ERYTHROCYTE, PLASMA, AND BLOOD VOLUMES OF "NORMAL" WEIGHT AND OBESE OSBORNE-MENDEL MALE RATS

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ABSTRACT

ERYTHROCYTE, PLASMA AND BLOOD VOLUMES OF "NORMAL" WEIGHT AND OBESE OSBORNE-MENDEL MALE RATS

By

Janet Grommet

The purpose of this study was to determine the effect of dietary obesity on total blood volume. Osborne-Mendel male rats fed a high fat diet (60% fat, w/w) served as the obesity model. Rats of the same strain and sex fed grain (3% fat, w/w) served as "normal" weight or control rats for the study.

Erythrocyte volume was determined using Cr^{51} tagged erythrocytes; plasma volume was determined using I^{125} rat serum albumin. The summation of erythrocyte and plasma volumes yielded blood volume.

Twenty-four rats were fed grain and another group of 24 littermate rats were fed a high fat diet throughout their post-weaning life. The volume of erythrocytes, plasma, and blood was determined in 6 grain-fed and 6 high fat-fed rats at each of the following ages: 6, 12, 24 and 48 weeks of age. In addition, the volume of erythrocytes,

plasma, and blood was determined in 18 grain-fed rats (6 rats per group) when these grain-fed rats were the same body weight as the 6, 12 and 24 week old high fat-fed rats. At these ages, the high fat-fed rats had averaged 245, 442, and 616 g, respectively. To establish base line values, the 3 volume measurements were determined in 6 weanling rats. Thus, the volume of erythrocytes, plasma and blood was calculated for weanling, high fat-fed rats, and grain-fed rats of the same age and grain-fed rats of the same body weight as the high fat-fed rats.

As the rats increased in age and body weight, milliliters of erythrocytes, plasma, and blood increased regardless of the diet fed the rats. In rats fed grain the increase from weanlings to 48 week old rats was from 1 to 13 mls, from 4 to 25 mls, and from 4 to 37 mls for erythrocytes, plasma, and blood, respectively; in rats fed the high fat diet, the increase was from 1 to 16 mls, from 4 to 39 mls, and from 5 to 56 mls for erythrocytes, plasma, and blood, respectively. Thus, the increase was greater in the high fat-fed rats than in the grain-fed rats of the same age. By 48 weeks of age, the blood volume (56 versus 37 mls) of the high fat-fed rats was 50% greater ($P < 0.01$) and the body weight (1076 versus 566 g) was 100% greater ($P < .001$) than grain-fed rats of the same age. In contrast the grain and high fat-fed rats of equal body weight had similar erythrocyte, plasma, and blood volumes ($P < 0.05$),

but these grain-fed rats were much older than the high fat-fed rats.

Plasma and blood volumes expressed relative to body weight (milliliters per kilogram body weight) were found to decrease from weaning to 12 weeks of age and plateau. Specifically both the plasma and blood volume of the weanling and 6 week old rats was 20 to 30 mls greater than the plasma and blood volume of the 12 week and older rats ($P < 0.05$ and better). In the grain-fed rats erythrocyte volume remained fairly constant (22 to 25 ml/kg body weight) whereas erythrocyte volume of the high fat-fed rats decreased from 6 to 48 weeks of age (26 ml/kg to 15 ml/kg body weight). Relative volumes of erythrocytes, plasma, and blood of the high fat-fed rats were actually lower than the grain-fed rats of the same age. By 48 weeks of age relative blood volume of the high fat-fed rats was significantly lower ($P < 0.01$) than grain-fed rats of the same age. Grain and high fat-fed rats of the same body weight contained nearly equal volumes of erythrocytes, plasma and blood volume.

Following the blood volume determinations, the spleen, liver, heart, and three fat depots (inguinal, testicular, perirenal-retroperitoneal) were dissected from the carcass and weighed. Weights of the fat depots were required to calculate total body fat from the fat prediction equation.

Simple correlations were calculated between blood compartments (erythrocyte, plasma or blood volume) and physiological parameters (total body fat, lean body mass or body weight). The correlations with the highest coefficients involved body weight (body weight and plasma volume correlation, r=0.83; body weight and blood volume correlation, r=0.84).

Weight of the organs increased with advancing age and increasing body weight: spleen, 0.215 to 1.001 g; liver, 2.645 to 29.328 g; heart, 0.318 to 2.179 g. High fat-fed rats had heavier livers than either the grain-fed rats of the same age or of the same body weight. Grain and high fat-fed rats of the same body weight had the same heart weights.

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In memory of my mother

RUTH ELLEN KOLMER

whose life was prolonged by

the current research of others.

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REVIEW OF LITERATURE

The following review includes (1) progress in the methodology of quantifying blood in an organism, (2) clinical application of blood volume determination, (3) discussion of blood volume and its relationship to other physiological parameters, and (4) enumeration of factors which may alter blood volume.

Accurate measurement of the total blood volume in the living organism is advantageous for several reasons. It can lead to an understanding of the work load of the heart, a better comprehension of total circulating blood substances, and an increased ability to identify factors which affect changes in the quantity of blood.

Direct Method of Measuring Blood Volume

Early attempts at quantifying blood in an organism utilized the direct method of determination. Welcker, as quoted by Rowntree, et al. (1929), in the mid-1800's exsanguinated dogs and concluded that the blood volume constituted 7.7% of the body weight.

A few years later, Bischoff (1855) confirmed this value for man using two guillotined criminals. The

weight of the blood lost was calculated by weighing the criminals just before being guillotined and then weighing the head and body afterwards. The blood vessels were then flushed with water and the tissues were minced and extracted with water until free of hemoglobin. The amount of hemoglobin in the washings and extract was then determined colorimetrically and compared to the amount of hemoglobin in a defibrinated blood sample which had been taken before the execution.

Thus, by correcting for the amount of water which had diluted the hemoglobin and consequently the blood, blood volume could be determined. Much can be said for the accuracy of the direct method, but obviously an element of practicality was lacking.

Indirect or Dilution Method of Measuring Blood Volume

Fundamental Principles

In simplest terms, the indirect method consists of introducing a known quantity of a foreign substance into the blood stream. The substance introduced into the blood must either adhere firmly to the erythrocytes or remain selectively within the plasma at least until it has become uniformly distributed. Fulfillment of the first criteria will permit determination of the volume of erythrocytes and fulfillment of the second will permit determination of plasma. Ideally, the substance should not be lost from the circulatory system, should be preserved with no

metabolic change, should be able to be measured readily and quantitatively, and should exist in the blood as a single molecular species (Underwood and Howland, 1964).

After the foreign substance has become evenly distributed in the circulatory system, a blood sample is taken and dilution of the foreign substance in the blood is determined. By knowing the quantity (N) of the foreign substance introduced into the blood and the dilution (n) of the foreign substance in the blood, the only unknown value is blood volume (V). Blood volume can thus be solved from the following equation (Williams, 1961):

$$n \text{ (concentration of foreign substance in blood)} = \frac{N \text{ (amount of foreign substance injected)}}{V \text{ (volume)}}$$

Early Attempts at Indirect Method

Throughout the latter quarter of the 1800's and early 1900's, a variety of foreign substances were utilized in an attempt to find a substance acceptable for use in the indirect method of blood volume determination. The use of isotonic chloride solution (Cohnstein and Zuntz, 1888), tetanus antitoxin, and gum acacia (McQuarrie and Davis, 1920) were among the methods attempted in the early days of perfecting the indirect method. For one reason or another none of these early methods became a generally accepted procedure. Either the method required

elaborate apparatus, necessitated exquisite chemical skill, could not be repeated often in an organism, or was simply unsuitable for use in humans.

