1-1-1966

Plasma, interstitial, and total body water of pigs from birth through six weeks of age with and without iron.

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PLASMA, INTERSTITIAL, AND TOTAL BODY WATER OF PIGS FROM BIRTH THROUGH SIX WEEKS OF AGE WITH AND WITHOUT IRON

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Physiology

Iowa State University
Of Science and Technology
Ames, Iowa

1966

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I. INTRODUCTION

The regulation of the distribution of water is a complex mechanism in the animal body as evidenced by its functioning in maintaining temperature, composition and volume of blood, interstitial fluid and intracellular fluid as well as being necessary to carry nutrients to and waste products from every organ and tissue in the body. In the healthy adult animal the quantity of water in the various compartments is believed to remain quite constant from hour to hour and from day to day. The influence of growth or pathologic changes, such as anemia, may influence the regulatory mechanism in such a way that there is a shift in the water distribution and this distribution may not remain constant.

Information to date has shown the porcine species to be a good experimental animal for hematologic and cardiovascular studies. The literature is lacking in basic information concerning physiological changes in water distribution during the first few weeks of life as well as in information concerning shifts in water distribution 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 on their dam as their only source of nutrients they become clinically anemic by the time they are 7 to 14 days of age. This anemia has been termed "physiological anemia" or "baby pig anemia". It has been well documented that a deficiency of iron is responsible for at least a portion of the anemia seen in baby pigs.

Water is the most abundant component of the living organism. It is distributed between two main compartments, extracellular and intracellular. The extracellular compartment is easily divided into plasma water and interstitial water. The individual existence of these compartments is assured by the presence of an active selective cell membrane which maintains their separate chemical and physiological characteristics.

Despite the obvious importance of the physiologic variations in the distribution of body water between the body water compartments, the methods available do not permit a simple, direct and quantitative analysis of these changes. In spite of these shortcomings the methods in use offer several advantages: accurate measurement of the space involved, simplicity, and the possibility of serial observations. The goal has been to measure simultaneously and independently the plasma, extracellular, and total body water and subsequently to calculate the volume of interstitial and intracellular water by simple difference.

The measurement of any body water compartment depends upon the use of a substance, usually foreign to the organism, which satisfies the primary criteria of uniform and exclusive distribution throughout the space being measured. If the amount of substance so distributed (T) and its concentration (C) are known, the volume (V) of the compartment is $V = T/C$.

When used in the intact animal or human, the substance should ideally also fulfill the following conditions: (1) fairly rapid and uniform distribution throughout the compartment, (2) no formation or destruction in the organism, (3) no specific influence on water distribution, (4) slow

elimination from the body, (5) no toxicity, and (6) accurate and easy determination.

The usual technique involves the single intravenous injection of a known amount of the measuring substance. Immediately after injection the plasma concentration will fall at a rate which is a function of (1) the velocity of diffusion throughout the space being measured, and (2) its rate of elimination or metabolism (Gaudino, 1949).

If diffusion is rapid and elimination slow, uniform distribution will rapidly occur and the plasma level will approach constancy. If a plasma sample is then analyzed, the volume of distribution may be calculated from the formula ($V = T/C$). The volume of distribution of the substance will then be equal to the volume of the compartment being measured, if the stated conditions have been fulfilled.

Since most substances are eliminated from the body at a constant rate, the simple formula ($V = T/C$) must be altered to account for this elimination and the formula then becomes $V = \frac{T-kt}{C}$ where: t = time and k is the loss per unit time.

The objectives of this study were to compare the volumes of water in the various water compartments of iron deficient pigs with pigs that have received an exogenous source of iron. These comparisons were designed to accomplish the following goals:

1. Verification that there is a difference in the ratio of plasma volume to body mass in control versus iron injected pigs.

2. Ascertain whether or not there is a difference in the ratio of extracellular fluid volume to body mass in control versus iron injected pigs.

3. Ascertain whether or not there is a difference in the ratios of total body water and intracellular water to body mass in control and iron injected pigs.

4. Establish elimination rates for N-acetyl-4-amino-antipyrine and sodium thiocyanate in measuring total body water and extracellular fluid respectively in baby pigs.

II. REVIEW OF LITERATURE

A. Definition of Blood and Plasma 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. Plasma is the fluid portion of the blood in which the corpuscles are suspended. These definitions have been followed in this thesis. It should be stated that no substance used to measure blood volume measures total blood volume. Each measures either plasma or cell volume. Total blood volume is then calculated from the values found.

B. Techniques for Measuring Plasma Volume

A chronologic listing of methods and procedures for measuring blood volume has been compiled by Gregersen and Rawson (1959). While not complete, this synopsis of methodology shows trends in technique development in the field and the order of this development and improvement as well as the periods of common usage for each technique.

The basis of methods currently used is the dilution technique which introduces a known quantity of a test substance into a fluid chamber of unknown quantity. A sample of the fluid containing the test substance is removed from the chamber following uniform dispersion and the concentration can be determined.

The T-1824 dye, radio-iodinated albumin, and chromic chloride methods are three most commonly used for measuring plasma volume. The most widely accepted method of measuring plasma volume is the T-1824 dye

dilution technique. Since the spectral absorption curve of T-1824 is not the same in plasma from all species (Allen et al., 1953), the dye should be standardized for each species. Lindhard (1926) disclosed large errors made by early investigators using water standards to determine plasma dye volume due to the differences between special characteristics of various dyes in aqueous and plasma solutions. Despite these findings, reports still appear comparing plasma samples containing dye with dye standards diluted in water (Stahl and Dale, 1958). Gregersen (1944) has published a concise and subsequently well verified summary of the basic points of the T-1824 technique stating that the dilution volume of T-1824 represents the true plasma volume.

Plasma volumes calculated from extrapolation of the time-concentration curve on a semi-log plot are essentially the same in simultaneous determinations of volume distributions of T-1824 and the antigens, bovine albumin, bovine globulin and the polysaccharide SIII (Gregersen et al., 1945; Gregersen et al., 1950) and similar tests with T-1824 and albumin I^{131} (Franks and Zizza, 1958; Gibson et al., 1946) and hemoglobin.

A technique which eliminates the concern with affecting factors of spectral absorption of T-1824 in plasma including the species difference was devised by Allen (1953). This process removes T-1824 dye from the serum by a simple paper pulp extraction and subsequent determination of concentration. This eliminates errors from hemolysis, lipemia, and from changes in inherent plasma color. Modifications speeding up elution were made by Campbell et al. (1958).

Because of the loss of a certain amount of the test substance during mixing and uniform distribution, the calculation of the volume of

the fluid chamber must take this into account to be valid and accurate. 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 value needed for calculation is the amount of test substance that would have been present if the test substance were completely mixed at the time of injection. 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. This assumption has been supported for the dye, T-1824, in the cow (Reynolds, 1953), the dog (Allen and Gregersen, 1953), and the pig (Talbot and Swenson, 1963). The need for validation in each species in which the dye is going to be used is pointed out by evidence that T-1824 overestimates plasma volume in the rabbit (Zizza and Reeve, 1958). Loss and unequal distribution may be factors.

The widely used I^{131} - albumin technique, evidently first used by Gibson et al. (1946) to estimate plasma volume, has limitations not always recognized. Absorption of iodinated protein by some types of glassware (Reeve and Franks, 1956) and unaccounted for free I^{131} in preparations of I^{131} - albumin (Franks and Zizza, 1957) will yield false plasma volume estimates. Another cause of false estimates can be the development of an immune reaction following injection of a foreign protein (Gregersen and Rawson, 1959).

Gray and Sterling (1950) introduced a method for measuring plasma volume that utilizes radioactive chromic chloride. 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 may be used for plasma volume measurements. The original results have been subsequently verified (Gray and Frank, 1953; Small and Verloop, 1956).

C. Plasma Volume of Swine

Reports of plasma volumes in swine are not abundant in the literature.

Using the Fe^{59} plasma packed cell volume technique, Jensen et al. (1956) reported a mean erythrocyte volume of 30.4 ml. per kg. and a mean plasma volume of 47.7 ml. per kg. in 18 normal swine ranging in weight from 8.6 to 97.0 kg. The erythrocyte volume values are calculated, not measured.

The same data were reported by Bush et al. (1956b) as well as 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.

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

Measuring the plasma volume of newborn pigs by the T-1824 technique, McCance and Widdowson (1959), with seven fasted pigs and seven allowed to suckle, found the plasma volume of the sucklings increased from 55 ml. per

kg. of body weight before suckling to 81 ml. per kg. 24 hours after suckling. The plasma volume of the fasted pigs stayed constant over the same 24 hour period.

Talbot (1963) measured plasma volume of pigs from birth through six weeks of age and found an increase in plasma volume per kilogram of body weight in control pigs while iron dextran treated pigs showed a progressive decrease as age increased.

D. Plasma Volume of Other Species

There are many reports of plasma volume determinations in the literature and only a few will be included in this study.

Baker and Remington (1960) used T-1824 dye and showed a mean plasma volume of 52.8 $\pm$ 8.7 ml. per kg. of body weight in 16 normal dogs. Later Baker (1963) reported a mean T-1824 plasma volume of 48.3 $\pm$ 7.1 ml. and a mean I^{131} plasma volume of 41.6 $\pm$ 4.2 ml. for 10 normal dogs. Chien (1960) found average plasma volume values of 54.8 ml. per kg. for sympathectomized - spleenectomized dogs. Parkinson and Dougherty (1958) reported plasma volume as determined on 21 normal beagle dogs to be 51.4 $\pm$ 12.4 expressed as a ml. per kg. of body weight basis. Deavers et al. (1960) reported data on 100 mongrel dogs and showed a mean T-1824 plasma volume of 50.2 $\pm$ 1.11 ml.

Reports of plasma volume determination in the human are very numerous but many are lacking in detail and are difficult to interpret. Schmidt et al. (1956) presented data collected on aged men and women. They found 40.6 and 42.7 ml. per kg. for 70-94 year old females and males, respectively.

Wadsworth (1954) reported on T-1824 determined plasma volumes of eight women and showed an average of 43.1 ml. per kg. of body weight. Wang (1959) reported a mean T-1824 plasma volume of 39.0 ml. per kg. in 50 unanesthetized rats after correcting for the F_{cells} factor. The guinea pig was found to have a mean T-1824 plasma volume of 72 ml. per kg. prior to correction for the F_{cells} factor, (Ancill, 1956). This value might be slightly lower if the correction had been made.

Gregersen et al. (1959) report on plasma volume of 18 Rhesus monkeys as determined with T-1824. A mean value of 36.4% $\pm$ 3.98 was given. Klement et al. (1955) measured T-1824 plasma volume in 20 goats and reported a mean value of 55.9 ml. per kg. of body weight. Courtice (1943) measured plasma volume in 30 goats using T-1824 and reported an average figure of 53 ml. per kg. In this same publication he gave T-1824 plasma volume values found in the rabbit. These probably should be disregarded since the work of Zizza and Reeve (1958) shows that plasma volumes measured with T-1824 in the rabbit are erroneous. The reason for this is unknown but unequal distribution, loss, and incomplete binding of T-1824 to rabbit plasma proteins may be factors.

E. Definition of Interstitial Fluid Volume

Interstitial fluid volume is the extracellular fluid water, which includes all body water which is not within cell membranes, less the plasma volume. There are at least two types of noncellular water which complicate a simple definition of extracellular fluid. The first of these is so called "transcellular water", which is fluid outside of the

tissues in such cavities as the gastrointestinal tract, glandular lumina, cerebrospinal fluid, water in bile, synovial sacs, and the upper and lower urinary tract (Power and Keith, 1936). The second includes extracellular water in connective tissue, including bone, that is not in simple diffusion equilibrium with the rest of the extracellular fluid (Edelman et al., 1952).

