotropic hormone resulted in significant decreases in the serum iron level, per cent saturation of transferrin, total iron-binding capacity, and the β- and γ-globulin percentage. Statistically significant increases occurred in the erythrocyte sedimentation rate and total leukocyte number. Due to the relative magnitude of the changes, it is felt that the changes in serum iron content, per cent saturation of transferrin, and total leukocyte count were probably primary changes due to specific effects of the adrenocortical hormones which were released by ACTH stimulation. The mechanisms of such actions are unknown. The decreases in the packed cell volume and total iron-binding capacity are relatively small, and it is proposed that these may be secondary to the plasma dilution that might have re-

sulted from the secretion of aldosterone from the adrenal cortex. Also, the increase in the sedimentation rate is relatively small and this is thought to reflect the decreased packed cell volume, rather than the decreased serum iron content. No explanation is proposed for the decrease in the β- and γ-globulin percentage.

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