Intravenous Injection of Dyes

In the early 1900's Keith, et al. (1915) introduced the dye dilution method, another indirect method. Evans brilliant vital red dye was probably the first dye to be used. The dilution of the red dye was measured in a simple colorimeter by comparison against standards of known amounts of dye. The most serious criticisms of Evans brilliant red were its rapid loss from the blood stream and its color which made hematocrit determinations difficult.

A series of over 60 dye substances was tested by Dawson, et al. (1920) for acceptability in determining blood volume. This resulted in distinguishing a blue azo dye, Tolidine-1824 (T-1824) or Evans blue, as possessing the most desirable characteristics for blood volume work. Evans blue is non-toxic, firmly binds to plasma albumin; and thus it is prevented from being rapidly eliminated from the circulatory system. Evans blue dye has been widely used for determining plasma volume and is still considered an acceptable method.

Single Radioisotope Dilution Methods

The introduction of radioactive elements into physiological studies made available an additional means of measuring blood volume. In the single radioisotope dilution method, a radioisotope is injected intravenously tagging either erythrocytes or serum albumin. In the case of an erythrocyte tag, erythrocyte volume is measured and plasma volume is determined indirectly based on plasmacrit (100 - hematocrit). With the injection of a plasma tag, plasma volume is measured and erythrocyte volume is determined indirectly based on hematocrit. Radioactive iron (Fe^{55} and Fe^{59}), radioactive phosphorus (P^{32}), radioactive potassium (K^{42}), and radioactive chromium (Cr^{51}) are noted for tagging erythrocytes; radioactive iodine (I^{125} and I^{131}) and the chromic as opposed to the chromate form of radioactive chromium (Cr^{51}) tag serum albumin.

The radioisotopes of Fe and P^{32} were used as early as the 1940's for determination of circulating erythrocyte volume. To use a radioisotope of Fe for blood volume work, it must be administered for one or more weeks to volunteer donors who incorporate it into the hemoglobin of their erythrocytes (Gibson, 1946). A blood sample from the donor is then injected intravenously into the experimental subject. Thus, the use of Fe^{55} and Fe^{59} requires *in vivo* tagging which may limit the use of these radioisotopes in routine measurements of blood volume.

Radioactive phosphorus as dihydrogen-sodium phosphate ($Na_2HP^{32}O_4$) may be added *in vitro* to a small volume of the experimental subject's blood and thus may be considered to have a technical advantage over radioactive iron (Sterling, 1950). The major drawback of P^{32} is its short half-life (14 hours) which necessitates a ready, fresh supply of the radioisotope. Due to the short half-life, the P^{32} diffuses from the erythrocytes with a loss of 50% in one day (Reeve, 1953). Nevertheless, it can be used with accuracy for blood volume determination since little of it is lost from the circulatory system during the equilibration period of 8 to 20 minutes (Berson and Yalow, 1952).

Radioactive potassium is also an *in vitro* erythrocyte tag, but its tagging of the erythrocyte is limited since erythrocyte potassium has an exchange rate of 1.5% per hour with plasma potassium (Schreiber and Rothschild, 1962). Consequently, relatively large quantities of K^{42} must be used in order to achieve a level of radioactivity in the erythrocyte that will allow for accurate measurement following dilution in the circulatory system. Berson, *et al.* (1952) stated that the use of 100 micro Curies of K^{42} would produce only 15 micro Curies in the erythrocytes. As with P^{32}, the half-life is very short (12 hours).

In 1950, Sterling and Gray (1950) reported the use of a new biological tracer, Cr^{51} (chromate ion). This radioisotope appeared to be ideal for tagging erythrocytes and measuring circulating erythrocyte volume since it could be tagged *in vitro* as could P^{32}, but unlike P^{32} the exchange of Cr^{51} between erythrocytes and plasma was negligible for 24 hours. Due to its technical advantages, Cr^{51} can be used with an accuracy of 3 to 5% (Sterling, 1960) and has emerged as the first choice as an erythrocyte tag for blood volume determination (Doornenbal, *et al.*, 1962).

The radioisotopes of iodine opened a new era in the measurement of plasma. In general, plasma volumes obtained from the use of Evans blue and radioisotopes of iodine are not statistically different (Crispell, *et al.*, 1950; Huang, 1956). Radioactive iodine is tagged *in vitro* to albumin, a plasma protein, and is readily available commercially as radioactive iodine human serum albumin (RISA). Today it is frequently used in hospitals for blood volume work since it can be purchased pre-measured in disposable syringes.

Each of the radioisotopes of iodine is a gamma-ray emitter. I^{131} has a half-life of 8 days and emits hard gamma rays plus beta rays. I^{125} has a half-life of approximately 60 days and emits very soft gamma rays

(Free, 1970). Until counters were equipped to count these softer gamma rays, I^{125} had limited use. Today, however, the longer half-life and soft gamma rays of I^{125} are considered advantageous in blood volume work.

Double Radioisotope Dilution Methods

Although apparently acceptable foreign substances (e.g. dyes, radioisotopes) had been identified for quantifying the blood volume in the live animal, a problem still existed in obtaining accurate blood volume data. An explanation of this problem and its solution follows.

When employing an erythrocyte label, the total blood volume is calculated from the following formula:

$$\text{Total blood volume (mls)} = \frac{\text{Total units of labeled blood injected}}{\text{Units of label/ml blood}}$$

For example, 1 ml of Cr^{51} tagged erythrocytes with an activity of 10,000,000 counts per minute (cpm) is injected intravenously. A post-injection sample of blood is then taken 10 minutes later and found to contain 2,500 cpm per milliliter of whole blood with a venous hematocrit of 40. The space in which the labeled erythrocytes were apparently distributed, i.e. blood volume, would be as follows:

$$\text{Total blood volume} = \frac{10{,}000{,}000 \text{ cpm}}{2{,}500 \text{ cpm/ml whole blood}} = 4{,}000 \text{ mls}$$

$$\text{Erythrocyte volume} = 4{,}000 \text{ mls X venous hematocrit of } .40$$

$$= 1{,}600 \text{ mls}$$

$$\text{Plasma volume} = \text{Total blood volume} - \text{erythrocyte volume}$$

$$= 4{,}000 \text{ mls} - 1{,}600 \text{ mls} = 2{,}400 \text{ mls}$$

Thus, erythrocyte volume was measured, and the unmeasured plasma and blood volume were determined based on the hematocrit value.

Plasma tags may be used in the same manner. For example, 1 ml of a solution of I^{125} with an activity of 10,000,000 cpm is injected intravenously. The post-injection sample is found to contain 2,000 cpm with a hematocrit of 40.

$$\text{Total blood volume} = \frac{10{,}000{,}000 \text{ cpm}}{2{,}000 \text{ cpm}} = 5{,}000 \text{ mls}$$

$$\text{Plasma volume} = 5{,}000 \text{ mls X } (1 - 0.40) = 3{,}000 \text{ mls}$$

$$\text{Erythrocyte volume} = \text{Total blood volume} - \text{plasma volume}$$

$$= 5{,}000 \text{ mls} - 3{,}000 \text{ mls} = 2{,}000 \text{ mls}$$

In this case plasma volume is measured, and the unmeasured erythrocyte and blood volume are determined based on the hematocrit.

It would appear that blood volume data derived from erythrocyte labels and blood volume data derived from plasma labels do not agree. Actually, the most accurate

estimate of total blood volume is obtained by adding the erythrocyte volume determined by using the erythrocyte label (1,600 mls) and the plasma volume determined by using the plasma label (3,000 mls) resulting in a total blood volume of 4,600 mls.