The extent to which thiocyanate or other substances used to measure ECF (extracellular fluid) penetrate these areas is not clear, although thiocyanate is known to be distributed into saliva and gastrointestinal secretion (Crandall and Anderson, 1934) and into saliva, pancreatic juice, bile, and urine (De Souza, 1907). Also Corper (1915) found that when given intravenously, it attains concentrations in the normal lung, eye, kidney, heart and testes not far from that of blood while its concentration was low in the liver and practically absent in muscle. From the foregoing it is assumed that the thiocyanate space includes a large part of this "transcellular water". Variations of ECF measurements, using various test substances, may be explained on the basis of the extent of their diffusion into the transcellular water spaces. Thus, there is no exact correlation between a distinct anatomical ECF and an ECF determined physiologically by the use of dilution techniques.

This basic limitation dictates that the types and amount of indicator substance used and the conditions of measurement be clearly specified in any determination of ECF. It also indicates that the derivation of intracellular water as the difference between total body water and an operationally determined ECF represents, at best, an approximation.

Despite such limitations the determination of water spaces by the dilution techniques is widely accepted and gives rise to data of great physiologic value.

F. Techniques for Measuring Interstitial Fluid Volume

All techniques used for the estimation of extracellular fluid volume employ the dilution technique. The interstitial fluid volume is then determined by subtracting the plasma volume from the ECF. As previously stated, by using this technique only an approximation is obtained.

There are many test substances used to estimate extracellular fluid volume. They include thiocyanate, thiosulfate, radioactive bromide, radioactive sodium, sodium, bromide, radioactive chloride, chloride inulin, sucrose, and mannitol.

The interstitial fluid space has great physiologic significance because it provides the environment in which the cells are constantly bathed. Its absolute measurement in the intact animal or man is not possible because the substances used do not fill the primary requisites of absolute exclusion from the cells or complete diffusion throughout the extracellular fluid including the transcellular water.

Fenn (1936) reports that the earliest attempt to measure extracellular fluid was made by Hermann (1888). This was done by histological examination of frozen sections of frog muscle. Hermann found a value of 14.5% of the muscle and Fenn attempted to repeat this work using a planimeter on camera lucida tracings and the space so measured represented 15-17% of the muscle mass. The fact that chemical analysis of muscle

gives a value for total chloride which is equal to the concentration in the plasma if it is considered to be limited to the extracellular fluid led to the conclusion that all chloride was extracellular (Fenn et al., 1934). Sodium on the same presumption was also considered extracellular (Harrison et al., 1936). Based on these two assumptions, and by assuming that sodium and chloride were equal in concentration throughout the body, Harrison et al. (1936) and Hastings and Eichelberger (1937) estimated extracellular fluid by measuring total body chloride or sodium and the serum concentration of chloride or sodium and then applying these values in the equation $V = T/C$. However, this method required that the animal be killed and as a result could not be applied to the intact animal or human. Complex systems for studying sodium and chloride balances were therefore developed and used to estimate extracellular fluid (Lavietes et al., 1935b). Subsequent work showed that the assumptions made before these values were obtained were incorrect and therefore the values found are questionable (Harrison et al., 1936; Manery and Hastings, 1939; Amberson et al., 1938; Wilde, 1945; and Boyle and Conway, 1941).

In spite of these findings, radioactive sodium and chloride were used to determine extracellular fluid volumes (Manery and Bale, 1941; Manery and Haege, 1941; and Kaltreider et al., 1941). The use of radioactive chloride (CL^{38}) is complicated by its half life which is only 37 minutes. Radioactive sodium is not desirable to use because complete diffusion throughout the sodium space takes approximately 24 hours. A fair estimate of the space can be made after 3 hours however (Levitt and Gaudino, 1949).

Bromide has been used to estimate extracellular fluid since its distribution is believed to be the same as that of chloride (Weir and Hastings, 1939). This work was done by Brodie et al. (1939), Wallace and Brodie (1939), Goudsmit et al. (1941), and Berger et al. (1950). Measurements of extracellular fluid volume using radioactive bromide in man were also made by Staffurth and Birchall (1960).

Probably the most widely used substance as indicator of extracellular fluid volume has been sodium thiocyanate (Crandall and Anderson, 1934; Ashworth et al., 1943; Elkinton and Taffel, 1943; Gregersen and Stewart, 1939; Medway and Kare, 1959; and Bowler, 1944). It offers the advantages of simplicity of determination, rapid diffusion and equilibration, and extremely slow renal elimination. However, it does have the following disadvantages; it does enter the red cells, the gastric mucosa, and possibly some other cells and therefore gives only an index of the change (Crandall and Anderson, 1934) in extracellular fluid volume rather than the absolute volume. It has one advantage over several other substances used and that is it diffuses into the transcellular spaces. In certain pathologic states (Overman, 1946 and Overman and Feldman, 1947) the cell permeability of thiocyanate is markedly increased. It must be stated that thiocyanate measures a volume which is slightly greater than the true extracellular volume.

The simultaneous use of thiocyanate, sodium ^{24} and Cl^{38} has given spaces of 35.6, 27.6, and 24.7 per cent of the body weight, respectively, in dogs (Winkler et al., 1943).

Sulfate has been used as an indicator substance, but it has the

disadvantage of rapid renal excretion and corrections must be made for normal sulfate levels in the serum and excretion of these normal sulfate levels (Lavietes et al., 1935b; Lavietes et al., 1936).

In an attempt to find a substance which would not permeate the cells even to the smallest degree, attention was turned to the carbohydrates. Sucrose was investigated by Lavietes et al. (1935a) and Lavietes et al., (1936), mannitol by Newman et al., (1944), Dominguez et al., (1947) and Elkinton (1947), and inulin by Wilde (1945), Gaudino et al., (1948), Gaudino and Levitt (1949), and Schwartz et al., (1949). Generally the space measured with the carbohydrates is smaller than with other indicator substances because they may not diffuse into the cells nor into the transcellular water. They offer the disadvantage of rapid renal excretion, difficult analytical methods and incomplete recovery which indicates some degree of utilization by the individual for which no accurate correction can be made (Dominguez et al., 1947; Keith and Power, 1937; and Smith et al., 1940).

Inulin offers many advantages as an indicator of extracellular fluid volume. It has a large molecular weight, it is not an electrolyte and is lipid-insoluble, all characteristics which make it less able to permeate the cell membrane. It does not permeate the erythrocyte according to Smith, (1937) diffuse through the renal tubule (Smith, 1937; Richards et al., 1934; Shannon and Smith, 1935) nor does it undergo concentration by the liver cells of the frog (Haywood and Hober, 1937). According to Shannon and Smith (1935) and Smith et al. (1938) it is physiologically inert and therefore exerts negligible osmotic pressure which prevents its drawing water from cells.

Inulin has one very great disadvantage. It cannot be used in the intact unanesthetized animal. It must either be used in nephrectomized animals or by continuous infusion into the subject. Nephrectomy does not allow usage of the subject for prolonged studies as are necessary to study changes due to age, and continuous infusion requires that the animal be anesthesized for prolonged periods which is undesirable as well as being very time consuming. The ECF as measured with inulin is significantly smaller than that of chloride or thiocyanate according to Wilde (1945).

Sodium thiosulfate was studied by Gilman et al. (1946). They found its volume distribution to be in the range of extracellular fluid volume, it diffuses rapidly, is metabolized slowly at an exponential rate and its rate of elimination is proportional to its concentration.

These properties suggested (Gilman et al., 1946) that the thiosulfate ion would be a suitable and convenient indicator agent to use for measuring ECF. Cardozo and Edelman (1952) undertook a study to determine if the thiosulfate ion distributed itself in the volume of the ECF. These authors cite Brun (Scandinav, Acta Med 1949) and state that Brun, "unequivocally asserted that the thiosulfate ion was confined to the extracellular space and not absorbed by the renal tubules". However, Cardozo and Edelman found that the thiosulfate ion does penetrate the erythrocyte after 70 minutes, does cross some cell membranes, and only 65% recovery of injected material in man could be accomplished. Gilman et al. (1946) reported 70-80% recovery in dogs.

G. Interstitial Fluid Volume of Swine

In searching the literature, no publications on the interstitial body fluid volume or on the extracellular fluid volume of swine could be found.

H. Interstitial Fluid Volume of Other Species

Medway and Kare (1959) found the thiocyanate space to be 61.0% of the body weight in the 1 week old chick and 26.2% in the 32 week old chick. The interstitial fluid space was 52.3% of the body weight in the 1 week old bird and 21.7% in the mature hen. This clearly shows a decrease in the interstitial fluid volume as age increases. These values compare very closely to those found by Hegsted et al. (1951) for birds of the same age.

Cardozo and Edelman (1952) determined the ECF volume on 7 dogs and obtained a value of 24.4% of the body weight with a range of 20.3 to 28.1%. They also reported the average thiosulfate space as 16.6% of the body weight in 6 adult men. In addition, they studied 5 patients with obvious edema and found thiosulfate space volumes to range from 21.5 to 28.7% of the body weight and stated, "It is of interest that none of these edematous patients showed thiosulfate spaces in the 'excessive' range (over 35% of body weight) often found in observing the radiosodium space and thiocyanate space in sick patients". This difference may be partially explained in that thiosulfate is not believed to diffuse into the "transcellular water" while thiocyanate does.

Crandall and Anderson (1934) observed a mean extracellular fluid

volume equal to 32.5% of the body weight with a range of 27-36% in 33 normal adult dogs, 24.2% with a range of 21-26% in 19 normal adult men, 26.5 with a range of 26-29% in 4 normal horses, and a figure of 35% of the body weight with a range of 32-37% was given for normal rabbits using sodium thiocyanate as the measuring substance.

Crandall and Anderson (1934) also reported on extracellular fluid volume on 17 dogs which were either superhydrated or showed spontaneous edema of varying degrees and obtained values ranging from 37 to 60% of the body weight depending on the degree of superhydration or edema. However, the largest values were found in animals showing spontaneous edema. In man they observed 3 cases of spontaneous edema or ascites and measurements ranged from 34-37% of the body weight. They then measured the ECF in one of these patients following a salt-free diet and ammonium nitrate diuresis. The volume fell from 34% of the body weight prior to treatment to 21% following therapy. By using sodium thiocyanate as an indicator, a value of 21% could indicate slight dehydration.

Extracellular fluid volumes as estimated in the dog by using various measuring substances were as follows: Inulin gave a space corresponding to 20% of the body weight (Gaudino and Levitt, 1949). The bromide space is given as being somewhat larger, 31% (Manery and Hastings, 1939) and 30% (Gaudino et al., 1948). The chloride space is given as 25, 23 and 27% by Winkler and Elkinton (1943), Amberson et al. (1938), and Harrison et al. (1936), respectively. The sodium space very closely approximated the chloride space as shown by Winkler and Elkinton (1943) to be 28%, Gaudino et al. (1948) to be 30%, and by Gaudino and Levitt (1949) to be 30%. The extracellular fluid volume of the dog as measured with sulfate

approximated 26% of the body weight (Smith et al., 1939). Thiocyanate measures a volume which is greater than that measured by any of the other measuring agents used. Values given for the dog are as follows: Gaudino et al. (1948) 32%, Gaudino and Levitt (1949), 34%, Goudsmit et al. (1941) 32%, Sunderman and Dohan (1941) 30%, and Winkler and Elkinton (1943) 36% while Elkinton and Taffel (1943) gave values of 33-35%. Crandall and Andersen (1934), who originally worked with thiocyanate, gave a value of 32.5%.

Cardozo and Edelman (1952) explored the use of sodium thiosulfate in the dog and reported an average figure of 24.4% of the body weight for seven dogs tested. Schwartz et al. (1949) obtained lower values in dogs. Their findings showed an average thiosulfate space of only 17%. This may have been due to a greater fat content in his animals. Gilman et al. (1946) obtained volumes of 22% of the body weight in dogs which agrees quite closely with Cardozo and Edelman (1952). Schwartz et al. (1949) studied two normal human subjects and obtained values of 15.7 and 19.5% by using thiosulfate.

As one can readily see, the extracellular fluid volume reported varies greatly with the test agent used. No one agent is believed to estimate the space more accurately than any other. Values reported for any one technique compare very favorably with values given by other workers using the same technique.

Morrison (1961) reported on the inulin and thiocyanate space in rats. They reported on two groups of normal rats in which they found a value of 16% of the body weight with a standard error of $\pm$ 0.2% in a group of

11 rats and a value of 16.6% with a standard error of ± 0.6% in a group of 6 rats. These same rats showed values of 27.8% with standard error ± 0.5, and 26.5 with standard error ± 0.9 respectively with sodium thiocyanate. These two measurements were made simultaneously. White and Rolf (1957) reported an inulin space of 20.0 ± 1.1% of the body weight in rats. Wang and Hegsted (1949) reported that the thiocyanate space occupied 17% of the body weight of rats, however, they allowed only 10 minutes for complete mixing and this may have resulted in incomplete diffusion with a subsequent high concentration and a lower percentage on a body weight basis. Huang and Bondurant (1956), in another rat study, reported the mean thiocyanate space to be 33 ± 1.0% of the body weight.

Many studies on the distribution of body water in man have been made. The extracellular fluid volumes for the various indicator substances used vary just as they do in other species. Values reported for the inulin space are 16% of the body weight as given by Gaudino et al. (1948) and Schwartz et al. (1949) and 15% given by Berger et al. (1949). Values given for mannitol are slightly higher, 18% reported by Newman et al. (1944), 16% Dominguez et al. (1947) and 23% given by Elkinton (1947). Sucrose values are given to be 20% by Lavietes et al. (1935a) and 19% by Lavietes et al. (1936).

Bromide spaces of 27 and 23% of the body weight are given by Brodie et al. (1939) and Schwartz et al. (1949), respectively. These values are somewhat higher than the chloride value reported by Moore (1946) who gave the chloride space as 18% of the body weight. The sodium space was given as 26% of the body weight by Moore (1946), Schwartz et al. (1949), and Kaltreider et al. (1941). The sulfate space was reported as

20% and 24% of the body weight by Lavietes et al. (1935a) and Lavietes et al. (1936), respectively, while Cardozo and Edelman (1952) found the thiosulfate volume to approximate 16.6% in normal adult men.

Thiocyanate has probably been used the most extensively for measurement of ECF in man and the following figures as a per cent of body weight have been reported: 22 and 23% (Lavietes et al. 1935a and 1936), 27% (Moore, 1946), 24% (Goudsmit et al., 1941 and Schwartz et al., 1949), 22% (Gaudino et al., 1948), and 25% (Kaltreider et al., 1941).

Radioactive bromine was used by Staffurth and Birchall (1960) to measure the extracellular fluid volume and they reported a mean of 26.4% with a standard deviation of 2.95 and a range of 20.8 to 32.8 for 21 normal patients consisting of 9 females and 12 males. They also showed that heavier patients tended to have lower ECF values as expressed on a percentage of body weight basis while lighter patients tended to have higher values. They also found that patients showed a higher value following a loss in weight. Seven adults (5 men and 2 women) were used for this study and they gave a mean figure of 32% with a standard deviation of 0.7% and a range of 31.3 to 33.3%.

Edelman et al. (1952) by using thiosulfate as the indicator substance reported a mean of 16.8% of the body weight with a range of 15.3 - 18.8% for normal adult men. They also observed a mean of 17.5 with a range of 15.3 - 21.0 for 13 normal adult women.

Extracellular water in sheep has been estimated by Hix et al. (1953). They reported that extracellular fluid volume in the sheep was equal to 30% of the sheared body weight as measured with sodium thiocyanate. In this same publication they reported "many simultaneous determinations of

ECF and TBW (total body water) on goats, sheep, and cattle, normally hydrated, reveal that the ECW is extremely consistent in volume at 30 per cent of body weight. Among these ruminants there exists a species difference in the metabolism of -SCN (unpublished data)".

Aikawa (1950) reported average ECF values of 26 and 22% of the body weight as determined on 39 rabbits, by using Na^{24} and -SCN, respectively, as the indicator substance.

I. Definition of Total Body Water and Intracellular Water

The terms "total body water" and "intracellular water" will be used to designate the quantities which simple examination of the term would suggest. That is, "total body water" will mean all of the water in the entire body and "intracellular water" will mean water which is contained within the cells of the body.

Intracellular water is obtained by subtracting the extracellular fluid volume from the total body water. As previously discussed this will give only an approximation of the intracellular water because of the variation in extracellular fluid volumes as determined with the various indicator substances.

J. Techniques for Measuring Total Body Water and Intracellular Fluid

The earliest methods used for the measurement of total body water depended on total or partial desiccation of the body (Mitchell et al., 1945). This procedure had obvious limitations which led to a search for

a practical approach to the measurement of total body water in the intact, living animal.

Newburgh et al. (1930) tried to study total body water by accurately accounting for all avenues of water intake as well as all avenues of water excretion and loss. The errors involved in this technique were discussed by Lavietes (1935), and can be summed up by saying it is not usable.

Gamble et al. (1923), operating on the observation that total base and water excreted are in the same proportion as in the plasma, suggested the use of cation balance studies to estimate total body water. This method required tedious balance experiments and was very subject to errors. Also, the basic assumptions that the cation concentration was the same all over the body and that this concentration remained constant in spite of changes in volume of total body water have been questioned by Elkinton et al. (1945).

Many attempts have been made to measure total body water by determining specific gravity of the whole body (Rathbun and Pace, 1945; Morales et al., 1945; Pace and Rathbun, 1945). The determination was made by using a formula derived from independent measurements of body specific gravity, total body water, and total body fat in the slaughtered or killed animal. However, in the living animal this method is unusable because it is complicated by the presence of gases in the respiratory and digestive tracts and no evidence has been shown to substantiate the use of this method in various physiologic and pathologic states.

In 1937, Marshall et al. studied the distribution of sulfanilamide in the dog. In 1938, Painter reported on the simultaneous determination

of total body water by using sulfanilamide and urea as indicator substances. Danowski (1944) reported on the use of thiourea in determining changes in body water. These substances have all been discarded as indicator substances because they failed to meet the requirements of a good indicator substance for measuring total body water. Urea, thiourea, and sulfanilamide have all been found to be unequally distributed in the body, and urea also varies greatly due to variation in endogenous formation. These observations have been made by Sise (1939), Waterhouse and Shannon (1942), Ralls (1943), Chesley (1944), and Williams and Kay (1945).

In 1938, Winkler and Smith reported on the use of potassium as an indicator substance but the use of this agent is questionable in view of work done by Fenn in 1936 when he reported that cells can actively concentrate potassium. This is generally accepted as being true today. Therefore, this method is no longer used.

Deuterium oxide (heavy water) is very similar to water chemically and biologically. These properties would appear to make it an ideal indicator substance for measuring total body water as observed by Hevesy and Hofer (1934). They also observed that it is nontoxic to the living individual. It has the advantage of rapid diffusion through the cell membrane with complete equilibrium being established rapidly after a single injection as reported by Lucke and Harvey (1935), Brooks (1936), Parpart (1936) and Govaerts and Lambrechts (1946).

Deuterium has the disadvantage that a certain per cent is believed to interchange with hydrogen atoms in organic compounds of the body. The actual amount that centers into the metabolic processes is not known

but is estimated by Moore (1946) to be no more than 5% of the total heavy water injected.

Pace et al. (1947) reported on the use of tritium as an indicator substance for measuring total body water. These workers stated that the body water as measured with tritium agrees very closely with that found by whole body desiccation. Tritium has the disadvantage of requiring very elaborate procedures for preparation and quantitation of samples.

In 1949, Soberman et al. reported on the uniform distribution of antipyrine throughout the body after a single injection, and its use for measuring total body water was recommended. This work showed that simultaneous determination of body water using deuterium and antipyrine gave good correlation for the human although the values found with deuterium were slightly higher possibly due to its entering into metabolic reactions.

Antipyrine has the advantage that it is much simpler and less expensive to use than any of the other indicator substances discussed which give valid results. However, it too has several disadvantages. A small undetermined amount is bound to the plasma proteins and no exact correction can be made for this. Antipyrine is believed to be metabolized at a constant rate, so extrapolation to zero time must be made for proper correction. The time required for equilibrium to be attained is prolonged in the clinically edematous individual. This disadvantage is true of all indicator substances discussed. Tissue analysis indicates that tissue levels and plasma levels are not the same. All these disadvantages were pointed out by the original workers who proposed its use. It has the further disadvantage of a rather complicated chemical determination

procedure which is subject to error. This last disadvantage led these same workers (Brodie et al., 1951) to report on the use of NAAP (N-acetyl-4-aminoantipyrine) as an indicator substance for measuring total body water.

N-acetyl-4-aminoantipyrine has the advantage of not binding to plasma proteins to any great extent, having a rate of metabolism which is practically nil, and a simple chemical analysis which does not require a spectrophotometer in the ultraviolet (1800-3900A) range (Brodie et al., 1951).

Ninty-two per cent of the disappearance of NAAP from the blood stream can be accounted for by its renal excretion (Brodie et al., 1951) and less than 3% of the injected NAAP is bound to the plasma protein.

NAAP has the disadvantage of being eliminated from the body via the urine so extrapolation must be done to get zero time concentration. NAAP has been reported not to cross the rumen wall from the blood stream to any great extent and thus measures only the true body water (Whiting et al., 1960). This was believed to be true for other parts of the digestive tract as well which would make NAAP the indicator of choice for measuring true body water content.

K. Total Body Water and Intracellular Fluid of Swine

The number of reports in the literature which give total body water or intracellular water values for swine is small, and all those which were found dealt with pigs which were much older than those used in this study.

Hansard (1964) reported values for the total body water of swine as follows: 67.3 ± 8.5% as determined with antipyrine on 19 animals weighing 39 ± 6 kg.; 63.8 ± 6.9% was determined with iodine tagged antipyrine on 23 animals weighing 40 ± 6 kg.; 64.1 ± 4.3% as determined with tritium on 11 animals weighing 42 ± 4 kg.

Hansard (1964) also reported on adult swine in which he found values of 48.2 ± 7.8% as measured with antipyrine on 15 animals weighing 93 ± 4 kg.; 44.8 ± 6.3% as measured with iodine tagged antipyrine on 14 animals weighing 98 ± 8 kg.; and for tritium he reported 45.5 ± 5.7% as measured on 7 animals weighing 96 ± 7 kg.

Kraybill et al. (1953) reported on 24 head of swine weighing 29 to 155 kg. at 90 to 466 days of age. They gave a mean total body water of 46.8% with a range of 36.7 - 64.5% as determined with antipyrine and 44.1% with a range of 33.8 - 59.6 as determined by specific gravity methods. This tremendous range can be partially explained by the difference in age and degree of fatness of the pigs as pointed out by the author.

L. Total Body Water and Intracellular Fluid of Other Species

By using deuterium as the indicator Flexner et al. (1942) found TBW value of 65% of the body weight for the guinea pig.