In other words, blood volume is most accurately determined using both an erythrocyte and plasma tag. This is commonly referred to as the double radioisotope dilution method. The use of only an erythrocyte label underestimates blood volume and the use of only a plasma tag overestimates blood volume (Huang, 1956).

The reason for the difference in blood volume data based solely on the use of an erythrocyte label or plasma label concerns the distribution of erythrocytes and plasma throughout the body. In determining blood volume based on the use of only one label, the volume for the unmeasured compartment of blood is derived from the venous hematocrit value.

Although venous hematocrit may be acceptable for routine hospital work, it may introduce significant errors in blood volume data since the proportion of erythrocytes to plasma varies with vessel diameter (Schreiber, et al., 1954; Rothschild, 1954). As the diameter of the vessels decrease, the proportion of erythrocytes to plasma also decreases. Thus, blood in the capillaries has a much

lower hematocrit than blood in the large vessels where venous hematocrit is taken (Fahraeus, 1929).

This "overall" hematocrit is known as body hematocrit and can be determined when erythrocyte volume and plasma volume are determined separately. In the previous example where the erythrocyte label yielded a volume of 1,600 mls erythrocytes and the plasma label yielded a volume of 3,000 mls, the body hematocrit was 35, i.e. $\frac{\text{1,600 mls erythrocyte volume}}{\text{4,600 mls total blood volume}}$. In humans the normal ratio of body hematocrit to venous hematocrit is approximately 0.92 (Berson and Yalow, 1954). A value of 0.74 has been reported for rats (Wang, 1959).

Adaptation of Radioisotope Dilution Techniques to Instrumentation

In the 1960's a semi-automatic device for measuring erythrocyte, plasma, and blood volumes by means of the double radioisotope dilution principle was developed. Considering the principle of the dilution method, $n = \frac{N}{V}$ (refer to page 3), the confidence limits of V depend on how accurately N (amount of foreign substance injected) is known and how accurately n (concentration of foreign substance in blood) can be determined. The prime consideration in designing the semi-automatic device was to increase the accuracy of both N and n (Williams, 1961).

The determination of blood volume by using the apparatus involves three steps. In the first step the instrument measures the amount of radioactivity in the syringe loaded with the injection dose of radioisotope. After injection of the dose, the syringe is reinserted into the apparatus and the remaining radioactivity is recorded. The final step consists of simultaneously measuring fixed volumes of pre-injection and post-injection blood samples. The ratio $\frac{N}{n}$, which equals V or blood volume, is then computed by the appartus.

Clinical Application

Surgery

Blood volume determination is useful for monitoring blood loss during surgery. Prior to the radioisotope dilution method, blood loss during surgery was estimated by collecting and measuring the blood in suction bottles, by extracting hemoglobin from sponges, or by comparing body weight before and after the operation. The values obtained were helpful, but they did not reflect the amount of blood actually present within the intravascular space at a given time (Albert, 1963). Measuring blood volume of the patient by way of the double radioisotope dilution method as opposed to the use of dyes or a single radioisotope more accurately estimates the blood loss. This narrows the chance of under- or over-infusion and

thereby improves the post-operative condition of the patient.

In some surgical procedures, the blood loss may not be of sufficient magnitude to require infusion during surgery, but a post-surgery infusion may be required. Measurement of blood volume prior to the operation can serve as a replacement guide to avoid under- or over-infusion.

Pre-operative blood volume is frequently estimated on the basis of the formula of 75 mls of blood per kilogram of body weight (Williams, 1961). Disease states and variation in body weight can cause significant deviations from this formula such that replacement therapy can be unintentionally withheld or misdirected. This is especially so for pediatric, geriatric, and cardiac patients. Thus, accurate knowledge of the blood volume is extremely valuable in guiding correct replacement of blood.

Pathological Conditions

In addition to the measurement of blood volume in surgery, the assessment of blood volume is also advantageous after extensive burns and during therapy for cardiac decompensation, acute renal failure, and other major disorders. Until the development of the double radioisotope dilution method, the results of total blood volume measurement on one individual would frequently vary to such an

extent that the information was of little value in evaluating fine changes in the condition of the patient.

Blood volume measurements are also valuable in diagnosing and studying blood-related diseases. Anemia has been classically defined as a reduction in erythrocyte number and has been ascertained by venous hematocrit. By determining the total blood volume of a patient by summation of erythrocyte and plasma volumes, initially what might appear to be anemia may actually be elevated plasma volume (Fudenberg, 1961).

Likewise leukemia, commonly thought of as uncontrolled proliferation of leucocyte production, may be accompanied by an increase in plasma volume yet with the absence of anemia (Rothschild, 1954). Thus, blood volume determinations can be performed to better analyze the physiological situation and thus improve medical care.

Predicting Blood Volume from Physiological Parameters

Body Weight

Blood volume data is generally expressed as a percentage of body weight with the specific gravity of blood being ignored, e.g. 1 ml blood is assumed to equal 1 g (Moore, 1959). The advantage of expressing blood volume in this manner is that in a clinical situation the blood volume of an individual can be readily estimated.

A disadvantage of expressing blood volume as a percentage of body weight is that it does not account for various body types (Scheldon, et al., 1940). As long as an individual's weight is ideal for his height, calculating blood volume as a percentage of body weight is fairly accurate. Beyond this range, blood volume calculated from body weight is an estimate.

Blood volume in mammals, birds, and reptiles is normally 6 to 10% of the body weight (Gordon, 1968). Normal blood volume in humans is considered to be 7.7% of the body weight (Scholer, 1965). Thus, for the 70 kg man, total blood volume is estimated to be 5,390 ml, i.e. 7.7% of 70 kg. Normal blood volume in the laboratory rat is considered to be approximately 6% of body weight (Huang and Bondurant, 1956; Wang, 1959).

Edwards and Whyte (1960) noted that discrepancies between measured blood volume and blood volume predicted as a precentage of body weight may occur since some tissues of the body contain more blood than others. That is, blood is not uniformly distributed throughout the body. The total amount of blood in the body according to Edwards depends upon the composition as well as the size of the body. He concluded that for the most accurate results, composition should be taken into account when comparing blood volume of different individuals or

when attempting to predict the normal blood volume of an individual.

Blood volume is not necessarily a linear function of body weight (Belcher, 1957), but linear correlations such as blood volume as a percentage of body weight have been attempted because of the simplicity of the prediction. Various attempts have been made to develop equations for predicting blood volume since it is generally agreed (although commonly disregarded) that over a range in body composition, blood volume as a percentage of body weight is inaccurate.

Body Composition

To ascertain the accuracy of predicting blood volume from various physiological parameters, Edwards and Whyte (1960) compared predicted blood volumes to measured volumes (Evans blue method). Measured values were obtained from 25 hospital patients (males and females) ranging in age from 14 to 76 years and in body weight from 69 to 165% of their standard weight.

Using an average volume of 69.1 mls of blood per kilogram body weight, predicted blood volume differed from measured blood volume by as much as 30%. Predictions based on body surface area had an error of 17%. By predicting blood volume from a regression equation based on body composition (calculated from method of

least squares) error was reduced to 14%. Body composition, however, required the quantification of lean body mass (calculated from Pace-Rathburn equation) and fat mass (Rathburn, 1945). Although prediction was improved by taking body composition into account, Edwards noted that quantifying lean and fat mass was more involved than measuring blood volume and suggested that simpler indices of body composition are necessary for prediction of blood volume.