Total body water volumes for man have been reported many times and only a few of the reports will be reviewed here. Soberman et al. (1949) observed a figure of 53 per cent of the body weight for man when measured with deuterium while they obtained a value of 52 per cent using antipyrine. These values were found by running simultaneous determinations

on the same individual. Pace et al. (1947) revealed that the space distribution of tritium in man is equal to 64.8% of the body weight. Dupertuis et al. (1951) using antipyrine measured total body water of men at different weights and found the following: twenty-three men averaging 86.2 kg. and 70 inches tall had an average body water content of 53.8% with a range of 43.0 - 58.0%; twenty-six men who averaged 72.2 kg. and 68.6 inches tall averaged 60.1% ranging from 58.1 and 63.0%; and thirty-two men averaging 70.7 kg. and 70 inches tall averaged 67.2% with a range from 63.1 to 73.0%. The average body water content was 61.1% for the 81 men sampled. As one can readily see, the per cent of body fluid is very closely correlated to the obesity of the individual.

Prentice et al. (1952) reported on 15 normal men using tritium as the indicator agent and found a mean of 52.1% of the body weight with a range of 47.9 to 56.7%. These data were collected on men ranging from 37 to 56 years of age. They also reported a comparison between volumes determined by using antipyrine and tritium as indicator substances. Five subjects showed a mean TBW of 58.9% with tritium and 56.1 with antipyrine.

Osserman et al. (1950) obtained TBW values on 81 men by the antipyrine and specific gravity methods. Antipyrine gave a mean total body water measurement equal to 61.1% of the body weight with a range of 43 to 72.9% while specific gravity methods gave a volume of 61.0% to the body with a range of 44 to 72.9% of the body weight. It is interesting to note that they got a very close correlation between specific gravity and antipyrine methods. This is not generally found to be true. Most authors agree that the specific gravity method leaves much to be desired.

Steele et al. (1950) reported a comparison of total body water in men and women. They found that the mean total body water of 51 men was 52.7% while that of 31 women was 44.6% as measured with antipyrine. Their work clearly shows that there is a difference in total body water between adult males and females. This is probably due to the effect of the female sex hormones which affect deposition of fat.

Berger et al. (1950) reported on the intracellular water of man. This work was done by subtracting the bromide space from the antipyrine space. Their findings indicate that there is a difference in intracellular water between males and females. The mean as found for 51 men was 46.6% of the total body water and for 31 women was 41.7%. Vaughan and Boling (1961) reported on the measurement of body fluids in men using three different methods employing tritium. They found that any of the three procedures was equally reliable and gave mean values for 12 patients so tested of 50.4%, 50.4%, and 51.5% of the total body weight as body water.

A comparison of the volume of distribution of antipyrine, N-acetyl-4-aminoantipyrine and I^{131} labeled 4-iodo-antipyrine in human beings was done by Talso et al. (1955). These workers reported the mean value for the volume of distribution of antipyrine was 49.7% of the body weight, for N-acetyl-4-aminoantipyrine 50.6% of the body weight and for 4-iodo-antipyrine 50.6% of the body weight. The difference between these values were not statistically significant.

The amount of literature available on the measurement of total body water of domestic animals is quite limited. Kraybill et al. (1951) and Kraybill et al. (1952) reported on the measurement of total body water in cattle using the specific gravity method on eviscerated carcasses and

compared this to the antipyrine method done just prior to slaughter. By using this method they obtained values of 54.4 and 54.3% for the specific gravity and antipyrine methods, respectively. There was also a very close correlation between the two methods and this can be explained by the fact that eviscerated carcasses were used, thereby eliminating the factors which make the specific gravity technique undesirable. Kraybill et al. (1951) also reported on 6 crossbred cattle and found a range of 45.4 - 61.0% with a mean of 52.2% of the body weight. For these same animals the specific gravity method gave a range of 46.2 - 59.2% with a mean of 51.9. Twenty-four Hereford cattle were also measured for TBW and values of 43.9 - 63.0% with a mean of 54.4 were found for antipyrine while specific gravity methods gave a range of 43.1 to 63.3 with a mean of 54.1%.

MacFadden and Richards (1956) reported on the measurement of total body water in calves at various ages from 1 to 16 weeks. They found values of 73.08% for one group and 70.36% for the other and explained the difference as being due to a lower per cent fat in the carcasses of the second group.

Whiting et al. (1960) compared the body water content of cattle as measured with NAAP and antipyrine. They consistently got higher values with antipyrine than with NAAP, 59.6% and 46.8%, respectively. They partially explained this difference due to the belief that NAAP does not pass into the digestive tract, particularly the forestomachs, to any great extent while antipyrine does and they present data to prove this point.

Hansard (1964) reported on measurements in young and mature beef cattle. The young animals were 129 days ± 30 days of age and the mature cattle were 1175 ± 110 days. In the young group, 20 animals measured with antipyrine gave a value of 71.1 ± .9% of their total body weight as water. Twenty-seven measured with I^{131} labeled antipyrine produced values averaging 68.4 ± 8.7% while sixteen measured with tritium produced results of 70.1 ± 4.1%. The mature animals showed a much lower value when measured with any of these indicator substances. The results were as follows: fifteen animals measured with antipyrine measured 51.6 ± 8.6% of the body weight, 13 measured with I^{131} labeled antipyrine gave 48.3 ± 8.1% of the body weight, and 8 measured with tritium showed that 50.7 ± 5.4% of their body weight was water.

Total body water measurements on sheep are not plentiful in the literature. Only one publication was found and that was by Hansard (1964). He compared volumes on young and mature sheep as measured with antipyrine, I^{131} labeled antipyrine, and tritium. He presented the following values for young sheep (137 ± 18 days). Forty-five animals measured with antipyrine averaged 61.8 ± 6.5%, 49 measured with I^{131} labeled antipyrine measured 61.4 ± 7.0%, and 12 measured with tritium gave 50.7 ± 5.4% of the body weight as total body water. The mature sheep averaged 350 ± 70 days of age. The TBW values reported for them by using antipyrine, I^{131} labeled antipyrine and tritium respectively were: 24 animals 56.7 ± 6.6%, 27 animals 55.8 ± 6.3%, and 13 animals 57.1 ± 5.4% of the total body weight.

The antipyrine dilution technique has been applied to estimation of total body water in the fowl by Weiss (1958) and he reports that in 7

white leghorn females an average of 61.4% of the body weight, ranging from 57 to 68% was total body water. He also found that the rate of transformation for the chicken was very high, averaging 46% per hour and ranging from 25 to 69% per hour. In this same publication, Weiss reports the change in water content of pullets during the first part of the laying year. He found that at 26 weeks of age, pullets averaged 66.0% body water with a standard error of $\pm$ 1.8 and their body water consistently decreased to 55 weeks of age when they averaged 52.9% of the body weight with a standard error of $\pm$ 1.

Herrald and Sapirstein (1952) measured total body water of the dog with antipyrine. Eight normal dogs were used and a range of 53.9 - 67.9% of the body weight was found to be body water. The average was 59.9%. This correlates very closely with Smith (1951) who reported 63.0% as measured with deuterium. They also found that three emaciated dogs showed values of 75%, 75.6%, and 75.9%. The elimination rate for antipyrine in dogs was determined by these workers to range from 20 to 60% per hour.

The great variability in the elimination rate of antipyrine from the body from individual to individual within a species makes it an undesirable indicator substance.

Huckabee (1956) investigated the use of 4-aminoantipyrine as an indicator substance for measuring total body water. This substance behaves much like NAAP, in fact NAAP must be converted to 4-aminoantipyrine by hydrolyzing it in acid (Brodie et al., 1951) before a colorimeteric determination can be made. Brodie and Axelrod (1950) observed that aminopyrine is metabolized to 4-aminoantipyrine and this is then rapidly

acetylated to N-acetyl-4-aminoantipyrine. Huckabee (1956) gave the following values as measured with 4-aminoantipyrine and antipyrine respectively, 49.4 and 50.3% in eight human subjects.

III. MATERIALS AND METHODS

A. Experimental Design and Methods

A total of 48 pigs were used to obtain the physiologic values reported in this study. Pregnant crossbred sows were obtained from farm herds in the Ames area and allowed to farrow in pens with concrete floors. The sow and pigs were housed in these same pens for the duration of the experiment. A 16.7% protein ration (Table 1) meeting the National Research Council requirements (1964) was fed to the dams prior to farrowing and during lactation. This ration, which contained no added iron, and the dam's milk were the only foods available to the pigs during the entire study.

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

Ingredient	Per cent of ration
Corn	46.0
Oats	20.0
Wheat bran	5.0
Soybean oil meal	16.4
Dried whey	10.0
Dicalcium phosphate	1.0
Feeding lime	0.6
Salt	0.5
Vitamin pre-mix[a]	0.5

[a]Vita-Plex, Vet-A-Mix, Shenandoah, Iowa.

Between 12 and 24 hours of age, the pigs in each litter were ear marked, weighed, and sexed. At this time pretreatment values were determined. The following determinations were made: body weight, erythrocyte number, leukocyte number, packed cell volume, hemoglobin, differential leukocyte count, T-1824 content of plasma, sodium thiocyanate content of plasma, and N-acetyl-4-aminoantipyrine content of plasma. From the data obtained, the following values were calculated: plasma volume, extracellular fluid volume, and total body water per kg. of body weight. Between 48 and 72 hours of age the litter was divided at random into two groups, one of which was treated with iron dextran.[1] Pigs used for controls were given no supplemental iron while treated pigs were given 2 ml. of iron dextran which supplied 150 mg. of elemental iron. All iron injections were given intramuscularly into the left rear leg. The determinations listed above were then repeated on the 7th, 14th, 21st, 28th, 35th, and 42nd day after birth.

B. Experimental Techniques

1. Plasma volume

The method used to determine the plasma volume was a modification of the T-1824 dilution method described by Gregersen (1944). The use of this method in the pig was validated by Talbot (1963). The following procedure was used for all plasma volume determinations. The T-1824 dye was prepared in a one per cent solution from the same lot for the

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

entire study and the same standard stock solution was used for the entire study. A Coleman Jr. spectrophotometer with matched cuvettes was used for all 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 concentration in the standard stock solution and hence the amount injected were predetermined by dilution procedures and spectrophotometric comparison with a known standard solution of T-1824 dye. Approximately 1 hour after injection of the T-1824 dye a 6 ml. sample of blood was drawn from the anterior vena cava and placed in a test tube with EDTA (disodium ethylenediamine tetraacetate). After thorough mixing to prevent clotting the samples were centrifuged for 15 minutes at 2800 rpm and plasma was harvested for determination of T-1824 sodium thiocyanate, and N-acetyl-4-aminoantipyrine content.

The dye was extracted from the plasma by the method of Campbell et al. (1958). Campbell's work was done on human serum but Talbot's work (1963) found this technique equally as accurate when used on swine serum.

After extraction of the dye, the concentration in mg./ml. of serum was determined spectrophotometrically. This concentration was corrected mathematically to compensate for the average rate of loss of dye from the circulatory system which was found to be 22.1 per cent/hour (Talbot, 1963).

The formulae used to correct the dye concentration were as follows:

$$\frac{\text{Optical Density of Unknown}}{\text{Optical Density of Solution}} \times \text{Conc. of solution (std.)} = \text{Concentration of Unknown solution}$$

(Min. post inj.) (Rate of loss/min.) (mg. inj.) = mg. loss

$$\frac{\text{(mg. inj.) - (mg. loss)}}{\text{Conc. of Unknown}} = \text{volume}$$

$$\frac{\text{Volume}}{\text{Body weight in Kg.}} = \text{ml./kg.}$$

2. Interstitial fluid volume

The method used to determine the interstitial fluid volume was a modification of the sodium thiocyanate dilution technique described by Bowler (1944).