In 22 healthy subjects ranging in age from 20 to 61 years and in body fat from 5 to 50% of body weight, Muldowney (1957) reported erythrocyte volume to be linearly correlated with lean body mass ($r = 0.99$). Erythrocyte volume was determined with Evans blue and lean body mass from the Pace-Rathburn equation (Rathburn and Pace, 1945). In theory there should be a close relationship between the oxygen carrying vehicle (blood) and those tissues consuming oxygen (lean body mass) (Gopolan, et al., 1955). Therefore, the difference in erythrocyte volume per unit body weight among persons of varying body composition is merely an expression of varying fat content. The results of Muldowney's experiment, however, have not been successfully repeated.

Doornenbal, et al. (1962), in an attempt to provide a direct check on the concept that erythrocyte volume is a valid index of lean body mass, fed one group of

experimental rats ad libitum and another restricted feed starting with approximately 5 grams of feed per day and gradually increasing it to 10 grams per day. This regimen was employed with hopes of obtaining rats with a range in body composition so the correlation of erythrocyte volume and lean body mass might be tested over a range of body composition. In Doorenbal's experiment the correlation of total lean body mass and total milliliters of erythrocyte had a correlation coefficient of r = 0.96. The correlation, however, of total body weight and erythrocyte volume also had a correlation coefficient of r = 0.96.

Doornenbal, et al. (1962) explained that the animals used in the experiment were relatively young and still growing when sacrificed. Fat depots were not well developed and consequently the range in body composition originally sought was not achieved. In fact, empty body weight and lean body mass had a correlation coefficient of r = 0.99 so it is not surprising that both correlated equally well with blood volume. Apparently in order to obtain a range in body composition in rats to check the theory, the rats should either be continued longer on the regimen established by Doornenbal or another regimen attempted.

Nadler (1962) determined blood volume in 155 human subjects (hospital staff, personnel, patients,

and prison inmates) using I^{131}-labeled albumin. He then compared the measured values with predicted values for blood volume based on (1) body weight formula (75 ml/kg body weight), (2) surface area formula (2.68 liters blood per square meter X surface area) and (3) Allen's (1956) height cubed-body mass formula. The subjects in this study ranged in age from 17 to 90 years, in weight from 80 to 390 pounds, and in height from 58 to 76 inches.

In both the body weight and surface area formulas, Nadler recognized major deviations from the mean and ascribed this deviation largely to differences in adiposity. Utilizing the height cubed-body mass formula, the major axis of the ellipsoid of plotted points was more nearly collinear with the 45 degree slope regression line than was the body weight or surface area when correlated with blood volume. This indicated that of the 3 formulas the height cubed-body mass formula had the best correlation with measured blood volume.

Nadler then predicted blood volume not only for the 155 subjects in his study but also for 83 subjects for whom Allen (1956) had measured blood volume. None of the 3 prediction equations fit both populations equally well. By submitting the data to a computer regression analysis, a computer-corrected surface area formula and computer-corrected height cubed-body mass formula were derived. These corrected formulas fit

several data groups from the literature equally well (Allen, 1956; Gibson and Evans, 1937; Wennesland, et al., 1959). Consequently, it was not possible to statistically distinguish between the results as determined by either of the corrected formulas.

Alexander (1963) in studying obesity and circulation measured blood volume (Evans blue and tagged erythrocytes) in 40 very obese subjects (average body weight of 300 pounds). Both total plasma and erythrocyte volumes were increased in these extremely obese individuals. The increase in plasma and erythrocytes was proportional such that the hematocrit remained normal.

The total blood volume of the individuals was correlated with their body weight (kilograms) in excess of their ideal weight. Although Alexander stated that it "correlated well" sufficient statistical information was not presented to calculate the correlation coefficient.

Potential Alteration of Blood Volume

Inactivity

A variety of factors suspected of altering blood volume have been investigated. Taylor, et al. (1945) studied the effect of inactivity on the blood volume of 5 young, adult men who were in good physical condition.

The experiment involved a 3 week period of bed rest followed by a 6 week period of normal physical activity.

With bed rest plasma volume (Evans blue method) was found to decrease an average of 518 mls or 15.5%. The average volume of circulating erythrocytes remained unchanged. Thus, blood volume loss associated with bed rest was calculated to be an average of 572 mls per individual or 9.3% of the initial blood volume. During the 6 week period of normal physical activity or reconditioning, blood volume returned to normal. Taylor suggested that bed rest or inactivity produced a decline in physical fitness which was accompanied by a deterioration of cardiovascular function as manifested in a change in blood volume.

Exercise

On the other hand, athletically trained subjects as well as those engaged in heavy physical labor have a considerably larger blood volume than untrained subjects. In Kjellberg's study (1949), the blood volume of 45 athletically trained adult men and women was compared to the blood volume of 266 untrained men and women. Blood volume in the trained men and women was increased 2.2 and 1.6 liters, respectively, or approximately 40%, compared to the controls.

In studying blood changes and physical training in 5 dogs, Davis (1935) noted that in the first week of exercise (swimming, running) a reduction of blood volume occurred. After the first week of exercise, blood volume not only returned to normal but exceeded the pre-exercise normal. The increased blood volume of the exercised dogs persisted for several weeks after the 4 to 9 weeks of exercise. Davis concluded that the oxygen debt of exercise was the stimulus to increased blood formation.

Temperature

Bazett, et al. (1940) studied the effect of temperature on blood volume under both natural and artificial conditions. In the former case, blood volume was determined (Congo red dye method) in 3 adult male subjects in the winter and in the summer following a period of cold and hot weather, respectively. A temperature controlled room provided the artificial conditions for the experiment. Changes in blood volume were measured by one of several methods (Congo red, Evans blue, carbon monoxide) in 6 adult male subjects.

Equivalent results were obtained under the natural and artificial conditions. That is, during 5 days in a warm room (32°C--dry bulb) or in the summer, plasma volume increased. Erythrocyte volume tended to increase, but the increase in erythrocyte volume

proceeded more slowly (thus causing a decreased hematocrit) and usually did not increase at all during the first two days in the warm room.

During 5 days in a cold room (23°C--dry bulb) or in winter, plasma volume decreased. The change in plasma volume was again more rapid than the accompanying change in erythrocyte volume thus resulting in a temporary rise in hematocrit values during acclimatization. The phenomenon of increased blood volume due to heat exposure and decreased blood volume due to cold exposure has been amply confirmed by other investigators (Barcroft, et al., 1922; Bazett, et al., 1940; Conley and Nickerson, 1945; Spealman, et al., 1947; Sunderland, et al., 1938).

The increased blood volume due to heat is thought to be due to vasodilation followed by a shift in interstitial fluid to the vascular bed (Bass and Henschel, 1956). The source of the additional erythrocytes has been vaguely attributed to "blood reservoirs," e.g. lungs, liver (Glaser, et al., 1950). Eliot, et al. (1949) attributed the loss of blood volume due to cold primarily to diuresis.

Anesthesia

Wang (1959) measured both plasma and blood volume in 2 groups of Sherman rats using the T-1824 dye method. Groups were treated identically except one group of 50

rats remained unanesthetized during the blood volume procedure whereas the other group of 10 rats was anesthetized with ether.

Expressed per 100 g body weight, the unanesthetized rats averaged 3.79 ± 0.33 mls plasma and 5.93 ± 0.51 mls blood. The etherized rats had an average of 3.62 ± 0.25 mls plasma and 5.75 ± 0.45 mls blood. Hematocrits were 47.8 and 49.5, respectively.