The sodium thiocyanate solution was prepared in a 10% solution from the same lot for the entire study and the same standard stock solution was used for the entire study. A Coleman Jr. spectrophotometer with matched cuvettes was used for all determinations of thiocyanate concentration.

The sodium thiocyanate was injected into an ear vein at an approximate dosage of 75 mg. per kg. of body wt. The exact dosage was determined by using a calibrated syringe. Exact amounts of NaSCN concentration in the standard stock solution and hence the amount injected were predetermined by dilution procedures and spectrophotometric comparison with a known standard solution. Approximately 1 hour after injection and each hour thereafter for five consecutive hours a 6 ml. sample of blood was drawn from the anterior vena cava and placed in a test tube with EDTA. After thorough mixing to prevent clotting the samples were centrifuged for 15 minutes at 2800 rpm and the plasma was harvested for determination of thiocyanate content, as well as for N-acetyl-4-aminoantipyrine.

This concentration was corrected mathematically to compensate for

the average loss of thiocyanate from the plasma as described in the Results and Discussion section. The formula used to correct the thiocyanate concentration was as follows:

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

where t = time elapsed between injection of NaSCN and withdrawal of sample

K = loss rate of thiocyanate from plasma

A = concentration of thiocyanate of time t

A_o = concentration of thiocyanate at time of injection if it were completely mixed in the thiocyanate space

2.303 = constant used to convert from log to the base ten to log to the base E.

The corrected plasma concentration was then used in the following formula to calculate extracellular fluid volume.

$$\text{extracellular fluid volume} = \frac{\text{amount of NaSCN injected}}{\text{concentration of NaSCN in plasma}}$$

3. Total body water and intracellular fluid

The method used to determine the total body water was a modification of the N-acetyl-4-aminoantipyrine technique described by Brodie et al. (1951).

The NAAP solution was prepared in a 10% solution from the same lot for the entire study and the same standard stock solution was used for the entire study. A Coleman Jr. spectrophotometer with matched cuvettes was used for all determinations of NAAP concentration.

The NAAP was injected into an ear vein at an approximate dosage of 150 mg. per kg. of body weight. The exact dosage was determined through the use of a calibrated syringe. Exact amounts of NAAP concentration in the standard stock solution and hence the amount injected were predetermined by dilution procedures and spectrophotometric comparison with known standard solutions of NAAP. Approximately 1 hour after injection and each hour thereafter for five consecutive hours a 6 ml. sample of blood was drawn and placed in a test tube containing EDTA. After thorough mixing to prevent clotting the samples were centrifuged for 15 minutes at 2800 rpm and the plasma was harvested for determination of NAAP and thiocyanate content.

This concentration was corrected mathematically to compensate for the average loss rate of NAAP from the plasma as described in the Results and Discussion section. The formula used to correct the NAAP concentration was as follows:

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

The corrected plasma concentration was then used in the following formula to calculate total body water:

$$\text{Total body water} = \frac{\text{amount of NAAP injected}}{\text{concentration of NAAP in plasma}}$$

4. Other hematologic determinations

Hemoglobin concentrations were determined by the cyanmethemoglobin

technique.[1] A Beckman B spectrophotometer with a set of matched cuvettes was used to make all spectrophotometric determinations. The readings obtained were then compared to a chart to convert them to hemoglobin concentration in grams per cent. All samples were run in duplicate.

Erythrocyte and leukocyte values were determined in duplicate using National Bureau of Standards certified pipettes, counting chambers, and cover glasses. The diluting fluids used were 0.90% NaCl for red blood cell determinations and 0.1 N HCl for leukocyte determinations.

Packed cell volumes were determined in duplicate by the micro-hematocrit method.

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[1]Method described in pamphlet (revised, 1962) from Hycel Inc., Houston, Texas.

IV. RESULTS AND DISCUSSION

A. Plasma Volume

After the original objectives of this project were defined, a review of the literature showed that only one method had been validated for measuring plasma volume in swine. This was the T-1824 dye dilution technique (Talbot, 1963).

The data obtained for plasma volume in pigs from birth through six weeks of age are presented in Table 2. The analysis of variance is presented in Table 3. Table 2 shows the ml. of plasma per kg. of body weight at each interval tested. The values are means plus or minus one standard deviation. The data are related to the physical size of the pig. This was done for all fluid compartments measured because of the great variation in size of the pigs used. As would be expected a larger pig has a larger total volume for any one given compartment. To compare this total compartment volume to that of a smaller pig is very apt to give a significant difference. This significance does not indicate anything other than larger pigs have more total water per compartment than smaller pigs. Volumes were expressed as ml. per kg. to adjust for the differences in body weights. By doing this a comparison on a per unit weight basis was made. Thus, plasma volume was expressed as ml. per kg. of body weight.

Visual analysis of the data (Table 2) shows that there is essentially no difference between treated and control pigs at 1 week of age. As the pigs increase in age the plasma volume per kg. of body weight tends to

Table 2. Effects of iron dextran and age on plasma volume of pigs

Age	CONTROLS			IRON DEXTRAN		
	Mean ml./kg.	s[a]	n[b]	Mean ml./kg.	s	n
Birth	81.5	8.4	12	77.3	7.0	12
1 wk.	79.9	4.6	21	78.9	10.6	19
2 wk.	76.3	10.0	18	72.5	9.7	16
3 wk.	73.9	8.6	15	61.0	4.4	14
4 wk.	78.8	13.5	15	58.9	11.9	14
5 wk.	72.3	6.7	15	59.0	6.0	14
6 wk.	67.2	14.8	13	56.2	12.3	14

[a]Standard deviation of the mean.

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

remain about constant for the control pigs while the plasma volume for the treated pigs progressively decreases. The analysis of variance verifies this observation statistically. Both age and iron play a part in this change in plasma volume as the interaction indicates (Table 3). However, a further breakdown of the data reveals that the age effects are significant only at the 10% level while the iron effect is significant at the 2.5% level. This indicates that although both iron and age are playing a part in this change the effect of the iron is much greater. The greater volume of plasma per kg. of body weight in control pigs was observed by Talbot (1963). His work was based on pigs of the same age as

Table 3. Analysis of variance of plasma volume of pigs

Source of Variation	df	S.S.	M.S.	F
litters	4			
iron	1	2,208.47	2,208.47	13.55**
error a	4	652.0	163.0	
age	5	4,749.0	949.8	5.83*
birth	1	1,614.75	1,614.75	18.57***
age x iron	5	1,721.0	344.2	3.95***
individuals	191	16,605.0	86.94	

*P 0.10

**P 0.025

***P 0.005

those used in this study. The values which he reports and those found in this study are comparable.

Talbot (1963) calculated total blood volumes and found that the total blood volume of control pigs and iron treated pigs were not significantly different. This observation is easy to understand since the PCV is lower in control pigs than in iron injected pigs and the total plasma volume is about equal. The injected pig has a greater total blood volume, since he is larger, but when this is expressed on a ml. per kg. basis the two groups compare favorably. This agrees with the theory advanced by Reeve et al. (1960) that a homeostatic control mechanism of

the body tends to keep the total blood volume constant. Talbot (1963) states that when red cell volume, plasma volume, and total blood volume are correlated, it is evident that plasma volume adjusts to compensate for varying amounts of erythrocyte volume. Talbot observed that after three weeks of age total blood volume and plasma volume expressed on a ml. per kg. basis were lower for iron treated than control pigs. It has also been observed by other workers that plasma volumes were lower in older swine (Jensen et al., 1956). These workers reported 47.7 ml. per kg. in 18 normal pigs ranging in weight from 8.6 to 97.0 kg. These data would indicate that as body weight increases and the amount of fat deposited increases, the plasma or total blood volume expressed on a body weight basis decreases.

McCance and Widdowson (1959) found that fasted newborn pigs had 55 ml. of plasma per kg. of body weight while those allowed to suckle had 81. The figure of 81 ml. per kg. of body weight is very similar to the values found in this study, 81.5 and 77.3 for the two groups of pigs at birth and before treatment. Plasma volumes found for pigs from birth through six weeks of age do not compare favorably with most other species. Values given for the adult dog average 49.9 ml. per kg. of body weight while the adult human averages 42.1. Other species have average plasma volumes in this same general range with the exception of the guinea pig which has 72 ml. per kg. of body weight (Ancill, 1956). However, it must be remembered that the values reported are for much older animals than those used in this study and when comparisons are made to mature swine the comparison is quite favorable. Adult swine have an average plasma volume of 47.7 ml. per kg. of body weight (Jensen et al., 1956).

Maclaurin (1966) measured the plasma volume in 46 infants ranging from 4 hrs. to 13 days of age with T-1824. He found a mean plasma volume of 47.2 $\pm$ 1.03 ml. per kg. His observations for infants agree quite closely with that of other workers. It is of interest that these findings are much lower than those found for the pig during this same period in life.

Valid comparisons are very difficult to make because many publications giving plasma volume fail to give enough information on the hemoglobin content, PCV, and RBC numbers. The plasma volume has been shown to increase as the degree of anemia increases (Talbot, 1963). Therefore, any comparison made must consider the data on erythrocytes and hemoglobin content. The data collected in this study show that control pigs were markedly anemic and the iron-injected pigs were somewhat anemic after the third week. Because of this, the values obtained cannot be considered to be comparable to values given for most normal individuals of any given species.

B. Extracellular Fluid Volume

In reviewing the literature, no report on the validation of any technique for measuring extracellular fluid volume in pigs could be found. Sodium thiocyanate was therefore selected as the indicator agent for measuring this space because of its many advantages as pointed out in the Review of the Literature. The concentration of thiocyanate was determined for each sample drawn and back. extrapolation using the "TARSIER" (Atkinson, 1966) method was used to estimate concentration at

zero time. The concentration at zero time was calculated separately for each pig weekly and the results reported in this study are based on these values rather than on an average elimination rate figure.

The data collected for extracellular fluid volumes in pigs from birth through six weeks of age are in Table 4. The values presented are means plus or minus one standard deviation and are expressed on a ml. per kg. of body weight basis. The statistical analysis of these data (Table 5) shows that there is no significant difference between iron injected and control pigs. The only significant difference seen is due to age as would be expected since the ECF decreased by approximately 40 per cent during the course of the six weeks study.

The validity of using sodium thiocyanate as an indicator agent in pigs at birth is questioned since at birth it measures a volume which is equal to or slightly greater than the volume for total body water as measured by NAAP (Table 6). In searching the literature no information could be found on the distribution of thiocyanate in the newborn pig or the newborn of any species. Work done on the "newborn" of other species has generally been done on individuals which are several days or even several weeks old. The following values have been reported for the "newborn" human infant. Ely and Sutou (1952) studied 14 infants under 6 months of age using thiocyanate as the indicator agent. They found an average space of 39.3% of the body weight but their youngest infant was 8 days old. Maclaurin (1966) measured ECF with thiocyanate in 26 infants from 4 hours to 3 days old and found it to equal 36.0 $\pm$ 0.7% of the body weight. Fellers *et al.* (1949) reported the average thiocyanate

Table 4. Effects iron dextran and age on extracellular fluid volume of pigs

Age	CONTROLS			IRON DEXTRAN		
	Mean ml./kg.	s[a]	n[b]	Mean ml./kg.	s	n
Birth	570	125.1	13	532	127.0	13
1 wk.	416	56.9	20	437	61.9	20
2 wk.	357	31.1	19	359	56.1	16
3 wk.	341	51.0	16	339	48.0	14
4 wk.	338	51.8	15	333	49.2	14
5 wk.	361	81.5	15	306	62.1	14
6 wk.	346	45.5	13	309	26.5	14

[a]Standard deviation of the mean.

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

space found in 16 infants to be 41.2% of the body weight. The range was 36.6 to 45.9% while the radiosodium space in these same infants averaged 42.6% and ranged from 31.0 to 51.8%. They do not give the age of the individuals used. Perley et al. (1951) reported that the sodium space, for 27 infants studied, ranged from 51.5% in a twenty-two day old, to 31.1, in a seven day old, with no age-volume relationship. They found that of the 27 infants studied the average for 11 premature babies was 43.5% while the average for 16 full term infants was 35.2%. Flexner et al. (1947) working with radioactive sodium reported an average value of 43.5% of the body weight for 3 infants. The ages of these infants were 1.5, 6 and 7 days.

Table 5. Analysis of variance of extracellular fluid volume of pigs

Source of Variation	df	S.S.	M.S.	F
litters	4			
iron	1	264.4	264.4	21
error a	4	5,011.0	1,252.75	
age	5	216,640.0	43,328.0	34.59***
birth	1	40,722.4	40.722.4	9.56***
age x iron	5	19,695.0	3,939.0	.93
individuals	195	8,302,738.0	4,257.8	

***P 0.005

Calcagno et al. (1951) observed ECF values ranging from 29.1 to 42.1 ml. per kg. in infants ranging from 6 - 34 days of age. The indicator agents used were insulin and sodium ferrocyanate.

The values found by individuals working with infants are similar to those in the pig from birth through six weeks. However, none of these workers worked with the infant at the time of or very shortly after birth and thus no light is shed on the distribution of thiocyanate on any other indicator at birth.

In searching the literature no information on the ECF value at the time of birth could be found for any animal species. Studies comparing the volume of the ECF as measured with various indicator substances in

the newborn pig should be done before any comparisons are made using the values found in this study.

It should be stated that the visible signs of edema occasionally seen in clinical cases of iron deficiency anemia in baby pigs were not observed in these pigs. Hix et al. (1959) observed in ECF for adult cattle to be 28.5 $\pm$ 0.9% of the body weight using thiocyanate as the measuring agent. This value is much lower than the values found in this study and no valid comparison can be made because of the difference in species and age.

Inglis et al. (1955) showed that the ECF was much higher in calves than in cows. They reported an ECF of 41.3 $\pm$ 2.3% for calves while that for cows was only 27.5 $\pm$ 4.5%. They used radiosodium as the measuring agent. It is of interest to note that the value which they give for calves is very similar to the values found in this study for pigs at 1 week of age, although they used a different indicator agent. Dalton (1964a) used thiosulfate to measure ECF in calves and reported an average ECF for 10 calves to be 24.2 $\pm$ 2.6% of the body weight. These calves were approximately 1 to 3 weeks of age. No comparison can be made with his work because he used a different species and indicator agent. Thiosulfate generally measures a smaller ECF volume than thiocyanate.

Anderson and Mixner (1960) measured the ECF volume in 1 cow and 1 calf. They used inulin and reported 15.3% for the cow and 29.3% of the body weight for the calf. Again the value for the calf is comparable to the values found in this study, being slightly lower than that found

for treated pigs at 5 and 6 weeks of age. Inulin is known to give a lower ECF volume than thiocyanate and this may explain some of the difference. Here too, no good comparison can be made because of species and indicator substance differences.

Thiocyanate space has been measured in the chick by Medway and Kare (1959) and by Hegsted et al. (1951). Medway and Kare found that the thiocyanate space of the week old chick was 61.0 $\pm$ 11.6%. This decreased rapidly during the first eight weeks to 42.4 $\pm$ 3.4%. Comparison of these data with those obtained for pigs is difficult because of the difference in species. The values found, however, follow the same general pattern as those for pigs and if extrapolation to birth time were made, essentially the same picture would be seen. The work done by Hegsted et al. (1951) was done with chicks from 13 to 35 days of age. The values they found were slightly lower than those found by Medway and Kare. Medway and Kare (1959) made no attempt to explain the large ECF obtained in the week old chick other than to say that the obligatory water loss is greater in the young than in the mature because the ratio of body surface area to body mass is considerably higher in the young. Thus, a greater extracellular fluid volume is required by the young to take care of this loss. It is very possible that sodium thiocyanate overestimates the ECF volume in chicks based on the observations made in this study. More work needs to be done in this area.

The newborn individual should not be considered to be in a pathologic state, but neither is there any reason to believe that its physiologic state is the same as that of an older individual. A good

example of this is the precipitation of proteins from plasma with trichloracetic acid. It has been observed in many laboratories that the plasma proteins of the newborn will not precipitate, forming instead a "milky" suspension. In view of this the findings of Overman (1946) and Overman and Feldman (1947) should be taken into consideration. They found that the permeability of cells to thiocyanate was markedly increased in certain pathologic states.

C. Total Body Water

As with extracellular fluid volume, no reports on the validation or use of any indicator substance in measuring total body water of young pigs could be found. NAAP was then selected as the indicator of choice because of its many advantages as stated in the review of literature. It was suggested by Whiting et al. (1960) that NAAP did not cross the rumen wall and therefore probably did not enter other parts of the digestive tract thus measuring only true body water. This did not prove to be true in the pig. In three pigs killed at three weeks of age the concentration in the fluid portion of the ingesta was found to be equal to that of the plasma while a greater concentration of NAAP was found in the urine.

The mathematical methods used to obtain total body water were the same as those discussed under extracellular fluids. The results of these calculations are in Table 6. This table shows that over the six week period the control pigs averaged 59.4% of the body weight as body water while the iron treated pigs averaged 58.5%. The statistical analysis (Table 7) shows that there is no significant difference in total body water content of pigs from birth through six weeks of age, nor is there

Table 6. Effects of iron dextran and age on total body water of pigs

Age	CONTROLS			IRON DEXTRAN		
	Mean ml./kg.	s[a]	n[b]	Mean ml./kg.	s	n
Birth	552	91.1	13	536	129.2	13
1 wk.	603	90.4	20	608	90.7	20
2 wk.	616	57.3	19	587	84.1	16
3 wk.	593	49.9	16	579	69.8	14
4 wk.	554	89.6	15	626	81.9	14
5 wk.	593	78.5	15	583	72.7	14
6 wk.	639	96.9	13	565	67.1	14

[a] Standard deviation of the mean.

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

any significant difference due to iron injection. Since there is little or no change in the total body water content per unit of weight during the first six weeks of life while the extracellular fluid volume per unit body weight steadily decreases, the only conclusion that can be drawn is that there is a shift of water from the extracellular fluid compartment to the intracellular compartment, assuming that thiocyanate accurately estimates the extracellular fluid space from the first through the sixth week of life. Again, it should be stated that the visible signs of edema occasionally seen in iron deficiency anemia in baby pigs were not observed in these pigs. The presence of edema would very likely show a different picture than the one seen here.

Table 7. Analysis of variance of total body water of pigs

Source of Variation	df	S.S.	M.S.	F
litters	4			
iron	1	5,152.8	5,152.8	.47
error a	4	44,241.0	11,060.25	
age	5	17,265.0	3,453.0	.31
birth	1	46,917.6	46,917.6	.71
age x iron	5	66,286.0	13,257.2	.20
individuals	195	1,289,738.0	66,140.4	

The mean TBW value found in this study was 59.0% of the body weight. This value is considerably lower than that found by Medway and Kare (1959) for chicks. They found 72.4 $\pm$ 1.3% at 1 week with a slow decrease to 68.7 $\pm$ 1.0% at 8 weeks of age. They do not state how these values were derived. The values found in this study are also much lower than those found by Hansard (1964) in pigs which were much older. He found values averaging from 63.8 to 67.3% of the body weight for pigs weighing approximately 40 kg.

Hansard (1964) and Kraybill et al. (1953) reported on TBW as measured with antipyrine in mature swine. Hansard found the TBW as determined with antipyrine to be 48.2 $\pm$ 7.8% of the body weight of pigs weighing 93 $\pm$ 4 kg. Kraybill et al. found TBW to average 46.8% for pigs ranging in weight from 29 to 155 kg. The total body water content in his

pigs ranged from 36.7 to 64.5% of the pigs' body weight. Normally it would be expected that the pigs used in this study should have a TBW value which is greater when expressed as a percentage of the body weight, than that found by Hansard and Kraybill et al. This is because as age increases the percentage of body water decreases. One possible explanation for the lower values found in this study is that the experimental design was such that it caused this to occur. The large quantity of blood drawn to establish elimination rates may have caused a slight dehydration.

The values found in this study are very similar or slightly higher than those values found in the adult of most species as reported in the review of literature.

Dalton (1964b) using Aryshire calves 1 to 3 weeks old obtained TBW values equal to 73.6 $\pm$ 6.4% by using urea as the measuring agent. This work agrees with that of MacFadden and Richards (1956) using antipyrine. They obtained 72.0 - 74.0% of the body weight in calves at 1 week of age. These values are also much higher than those found for pigs in this study.

Hanna (1960) measured TBW in 52 infants at 3 hours of age. He found the deuterium space of these individuals to be 77.2 $\pm$ 4% of the body weight. His work agrees quite closely with Maclaurin (1966) who reports values of 71.5 $\pm$ 1% for infants 4 hrs. to 3 days old when measured with antipyrine. The values reported for infants at birth are considerably higher than those found for pigs in this study.

D. Body Weight

The data on body weights are included in this thesis because of the

importance that body weight plays on quantitative data obtained from measuring body fluid compartments. As obesity of the individual increases body water expressed as percentage of the body weight decreases. This is due to the low water content of adipose tissue. The adult is found to have a lower water content per unit of body weight than is found in the young or immature.

Table 8 gives the mean body weights plus or minus one standard deviation. The analysis of variance of these data is given in Table 9. This table shows a significant age by iron interaction as well as

Table 8. Effects of iron dextran and age on body weights of pigs

Age	CONTROLS			IRON DEXTRAN		
	Mean ml./kg.	s[a]	n[b]	Mean ml./kg.	s	n
Birth	1.5	.2	14	1.4	.2	13
1 wk.	2.0	.5	22	2.0	.5	20
2 wk.	3.3	.7	19	3.2	1.0	16
3 wk.	4.6	1.1	16	5.0	1.3	14
4 wk.	5.6	1.6	15	6.9	1.7	14
5 wk.	6.6	2.3	15	9.1	2.2	14
6 wk.	8.4	3.2	13	11.3	2.9	14

[a] Standard deviation of mean.

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

Table 9. Analysis of variance of body weights of pigs

Source of Variation	df	S.S.	M.S.	F
litters	4			
iron	1	53.32	53.32	16.10**
error a	4	13.25	3.31	
age	5	1,066.61	213.32	64.42***
birth	1	579.54	579.54	174.96***
age x iron	5	41.67	8.33	3.82***
individuals	198	431.99	2.18	

**P 0.025

***P 0.005

significance due to age and to iron. The significance due to age is expected since there is approximately a 680 per cent increase in weight over the six week study period. The significance due to iron treatment is of greater interest because it indicates that 150 mg. of iron dextran injected at 48 - 72 hrs. of age significantly increases weight gain over non-injected control pigs. Since it is known that obesity plays a very important role in measurement of body fluid compartments, this observation must be kept in mind when comparing the values found for the various age groups. At the end of the six week study period the iron injected pigs weighed approximately 33% more than the non-injected pigs,

whereas, at the beginning of the study period their weights were approximately equal.

Comparison of data collected from iron injected and control pigs of the same weight (ignoring age) cannot be used to correct for this weight difference because of the shift in body fluids with increasing age. Restriction of food and water intake by the iron treated pigs to that of the non-injected controls in an attempt to keep weight gains equal would be very difficult to achieve with the baby pig. This would also be objectionable because it would not give the true values for iron injected pigs.