Using the "pooled" t-test (Sokal and Rohlf, 1969), statistical analysis of the above data revealed that there was no statistical difference in plasma or blood volume of the control and anesthetized rats. Wang thus concluded that etherization had little effect, if any, on plasma and blood volume.

Spleen

Wang's study (1959) also examined the effect the spleen might have on blood volume. The study was conducted on 11 unanesthetized control Sherman rats and 10 unanesthetized, splenectomized Sherman rats. To allow the rats a recovery period, the splenectomies were performed 2 or more weeks prior to determining plasma and blood volume (T-1824 and P^{32} methods).

Expressed per 100 g body weight, the control group had 4.04 ± 0.24 mls plasma and 6.39 ± 0.36 mls blood;

the splenectomized rats had 3.83 ± 0.39 mls plasma and 6.19 ± 0.42 mls blood.

Wang stated that the F cells factor for the control and splenectomized rats was not statistically different. Further analysis using a "pooled" t-test (Sokal and Rohlf, 1969) showed that at the 0.10 level there was no difference in plasma and blood volumes of the 2 groups of rats. This would lead one to conclude that the rat spleen had little effect on blood volume.

Gordon (1968) also mentioned that the spleen of the rat is similar to the spleen of man as opposed to that of the dog. In the dog the spleen may act as a significant reservoir of erythrocytes whereas in the rat and man splenic discharge of erythrocytes is apparently small. Thus, it would seem that the possibility of erythrocytes being lodged in the spleen and interfering with blood volume measurements would not occur.

Pregnancy

Pregnancy increases both erythrocyte and plasma volume, but values reported in the literature vary considerably. Erythrocyte volume in healthy pregnant women varied from an increase of 10% compared to non-pregnant women (Gahres et al., 1962) to an increase of 40% (Canton et al., 1951). Values reported for increased plasma volume during pregnancy varied from an increase of

22% (Adams, 1954) to 55% (Canton, 1951). Individual variation is much greater in pregnant than in non-pregnant women (Hytten and Duncan, 1956) and may account for the variation found between studies.

Erythrocyte volume is thought to gradually increase throughout pregnancy. The fluctuations in the increased blood volume associated with pregnancy are generally attributed to fluctuations in plasma volume (Hytten and Duncan, 1956). In some women the increased plasma volume results in anemia.

INTRODUCTION

Cardiovascular accidents are more prevalent in obese than ideal or underweight adults (Society of Actuaries, 1959). The relationship however between cardiovascular accidents and obesity still remains to be elucidated. Blood volume is thus one of many factors that can be investigated to further understand the relationship between cardiovascular complications and obesity (Schreiber, 1962).

Although human subjects are ideal for application of knowledge to human beings, there are restrictions on the types of experiments that can be done with human subjects. Therefore, some procedures are better done with experimental animals. In the following study Osborne-Mendel male rats fed a high fat diet served as the obesity model.

The use of a high fat diet as a means of producing experimental obesity in Osborne-Mendel rats is relatively free of complications (Mickelsen et al., 1955). The diet can be offered to the rats ad libitum thus avoiding the physiological aberrations of other experimental obesity techniques that involve force feeding (Ingle, 1949) or surgery (Brobeck, et al., 1943; Han, et al., 1965). In

contrast, rats of the same strain fed grain do not become obese and, therefore, provide "normal" weight controls (Schemmel, et al., 1969, 1970; Campbell, et al., 1966) for comparison.

Although rats are not considered to be an ideal experimental animal for studying cardiovascular diseases, e.g. atherosclerosis (Bragdon and Mickelsen, 1955), quantifying blood in obese rats and comparing the volumes to that of control rats should have application to human subjects. The primary purpose, therefore, of this study was to evaluate the effect of obesity on total blood volume. This was accomplished by quantifying the blood in Osborne-Mendel "normal" weight and obese rats at specified ages and weights.

METHOD

Experimental Diets

The rats were offered the experimental diets and water ad libitum. Details of the composition of the grain and high fat diets fed to the rats are presented in Appendix A (Schemmel et al., 1970).

The basic differences between the diets are in fat and energy content. The grain diet fed the control rats contained 3% fat, w/w, with 3.4 digestible kilocalories per gram; the high fat diet contained 60% fat, w/w, of a partially hydrogenated vegetable shortening (Crisco) with 6.7 digestible kilocalories per gram. Rats became obese when fed the high fat diet. Both diets were nutritionally complete.

Experimental Conditions

Since inactivity, exercise, temperature extremes, and pregnancy are known to influence blood volume, care was taken to control these factors in the study. Rats were housed in individual wire screen cages (18 x 18 x 25 cm). Their activity was neither forced nor restrained.

The temperature of the animal room was controlled at 23 ± 1°C throughout the study. Thus, the rats were not

exposed to extreme temperatures, but they were, in fact, acclimatized to a constant environmental temperature.

Experimental Design

Age-Diet Study

The experiment was organized as a split-plot design employing male Osborne-Mendel littermate rats as subjects. Rats were sacrificed at 3, 6, 12, 24, and 48 weeks of age (blocks) and fed either grain or high fat diet (treatments) as diagrammed in Figure 1. Each block or age level was composed of 6 pairs of littermate rats with diet "split" between the littermates, i.e., within an age group one littermate from each of the 6 pairs was fed grain and the other littermate of the pairs was fed the high fat diet. Thus, measurements were made in grain and high fat-fed littermates of the same age.

Since the 3 week old rats, or weanlings, were all fed the same diet (dam's milk and grain), 6 weanlings rather than 6 pairs were employed at 3 weeks of age. The total number of rats in the age-diet study was 54.

Weight-Diet Study

Whereas in the age-diet study erythrocyte, plasma, and blood volume measurements were made on grain and high fat-fed littermate rats of the same age, in the weight-diet study the same measurements were made on grain and high fat-fed littermates of the same body weight. Therefore,

for this study the grain-fed rats were older than the high fat-fed rats.

It had been shown by Schemmel et al. (1969) that approximately one year old grain-fed Osborne-Mendel rats seldom weighed more than 650 g, a weight considerably less than that of 48 week old high fat-fed rats. Consequently, the weight-diet study consisted of 4 rather than 5 blocks or weights, i.e., one block to match the weight of 6, 12, and 24 week old high fat-fed rats plus the weanling rats, and the 2 treatment effects of grain and high fat diet. The weight-diet study is also diagrammed in Figure 1.

Grain-fed rats of the same litter as the 24 week old high fat-fed rats never achieved their weight; therefore, grain-fed rats of the same weights but not of the same litters were substituted in this block. The weight-diet study was analyzed as a randomized block rather than split-plot design since rat littermates could not be used throughout the study. The total number of rats in the weight-diet study was 42.

Preparation of Radioactive Injection Solution

The double radioisotope dilution method using Cr^{51} as an erythrocyte tag and I^{125} rat serum albumin as a plasma tag was used in measuring blood volume.

Erythrocyte Tag[1]

In order to avoid immunological reactions and since erythrocytes must be tagged with Cr^{51} in an *in vitro* preparation, a blood sample was secured from each rat and the erythrocytes tagged with Cr^{51}. To avoid altering the blood volume of the animal, the smallest volume of blood that was needed for all analyses on one rat was drawn. This volume was calculated to be 0.3 ml blood and bore no relationship to the size of the rat.

The 0.3 ml blood sample was secured via cardiac puncture with a 1 ml capacity tuberculin syringe and a 26½ guage needle. The small needle size was used in order to prevent tissue damage to the heart. The blood sample was delivered to a glass blood tube containing 3 to 4 drops of heparin. To the whole blood sample, 5 micro Curie Cr^{51} (as determined from a Cr^{51} radioactive decay curve) was added as sodium chromate.

The blood was allowed to stand at room temperature for 30 minutes in contact with the radioisotope with intermittent mechanical shaking. At the end of the 30 minutes, more than 90% of the Cr^{51} had complexed with the beta chain of the hemoglobin molecule of the erythrocytes (Sterling, 1960); thus, the erythrocytes had acquired sufficient radioactivity for counting.

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[1]Robert W. Bull, personal communication, September, 1971.

To eliminate the Cr^{51} which did not complex with hemoglobin, the erythrocytes were washed three times with sterile saline. The washing process consisted of adding saline to the glass tube containing the tagged blood, centrifuging, and pipetting off the supernatant of plasma and saline which contained the uncomplexed Cr^{51}.

Plasma Tag

To use I^{125} as a plasma tag, it must be bound to albumin. Commercial preparations of tagged human serum albumin are available, but they are tagged human serum albumin. To avoid any immunological reaction which might occur with the use of the human albumin, a foreign protein as far as the rat is concerned, I^{125} was tagged to rat serum albumin. The procedure for iodination was adapted from Greenwood, et al. (1963). The I^{125} was purchased as I^{125}, carrier free, in sodium hydroxide (NaOH) solution.

The tagging of the I^{125} (1 milli Curie) to the albumin of the rat serum (5 microliter) was carried out in a sodium phosphate buffer (25 microliter 0.5 M PO_4 buffer, pH 7.5). The reaction was facilitated with the addition of chloramine-T solution (10 mg/10 ml 0.05 M PO_4 buffer, pH 7.5), an oxidant. This 2 minute tagging reaction was stopped by the addition of sodium metabisulfite solution (25 mg $Na_2S_2O_2$/10 ml 0.05 M PO_4 buffer, pH 7.5) which prevented further iodination by converting the iodine to iodide.

To separate the protein bound I^{125} (large molecules) from free I^{125} (small molecules), the reaction mixture was poured into a 1 x 15 cm bio gel P-60 [2] column which separated the fractions according to size. Twelve 1 ml eluates were collected from the column each in a small vial containing 1 ml of 2% PBS (phosphate buffer saline).

Ten microliters from each of the eluates was counted to locate the protein bound I^{125} peak. The elution pattern is reproducible and consisted of a protein peak (radioactive serum albumin) followed by a salt peak (sodium iodide). The protein peak always came within the second through sixth eluates. The eluates comprising the protein bound I^{125} peak were cumulated and used as the I^{125} rat serum albumin stock solution.

According to Free (1970) I^{125} albumin may be stored up to 120 days. Since with time the I^{125} is gradually released as free I^{125}, it may be desirable to "clean up" or repurify the stock solution by again pouring it through the bio gel P-60 column to separate the bound and free forms of the iodine.[3]

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[2]Bio gel was obtained from Bio-Rad Laboratories, 32nd and Green, Richmond, California.

[3]Harold Hafs, personal communication, May, 1972.

Radioisotope Injection Solution[4]

Five micro Curies of the I^{125} rat serum albumin stock solution and 0.4 ml sterile saline were then added to the heparinized glass tube (previously mentioned on page 32) containing the washed, Cr^{51} tagged erythrocytes. The 0.4 ml saline was added in order to assure an adequate quantity of radioactive injection solution for the procedure. The tube containing the Cr^{51} tagged erythrocytes, I^{125} rat serum albumin, and saline is henceforth referred to as the radioisotope injection solution.

From the tube containing the radioisotope injection solution, 0.3 ml was removed into a 1 ml tuberculin syringe. The filled syringe was weighed and injected into the femoral vein of the same rat as was used for the in vitro preparation of the Cr^{51} tagged erythrocytes. The 0.3 ml of radioactive solution injected into the rat was the same volume as the amount of blood withdrawn from the rat for the preparation of the tagged erythrocytes.

After injection, the syringe was again weighed (to determine net weight) so the volume of radioactive injection solution could be determined by weight. Volume calculated from weight provided an accurate means of determining the volume of radioactive solution injected into the rat.

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[4]Robert W. Bull, personal communication, September, 1971.

In addition to loading the syringe for injection, 0.1 ml of the radioactive injection solution was counted for radioactivity and served as the standard. Since a radioactive injection solution was prepared for each rat, a standard also needed to be prepared for each rat.

For accuracy in counting radioactivity, the well-type gamma counter should be exposed to the same volume in each counting tube. A volume of 1 ml was used consistently throughout the experiment. Consequently, the 0.1 ml of injection solution used as a standard was diluted with 0.9 ml distilled water to yield a 1 ml volume for counting. Also, the addition of water lyzed the erythrocytes and thus facilitated a homogeneous distribution of the tagged erythrocytes as opposed to settling of tagged erythrocytes on the bottom of the counting tubes.

Using a microhematocrit high speed centrifuge, the hematocrit and plasmacrit (100 - hematocrit) of the radioactive injection solution for each rat was determined. These values were necessary for determining the specific gravity of the injection solution.

Specific gravity of erythrocytes is 1.06; specific gravity of plasma is 1.02. By multiplying erythrocyte and plasma specific gravity by the percentage of erythrocytes and plasma present in the radioactive injection solution (hematocrit and plasmacrit,respectively) and summing the

two products, specific gravity of the radioactive injection solution could be determined:

$$\text{Specific gravity} = 1.06 \times \frac{\text{hematocrit}}{100} + 1.02 \times \frac{\text{plasmacrit}}{100}$$

Specific gravity in turn was necessary in order to determine the volume of the injection solution by weight:

$$\text{Volume} = \frac{\text{net weight}}{\text{specific gravity}}$$

Intravenous Injection of Radioactive Solution

Since the radioactive injection solution contained both the Cr^{51} and I^{125}, only one intravenous injection was required. If the entire volume of the injection solution did not enter the vein directly (for example, if a portion of the solution was accidentally injected intramuscularly rather than intravenously), results were invalidated since there was no way to accurately correct for the amount of radioactivity which did not enter the circulatory system. Consequently, injection directly into the vein was of utmost importance.

Prior to the actual injection, the body weight of the rat was recorded. Blood volume data could then be expressed in absolute terms as well as relative to the body weight of the rat. The femoral vein was selected as the injection site as it could be rather easily exposed with a minimum amount of dissection. The position of the

needle in the vein could then be seen and thus the risk of missing the vein was decreased.

To prepare the rat for injection, each rat was anesthetized with ether in a closed chamber and remained anesthetized during the injection procedure by breathing ether from a cotton swab placed in a nose cone. The anesthetized rat was clipped of body hair in the area of the junction of the hind leg and body cavity on the right ventral side and a 1 inch incision was made through the skin exposing the inguinal fat depot. This fat depot was then laid back to uncover the femoral vein, artery, and nerve complex. The radioactive injection solution was injected into the circulatory system via the right femoral vein.

To prevent the loss of blood or seepage of radioactive solution, the needle was left "in situ" for a few seconds to allow the circulating blood to flush the radioactive solution from the injection site. Then placing a cotton swab over the injection site, the needle was removed and pressure applied with the cotton swab for a few seconds to prevent bleeding.

Following injection, the radioactivity remaining in the syringe was counted to correct for the radioactivity which did not enter the rat. The 1 ml tuberculin syringe was washed 10 times with distilled water (total wash of 10 mls). Since a 1 ml volume for the counting tubes was

consistently used throughout the experiment, 1 ml of the wash was delivered to a counting tube to be counted. The radioactivity of 1 ml of wash was then multiplied by a factor of 10 to account for the entire residual radioactivity remaining in the injection syringe. Total residual activity was then subtracted from total activity in the injection syringe (as determined from the standard) to determine total radioactivity actually injected. Calculation of residual activity is merely a correction term.