Because of the foregoing, any comparison of body fluid compartments between iron treated and control pigs is not completely valid unless some consideration is given to the difference in weight and percentage of body fat of the two groups.

E. Other Hematologic Observations

The hemoglobin values (Table 10) in this study compare quite favorably with the values found by Coulter (1965) and to those found by Talbot and Swenson (1963) during the first three weeks of their study. They exceed the upper and lower limits set by Schalm (1961). The conditions under which pigs are raised play an important role in the degree of anemia which is attained. The pigs in this study had access to no exogenous source of iron other than that which was injected and a small amount from the sow's ration (Table 1) and dam's milk. The same experimental situation was present for the work done by Coulter and Talbot. This may explain the close correlation.

Table 10. Effects of iron dextran and age on hemoglobin values of pigs

Age	CONTROLS			IRON DEXTRAN		
	Mean (gms./100 ml.)	s[a]	n[b]	Mean (gms./100 ml.)	s	n
Birth	9.6	2.5	13	9.5	1.7	10
1 wk.	8.1	1.4	20	10.1	1.4	17
2 wk.	5.7	1.6	19	11.0	1.1	16
3 wk.	4.5	0.7	16	11.2	0.8	14
4 wk.	4.4	1.2	15	9.9	1.0	14
5 wk.	4.7	1.9	15	8.8	2.0	14
6 wk.	6.7	2.9	13	9.7	1.8	14

[a]Standard deviation of the mean.

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

After the third week of age the pigs in the iron injected group show a decline in the hemoglobin content of the blood. This may have been due to the extensive bleeding to which these pigs were subjected and very possibly could have been avoided by giving a second injection of iron dextran at about three weeks of age. The same can be said for the hemoglobin values of the control pigs which are generally much lower than those reported by Talbot (1963).

The statistical analysis showed an age by iron interaction. This is true because both groups of pigs showed a general decrease in hemoglobin content from the first through the fifth week of age. However, it is also

Table 11. Analysis of variance of hemoglobin values of pigs excluding birth data

Source of Variation	df	S.S.	M.S.	F
litters	4			
weeks	1			
iron	1	284.98	284.98	37.79***
error a	4	30.24	7.56	
age	4	43.43	10.86	1.44
age x iron	4	123.52	30.86	14.84***
individuals	168	349.69	2.08	

***P 0.005

noted that when the effects of iron alone are analyzed there is a significant difference at the 0.5% level between the two groups.

The packed cell volumes are very readily correlated with the hemoglobin values found in this study. It is of interest that although the hemoglobin values correlate quite closely to the values found by Coulter (1965), the PCV values average 3% less during the first three weeks, but they compare very favorably with Talbot (1963). The PCV's seen in this study in the treated pigs show a decline after the third week. Again this is believed to be due to the extensive bleeding to which these pigs were subjected.

The analysis of variance (Table 13) shows that the PCV values are significantly different at the 0.5% level. It also shows that there is

an interaction between age and iron. Examination of the data (Table 12) would indicate that this would be true because a regression line drawn through the values for the treated pigs would be almost horizontal while a regression line drawn through the data from the controls would slope rather sharply.

The packed cell volumes compare very well with those cited by Schalm (1961) for Duroc-Jersey pigs kept on concrete. He cited a mean of 26.7% at 6 days of age.

Table 12. 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	30.81	6.91	13	32.35	6.14	13
1 wk.	25.39	3.73	20	31.59	3.54	17
2 wk.	19.07	3.88	19	34.92	3.64	16
3 wk.	16.47	2.93	16	34.98	2.35	14
4 wk.	16.10	3.14	15	30.73	2.71	14
5 wk.	17.74	7.03	15	28.02	5.05	14
6 wk.	22.23	8.14	13	29.98	4.62	14

[a]Standard deviation of the mean.

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

Table 13. Analysis of variance of packed cell volume of pigs excluding birth data

Source of Variation	df	S.S.	M.S.	F
litters	4			
weeks	1			
iron	1	2,518.73	2,518.73	54.49***
error a	4	184.89	46.22	
age	4	354.82	88.71	1.92
age x iron	4	839.34	209.84	10.59***
individuals	168	3,329.32	19.82	

***P 0.005

The erythrocyte counts (Table 14) are below the values given by Talbot (1963) and above those given by Coulter (1965) during the first three weeks of age. However, after the third week the values found are considerably lower than those reported by Talbot. This again may be due to extensive bleeding which was necessary for this study. It is of interest to note that after the third week the hemoglobin and PCV values for the control pigs fell while the erythrocyte number increased. This indicates a change in the cell size. This is seen in cases of chronic blood loss in which case the bone marrow continues to produce erythrocytes but if insufficient iron is available to keep up with the rate of erythrocyte production the cells which are formed are low in hemoglobin content and a hypochromic, microcytic anemia results (Guyton,

Table 14. Effects of iron dextran and age on the number of 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	4.6	1.6	11	4.5	1.0	10
1 wk.	4.2	1.0	20	4.4	1.0	17
2 wk.	4.0	0.6	19	4.9	0.6	16
3 wk.	3.6	0.5	16	5.3	0.8	14
4 wk.	3.9	0.6	15	5.7	0.5	14
5 wk.	3.8	1.1	15	5.6	1.3	14
6 wk.	4.5	1.2	13	6.3	1.1	14

[a]Standard deviation of the mean.

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

1961). This could explain the decrease in hemoglobin and packed cell volume with an increase in the erythrocyte count. A second injection of iron at about 21 days of age would very probably prevent this situation. However, the amount of blood needed at each bleeding for this study (30 ml. at birth and 60 ml. at each subsequent bleeding) was not believed to be sufficient to cause this decrease in PCV and hemoglobin when the experiment was being planned.

Examination of the analysis of variance of the erythrocyte count (Table 15) reveals an age by iron interaction. This is due to the decrease in RBC numbers in the control pigs as compared with an increase

Table 15. Analysis of variance of erythrocyte numbers of pigs excluding birth data

Source of Variation	df	S.S.	M.S.	F
litters	4			
weeks	1			
iron	1	25.42	25.42	25.94**
error a	4	3.90	.98	
age	4	17.06	4.27	4.36*
age x iron	4	12.72	3.18	4.97***
individuals	168	107.24	.64	

*P 0.010

**P 0.025

***P 0.005

in the red cell count of the iron-treated pigs (Table 14). This same general observation was made by Talbot (1963). The control pigs used in his work did not decrease with age but increased much more slowly than the treated pigs.

The leukocyte count (Table 16) shows that from the second to the fifth week data from the control pigs tend to be slightly lower than the treated groups. During this same period the iron-injected pigs also show a slight decrease in leukocytes. Examination of the analysis of variance (Table 17) reveals that this is a large enough decrease to show significance due to age. The reason for this is very probably

Table 16. Effects of iron dextran and age on the number of 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	10.0	4.2	11	10.8	1.4	10
1 wk.	10.0	3.3	20	10.4	3.8	17
2 wk.	7.6	1.9	19	9.0	3.3	16
3 wk.	8.1	2.6	16	9.2	2.2	14
4 wk.	8.5	2.4	15	10.2	4.1	14
5 wk.	10.4	5.3	15	12.1	2.3	14
6 wk.	11.9	3.2	13	11.6	4.0	14

[a]Standard deviation of the mean.

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

coincidence. No other reports of this occurring could be found in the literature and no explanation for its occurrence here could be found. The values found are in the same range as those reported by Coulter (1965) and are in agreement with Seamer (1956) who reports a slight leukopenia should accompany iron deficiency. They are also in agreement with Ullrey et al. (1959) who reports no significant difference between iron injected and control pigs up to 5 weeks of age.

The values reported for this study were corrected for nucleated red cells as were those reported by Coulter (1965). He found a significant

Table 17. Analysis of variance of leukocyte numbers of pigs excluding birth data

Source of Variation	df	S.S.	M.S.	F
litters	4			
weeks	1			
iron	1	8.96	8.96	1.04
error a	4	35.52	8.88	
age	4	294.87	73.72	8.30*
age x iron	4	5.26	1.32	0.15
individuals	168	1,450.18	8.63	

*P 0.010

difference between iron injected and control pigs at 2 and 3 weeks of age. This probably was due to the type of statistical analysis which he used.

F. Elimination Rates

The rate of elimination of any substance used as an indicator agent in the measurement of any body fluid compartment is very important. Many reports in the literature completely ignore the amount of the indicator agent which is lost during the time from injection of the agent to the time when the sample to be analyzed is withdrawn. This loss can significantly alter the value obtained. The elimination rates of the

various agents vary from species to species and from individual to individual. Soberman et al. (1949) reported that antipyrine is eliminated from the dog at a rate of approximately 30% per hour while the elimination rate for man is 6% per hour. Obviously if the time lapse and elimination rate are not taken into account erroneous values are obtained.

No reports of elimination rates varying with age were found in the literature. In fact, no studies in this area were encountered. However, examination of the data (Tables 18 and 20) shows that there is no difference in the elimination rate of either sodium thiocyanate or N-acetyl-4-aminoantipyrine from birth through six weeks in the pig regardless of treatment. The analysis of variance (Tables 19 and 21) proves these observations statistically. Since there is no significant difference from week to week in pigs, the average elimination rate for the entire study period can be used to establish an elimination rate for use in any further studies which may be conducted. Thus, the average elimination rate for sodium thiocyanate in pigs from birth to six weeks of age based on 216 observations is $4.24 \pm 2.23\%$ per hour. The average elimination rate for N-acetyl-4-aminoantipyrine based on the same 216 individual pigs is $4.65 \pm 2.91\%$ per hour. The 2.23% and the 2.91% represent one standard deviation.

It should be stated here that these average elimination rate figures were not used to obtain the values reported in this study. To find the elimination rate using the method described by Atkinson (1966) it was necessary to extrapolate the data collected on each pig for each week to

Table 18. Effects of iron dextran and age on elimination rates of NaSCN from pigs

Age	CONTROLS			IRON DEXTRAN		
	Mean %/hr.[c]	s[a]	n[b]	Mean %/hr.	s	n
Birth	4.28	2.37	13	3.06	3.05	13
1 wk.	3.55	2.20	20	4.08	1.75	20
2 wk.	3.41	1.61	19	4.56	2.55	16
3 wk.	4.36	2.53	16	4.88	1.54	14
4 wk.	2.85	1.59	15	4.40	2.44	14
5 wk.	5.10	2.68	15	4.76	2.39	14
6 wk.	4.47	1.61	13	5.84	1.62	14
	Across Ages within Treatments					
	3.98	2.16	111	4.50	2.05	105
	Across Ages and Treatments					
	4.24	2.23	216			

[a] Standard deviation of the mean.

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

[c] Per cent of the remaining eliminated per hour.

zero time. Since the concentration at zero time was then known for each observation, the most accurate value for a given pig could be obtained by using this zero time concentration. Therefore, the values given in this study are based on each individual's elimination rate for each week.

Table 19. Analysis of variance of elimination rates of NaSCN from pigs excluding birth data

Source of Variation	df	S.S.	M.S.	F
litters	4			
weeks	1			
iron	1	3.76	3.76	0.87
error a	4	22.37	5.59	
age	4	47.32	11.83	2.12
age x iron	4	8.09	2.02	0.47
individuals	171	738.30	4.32	

Extrapolation to zero time is a very time consuming and costly process if done on an individual animal basis but it is the most accurate, especially if there is a wide variation in the elimination rate from individual to individual. Using the individual elimination rate tends to give a much smaller standard deviation and enables the researcher to estimate more closely the actual mean value of the population.