Securing Post-Injection Samples

At 10 minutes and at approximately 15 minutes post-injection, 0.3 ml blood samples were secured via cardiac puncture and delivered to glass blood tubes containing 3 to 4 drops of heparin. These time periods were selected because the radioisotopes are evenly distributed in the circulating blood 10 minutes after injection and remain so for several hours. Thus, the radioactivity in the two post-injection blood samples should be the same. The post-injection blood samples were prepared for the counter in the same manner as the injection sample, i.e., 0.1 ml blood was diluted with 0.9 ml distilled water to bring the volume to 1 ml for counting the radioactivity.

Disposable syringes, needles, blood tubes, and pipettes were used throughout the experiment to alleviate the possibility of radioactive contamination and to facilitate clean-up.

Counting Samples

The 4 counting tubes each containing 1 ml of solution (injection solution, S_I; wash solution, S_W; and two post-injection solutions, S_PA and S_PB) were counted twice. The first counting was to determine the radioactivity level of Cr^{51}; the second counting was to determine the radioactivity level of I^{125}. The well-type scintillation counter[5] used for counting was set as follows for the two isotopes:

Cr^{51}		I^{125}	
Coarse	= 800	Coarse	= 800
Fine	= 36	Fine	= 36
Base	= 5.42	Base	= 1.5
Window	= 2	Window	= 8.5
Attenuator	= 4	Attenuator	= 1
Wide differential		Wide differential	
10,000 counts		10,000 counts	
30 minutes		30 minutes	

With this setting for Cr^{51}, the photo peak of 0.321 milli electron volts (mEv) was counted. The wider window setting for I^{125} permitted the counting of two photo peaks in the range of 0.035 to 0.075 mEv. Photo

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[5] 1185 series of the Nuclear Chicago gamma counting system.

peaks for the two isotopes (Cr^{51} and I^{125}) are discriminant so that counting one isotope did not elicit interference from the second isotope (Diem and Lenter, 1971).

The Appendixes include a summary of the double isotope dilution method (Appendix B) as well as the mathematics of transforming the counts per minute to milliliters of blood (Appendix C).

Determination of Hematocrits

A portion of both of the post-injection blood samples was used for the determination of the venous hematocrit for each rat. Micro-capillary tubes were filled with blood, sealed, and centrifuged with a micro-hematocrit high speed centrifuge[6] (11,500 rpm; 13,000 g) for 5 minutes. Venous hematocrit values were determined in duplicate for each of the two post-injection blood samples. An average of the four readings represented the venous hematocrit for each rat.

In addition, body hematocrits were estimated by dividing the volume of erythrocytes as determined with Cr^{51} by the total volume of blood calculated by summation of the measured erythrocyte and measured plasma volume. F cells factors were then calculated for each rat by dividing body hematocrit by venous hematocrit.

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[6]International micro-capillary centrifuge, Model MB.

Procedure for Sacrificing Rats and Removing Organs and Fat Depots

After the post-injection blood samples were secured for radioactive analyses, rats were sacrificed with an overdose of ether. Immediately thereafter the spleen, liver, heart, and three fat depots (inguinal, testicular, perirenal-retroperitoneal) were dissected from the carcass. The fat depots were consistently dissected from the left side of the carcasses. During dissection, hemostats were used to limit the loss of blood from the organs.

After recording the fresh weights, organs and fat depots were packaged individually in self-sealing plastic bags and immediately frozen by submerging in an acetone-dry ice bath. Wedge-shaped pieces weighing 1 to 2 g were counted for radioactivity so the erythrocyte, plasma, and blood volume of the organs and fat depots might be determined. Since the spleen and the heart generally weighed less than 2 g, these organs were counted in entirety. Blood volume data for the organs and the fat depots will be reported elsewhere.

Determination of Body Fat and Lean Body Mass

In order to interpret the blood volume results in terms of body composition as well as in milliliters of blood and in milliliters relative to body weight, total body fat of the rats was determined using the body fat

prediction equations developed by Grewal, et al. (in press). The general form of the equations, $y = a + bx$, is the same as the equation for regression analysis or the equation for a straight line. In this case where total body fat in grams was predicted, y = body fat (grams), a = constant, b = coefficient, and x = fresh weight (grams) of a specific fat depot.

The equation for predicting body fat in weanlings, 6 to 12 week old high fat-fed rats, and all grain-fed rats of this study was as follows: $y = 2.328 + 3.958\ x_1 - 0.525\ x_2 + 9.681\ x_3$, where x_1 = inguinal fat depot weight, x_2 = testicular fat depot weight, and x_3 = perirenal-retroperitoneal fat depot weight. Body fat for the 24 week old high fat-fed rats was predicted from the equation $y = 33.165 + 8.218\ x$, where x = inguinal fat depot weight.

High fat-fed rats 48 weeks of age were not among the original sample of more than 250 rats on which chemical analysis of body fat was performed to determine the constants and coefficients for the fat prediction equations. However, the 6 high fat-fed rats 48 weeks of age did not have to be eliminated from the body composition interpretations since chemically determined body fat values for individual male Osborne-Mendel high fat-fed rats weighing within 40 grams of the body weight of the rats of this study were available for 4 of the 48 week old high fat-fed rats (Schemmel, et al., 1969). These body fat values were

used rather than extrapolating from the prediction equations which would be highly inaccurate. Two of the 48 week old high fat-fed rats were deleted from the body composition interpretations since body fat values of rats of the same sex, strain, diet, and near-equal body weight were not available from previous body composition studies in the laboratory.

Upon determining body fat for the rats, lean body mass was calculated by subtracting body fat from body weight: Body weight (grams) - Body fat (grams) = Lean body mass (grams). Thus, individual body fat and lean body mass values in grams were determined for the rats of the age-diet and weight-diet studies.

Analysis of Data

Erythrocyte and plasma volume were measured for each rat; the summation of these values then represented total blood volume for each rat. Means and standard deviations were determined for the groups of 6 rats at each of the ages and at each of the body weights employed in the study.

Significance of age, weight, and diet were determined by analysis of variance (Cochran and Cox, 1957). Actual calculation of the analyses was performed by the 3600 Controlled Data Corporation (CDC) computer, Michigan State University, according to the Agricultural Experiment Station STAT series description No. 14. AOV (Ruble, et al., 1966).

For the erythrocyte, plasma, and blood data Tukey's test was used for comparison of means (Steel and Torrie, 1960). Since the mechanics of Tukey's test varies for split plot and randomized block designs, an explanation of the test is presented in Appendix D.

Mean values and standard deviations were also calculated for body weight, hematocrits, organ weights, body fat, and lean body mass. Where it was appropriate, significant differences between means for groups of 6 rats were determined by Student's t-test (Sokal and Rohlf, 1969).

Simple correlations were determined between each of the blood compartments (erythrocyte, plasma, and blood volumes) and physiological parameters (body weight, body fat, and lean body mass). For example, correlation coefficients (r) were calculated for the simple correlation of erythrocyte volume and body weight, plasma volume, and body weight, blood volume and body weight, etc. The actual calculation of the correlations was performed by the 3600 Controlled Data Corporation (CDC) computer, Michigan State University according to the Agricultural Experiment Station, STAT series description No. 5. BASTAT (Ruble, et al., 1969).