Table 20. Effects of iron dextran and age on elimination rates of NAAP from pigs

Age	CONTROLS			IRON DEXTRAN		
	Mean %/hr.[c]	s[a]	n[b]	Mean %/hr.	s	n
Birth	2.92	2.09	13	3.60	2.22	13
1 wk.	5.41	2.32	20	5.02	2.79	20
2 wk.	4.30	2.28	19	4.91	3.36	16
3 wk.	3.99	2.69	16	5.19	3.99	14
4 wk.	3.82	3.30	15	3.26	2.84	14
5 wk.	5.88	2.86	15	5.43	2.52	14
6 wk.	5.46	3.16	13	5.51	3.20	14
	Across Ages within Treatments					
	4.58	2.77	111	4.73	3.07	105
	Across Ages and Treatments					
	4.65	2.91	216			

[a] Standard deviation of the mean.

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

[c] Per cent of the remaining eliminated per hour.

Table 21. Analysis of variance of elimination rates of NAAP from pigs excluding birth data

Source of Variation	df	S.S.	M.S.	F
litters	4			
weeks	1			
iron	1	0.10	0.10	0.01
error a	4	88.32	22.80	
age	4	81.23	20.31	0.89
age x iron	4	12.72	3.18	0.38
individuals	171	1,422.84	8.32	

G. Intracellular Water and Interstitial Fluid

By subtracting the means for extracellular water from the means for total body water the mean intracellular water was estimated. The values obtained in this manner are given in Table 22.

An obvious impossibility is noted upon examination of this table. Obviously it is impossible for a mammal to have more ECF volume than TBW at birth or at any time. A thorough search of the literature sheds no light upon the distribution of sodium thiocyanate in the newborn pig. Work done on the "newborn" of other species has been on individuals several days or weeks old as discussed earlier under extracellular fluid volume.

Table 22. Effects of iron dextran and age on estimated intracellular water determined by subtracting mean extracellular fluid space from mean total body water measurement

Age	CONTROLS Calculated Mean ml./kg.	IRON DEXTRAN Calculated Mean ml./kg.
Birth	- 18[a]	4[a]
1 wk.	187	171
2 wk.	259	228
3 wk.	252	240
4 wk.	216	293
5 wk.	232	277
6 wk.	293	256

[a] Estimating error believed to be due to distribution of sodium thiocyanate at birth.

It is also noted that the intracellular fluid tends to increase during the first four weeks of life in the treated pig and then begins to decrease while the control pigs increase for only the first two weeks and then begins to decrease. It is the belief of the author based on clinical observation, that this is due to the difference in the physical strength of the treated and the non treated pigs. The treated pigs tend to prevent the control pigs from nursing and the fluid distribution difference is very possibly due to dehydration in the control pigs. The decrease in intracellular water after the fourth week in treated pigs is very possibly due to the fact that the quantity

of sows milk is not sufficient to maintain the pigs and they are not yet consuming great enough amounts of food and water to compensate for this. This may also explain the increase in intracellular water in the control pigs at six weeks of age. They are forced to start consuming feed and water under the experimental conditions when they are about 3 weeks of age and are completely weaned by the time they are 6 weeks old. Thus, they appear to have corrected any dehydration by the sixth week of age.

Valid comparisons of the intracellular fluid volume in the pigs used in this study cannot be made because no work in this area could be found in reviewing the literature. Reports of intracellular water measurement are not plentiful in the literature. All of them have been done on animals which are much older than those used in this study except for work done on the chick by Medway and Kare (1959).

Berger et al. (1950) give values for total body water and extracellular water in man and dogs. Their work is based on measurements made with antipyrine and sodium bromide or inulin in adult individuals. Their findings indicate that there is a steady decrease in intracellular water expressed as percentage of total body water as age increases. They found 51.9% of the body water was intracellular in males between ages 20 and 39 while men over 80 had only 35.9% intracellular. The same trend was also seen in women; however, the amount of water which was intracellular expressed as a percentage of total water was considerably less. This can be explained on the basis of the female sex hormones which cause the female to deposit more fat. The overall study shows an

average intracellular fluid of 46.6 ± 7.1% of the total body water for men and 41.7 ± 7.5 for women. The work of Berger et al. (1950) does not compare very favorably with that done by Deane (1951) who used antipyrine for total body water measurement and sucrose for ECF measurement. Deane found that the average intracellular water content expressed on a percentage of total water basis was 70.2%. This is much higher than the figure observed by Berger et al. This may be partially explained by the fact that they used different agents to measure the ECF volume. Sucrose is known to give a smaller ECF than most ECF measuring agents. This would result in a higher intracellular fluid content after calculation. Deane (1951) also states that the intracellular water comprises 40.6% of the body weight.

The values found for intracellular fluid in this study (average 24.0% for controls, 24.4% for treated) are much lower than those found by Deane (1951). When compared with the findings of Berger et al. (1950) the correlation is much better. Berger obtained values of 24.6% of the body weight for men and 18.8% for women. Gaudino and Levitt (1949) give the average intracellular water content of normal adult dogs as 43.0% using deuterium and inulin as indicating agents. The choice of measuring agents may explain a part of the high value found by Gaudino and Levitt but there also must be some other contributing factor.

The work of Medway and Kare (1959) in chicks shows the same general trend as that found in this study. They found the intracellular water to equal 11.4 ± 11.7% of the body weight at 1 week, 21 ± 4.6 at 2 weeks, 24.6 ± 1.0 at 3 weeks, 24 ± 1.8 at 4 weeks, and 26.6 ± 3.6 at 8 weeks of age. These values compare very closely with those found for the

pigs in this study. Unfortunately, Medway and Kare did not work with newly hatched chicks. They also did not state how they determined total body water.

Maclaurin (1966) showed the intracellular fluid volume to equal $35.6 \pm 1\%$ of the body weight using antipyrine and thiocyanate in infants 4 hours to 3 days old. These values are much higher at this early age than those reported for the chick or those found for the pig in this study.

By subtracting the plasma volume from the extracellular fluid volume an estimate of the interstitial fluid volume can be made.

The data given in Table 23 were obtained by subtracting the mean plasma volume from the mean extracellular fluid volume for each age and treatment group. Gross examination of these data does not reveal any striking difference between the two groups but it shows a general tendency for the ml. of interstitial fluid per kg. of body weight to decrease as age increases. It is also interesting to note that the control pigs tend to have a lower interstitial fluid volume than treated pigs during the first four weeks of life but the reverse is true during the fifth and sixth weeks of life. There are two factors which may explain this. The first is that the treated pigs are growing much more rapidly and are laying down adipose tissue as well as having more bony tissue which is low in its fluid content. Therefore, when interstitial fluid is expressed on a ml. per kg. of body weight basis it will be lower in the treated pigs. The second possible explanation is that the control pigs clinically appear to be dehydrated during the third and fourth week of life which could explain their lowered interstitial fluid

Table 23. Effects of iron dextran and age on estimated interstitial fluid volume determined by substracting mean plasma volume[a] from mean extracellular fluid space

Age	CONTROLS Calculated Mean ml./kg.	IRON DEXTRAN Calculated Mean ml./kg.
Birth	488[b]	455[b]
1 wk.	337	358
2 wk.	281	286
3 wk.	267	278
4 wk.	259	274
5 wk.	289	247
6 wk.	279	253

[a]Value to nearest ml.

[b]May be overestimated due to distribution of NaSCN at birth.

volume at this time. These same pigs are seen to have a decreasing intracellular water during this same period of time (Table 22). At 5 and 6 weeks of age the control pigs have a greater interstitial fluid volume but it must be remembered that at this age they are much smaller and have much less adipose tissue than their treated littermates.

The values given for interstitial fluid volume in the chick (Medway and Kare, 1959) are similar to those seen in the pigs used in this study. They observed an interstitial fluid volume of 52.3 $\pm$ 11.6% of the body weight at 1 week of age and a value of 36.1 $\pm$ 3.5% at 8 weeks of age. They used sodium thiocyanate to measure ECF volume, but they do not state what was used to measure plasma volume.

V. SUMMARY

Comparisons of plasma, extracellular fluid, intracellular fluid, interstitial fluid, and total body water volumes as well as the classical hematologic observations of erythrocyte number, hemoglobin, packed cell volume, and leukocyte count were made between iron deficient pigs and pigs which had received one injection (150 mg. elemental iron) or iron dextran between 48 and 72 hours of age. In addition, comparison of the body weights of the two groups was made and fluid compartment data were expressed on a body weight basis (ml. per kg.) to compensate for differences in rate of gain for the two groups.

The plasma volume for the iron injected pigs was shown to decrease from 77.3 $\pm$ 7.0 ml. per kg. at birth to 56.2 $\pm$ 12.3 at six weeks of age. The anemic control pigs gave mean values that fluctuated from 81.5 to 67.2 during the same period.

The extracellular fluid volume was determined using sodium thiocyanate. No significant difference was seen due to iron injection; however, a significant difference was seen due to age. As age increased ECF volume steadily decreased. The mean for the control pigs was 570 ml. per kg. at birth and fluctuated slightly until it reached 346 ml. per kg. at six weeks. The iron treated pigs measured 532 ml. per kg. at birth and steadily decreased to 309 at six weeks of age. Some question as to the validity of using sodium thiocyanate as the indicator agent for estimating ECF volume in pigs from birth through six weeks of age was raised. Sodium thiocyanate gave an ECF volume equal to or slightly greater than the volume of TBW as measured with NAAP at 12-24 hours of age.

Mean values for interstitial fluid volumes and intracellular volumes are presented for both groups of pigs. The interstitial fluid volume shows a general decline from 472.5 to 265.5 (average of two groups) ml. per kg. during the six week period. The intracellular fluid volume shows a general increase during this same period. The average for the two groups being incalculable at birth but ranging from 179 at 1 week to 273.8 ml. per kg. at six weeks of age. These values are heavily qualified because of the questions concerning sodium thiocyanate due to its giving a larger ECF value at birth than is obtained with NAAP for total body water. Conclusions regarding the shift of fluids during the first several days of the pig's life cannot be made because of the apparent overestimation of ECF volume by thiocyanate at birth. From 1 week to 6 weeks of age there appears to be a gradual shift from the ECF space to the intracellular space.

Treatment with iron dextran and effect of age are shown to have little or no effect on total body water expressed on a ml. per kg. of body weight basis. The overall weighted mean is 583.6 ml. per kg. of body weight for the six week period.

Data obtained for erythrocyte count, hemoglobin, and packed cell volume showed significantly higher values for the treated than for the control pigs and a significant difference in the weight gain was seen. The iron injected pigs weighed approximately 33% more than the (non-injected) control pigs at six weeks of age. Iron injection caused no significant difference in leukocyte count.

The rates of elimination of NaSCN and NAAP from the plasma have

been shown to be 4.24 $\pm$ 2.23% and 4.65 $\pm$ 2.91% per hour respectively. It was further shown that the elimination rate was not significantly different for iron injected and control pigs. This was also true for pigs of different ages from birth through six weeks.

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VII. ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Melvin J. Swenson for his guidance and council during this study; to Mrs. Carole Ringham, Mr. Reuben Severson, Mr. Tom Olson, Mr. Douglas Hoefling, and Mrs. Rachel Shireman for technical assistance; to Dr. Donald Hotchkiss, Mr. Ralph Fulsum, and Mr. Joseph Atkinson for statistical consultation; to Mrs. Betty Apland, Miss Judy Nystrom, and Mrs. Iola Whitver for typing the rough draft of this thesis; to Mrs. Verona Johnson for typing the final copy of this thesis; to the National Institutes of Health for financial support from a General Research Support Grant; and to my wife, Judith, for her assistance in the preparation of this manuscript as well as for her patience.