RESULTS AND DISCUSSION

Body Weights and Ages

Mean ages and body weights of the weanling, grain-fed, and high fat-fed rats are presented in Table 1. At each age in the age-diet study, the high fat-fed rats were significantly heavier than the control rats ($P < 0.001$).

At each weight level in the weight-diet study, grain-fed rats took progressively longer to reach the weight of the high fat-fed rats. Only two additional weeks of growth were required for the grain-fed rats to reach the body weight of 6 week old high fat-fed rats. An average of 12 additional weeks and 36 additional weeks of growth, however, were necessary for the grain-fed rats to reach the respective body weights of 12 week old and 24 week old high fat-fed rats. Thus, at the higher weight levels grain-fed rats were considerably older than the high fat-fed rats.

Age-Diet Study

Analyses of variance using age and diet as sources of variance were calculated for each of the blood compartments (erythrocytes, plasma, and blood) for both absolute (Tables 3.1-3.3) and relative quantities (Tables 3.4-3.6). For each of the blood compartments, regardless if expressed

on an absolute or a relative basis, the F statistics for age ($P < 0.001$ or better) and diet ($P < 0.002$ or better) were highly significant.

Absolute Volume (mls) of Erythrocytes, Plasma, and Blood

Rats of the same age but fed different diets.--
At each age level in the age-diet study, the high fat-fed rats compared to the grain-fed or "normal" weight rats had a larger volume of erythrocytes and plasma and consequently a larger blood volume (Table 2, Figure 2). At 6, 12, and 24 weeks of age, the blood volume of the high fat-fed rats exceeded that of the grain-fed rats by approximately 4 mls, but according to Tukey's test (Table 4.1a-4.3a) this elevated blood volume was not significantly greater ($P > 0.05$). By 48 weeks of age, however, the blood volume of the high fat-fed rats was 18 mls in excess of the blood volume of the grain-fed rats and was significantly greater: erythrocytes ($P < 0.05$), plasma ($P < 0.01$), blood ($P < 0.01$).

Rats of different ages but fed the same diet.--
The volume of erythrocyte, plasma, and blood increased with advancing age regardless of the diet fed to the rats (Table 2, Figure 2). The weanling rats, the youngest age group in the study, had approximately 5 mls of blood whereas by 48 weeks of age the grain-fed rats had 37 mls and the high fat-fed rats had 56 mls of blood.

Tukey's test (Tables 4.1b-4.3b) was used to identify significant differences in erythrocyte, plasma, and blood volumes between age groups regardless of the diet. Significant differences in blood volume between adjacent age groups fed the same diet existed in only two instances: weanlings and 6 week old high fat-fed rats ($P < 0.05$); 24 and 48 week old high fat-fed rats ($P < 0.01$).

Numerous significant differences ($P < 0.05$ and better) in blood volumes occurred in comparing an age-diet group to a group fed the same diet but two or more age levels removed (e.g., 6 and 24 week old grain-fed rats, 6 and 24 week old high fat-fed rats, 12 and 48 week old grain-fed rats, 12 and 48 week old high fat-fed rats). This identification of significant increases in blood volume indicated that the increase in blood volume was a gradual increase with advancing age.

Relative Volumes (ml/kg) of Erythrocytes, Plasma and Blood

Rats of the same age but fed different diets.--Whereas on an absolute basis, high fat-fed rats had elevated volumes of erythrocyte, plasma, and blood compared to grain-fed rats of the same age, when data were expressed on a basis relative to body weight, high fat-fed rats had lower volumes of erythrocyte, plasma, and blood than did control rats (Table 2, Figure 3). The difference in the relative blood volumes of the grain and high fat-fed rats

progressively widened with advancing age. For example, at 6, 12, 24, and 48 weeks of age, blood volumes for high fat-fed rats were 2, 6, and 16 ml/kg body weight, respectively, less than grain-fed rats of the same age. By 48 weeks of age the relative erythrocyte and blood volumes (but not plasma volume) of the high fat-fed rats was significantly lower ($P < 0.01$) as indicated in Tables 4.3a-4.6a.

Rats of different ages but fed the same diet.-- From 6 through 48 weeks of age, there was little variation in the relative erythrocyte volume of the grain-fed rats (Table 2, Figure 3). Values ranged from 22 to 25 ml/kg body weight and were not significantly different ($P < 0.05$) as indicated in Table 4.4b. The relative erythrocyte volume of the high fat-fed rats, however, gradually decreased from a high of 26 ml/kg body weight at 6 weeks of age to a low of 15 ml/kg body weight at 48 weeks of age ($P < 0.01$).

Weanling rats had fewer erythrocytes (17 ml/kg body weight) than rats at any other age except the 48 week old high fat-fed rats (15 ml/kg body weight). These low values, particularly for the weanlings, were reflected in low venous hematocrits (Table 9) and are further discussed under Hematocrits.

The relative quantity of plasma in the body was more clearly related to age than to diet. As indicated before,

there were no significant differences between relative plasma volume of grain and high fat-fed rats of the same age. There was, however, a significant drop ($P < 0.05$ and better) in plasma volume from the weaning to 12 weeks of age, i.e., weanlings (65 ml/kg body weight), 6 week old grain-fed rats (61 ml/kg body weight), and 6 week old high fat-fed rats (57 ml/kg body weight) to 12 week old grain-fed rats (41 ml/kg body weight) and 12 week old high fat-fed rats (36 ml/kg body weight). These values are presented in Table 2 and graphed in Figure 2. From 12 through 48 weeks of age, plasma remained constant.

This rapid decrease in plasma volume is a reflection of the fact that the younger rats, i.e., 3 and 6 week old rats, still have relatively more body water than the older rats (Chanutin, 1963). In Osborne-Mendel rats 3 weeks of age, Schemmel, et al. (1969) reported that the lean body mass contained 78% water whereas in older rats water in the lean body mass had decreased to 70% - 72%.

The relative quantity of blood in the rats represented a composite of erythrocytes and plasma but mimicked the trends in plasma volume more so than erythrocyte volume. Thus, blood volume decreased from the value observed in weanling rats (82 ml/kg body weight), 6 week old grain-fed rats (85 ml/kg body weight), and 6 week old

high fat-fed (83 ml/kg body weight) to the level observed in 12 week old grain-fed rats (64 ml/kg body weight) and 12 week old high fat-fed rats (58 ml/kg body weight) and continued at this level throughout 48 weeks of age (Table 2, Figure 3). This indicates that relative blood volume is fairly constant (Table 4.6b, $P > 0.05$) in the rat after its body composition reaches chemical maturity.

Weight-Diet Study

Likewise in the weight-diet study, analyses of variance were calculated for each of the blood compartments (erythrocytes, plasma and blood volumes) for both absolute (Tables 6.1-6.3) and relative quantities (Tables 6.4-6.6). In each of the analyses, weight was highly significant ($P < 0.004$ or better). Diet, however, lacked the high level of significance it had displayed in the age-diet study and was not significant at the 0.10 level except where the dependent variable was milliliters of erythrocytes. It is reasonable to expect that diet *per se* would not be highly significant in this study since the rats, regardless of the diet consumed, were the same body weight but not of the same body composition.

Absolute and Relative Volume of Erythrocytes, Plasma and Blood

On an absolute basis, the volume of erythrocytes, plasma, and blood increased with gains in body weight.

For example, rats averaging 61 g body weight had an absolute blood volume of 5 mls whereas rats averaging 616 g had 36 mls of blood (Table 5, Figure 4). The relative volume of erythrocytes remained constant (approximately 22 ml/kg body weight), particularly in rats 12 weeks of age and older. Relative plasma and blood volumes were significantly higher in the younger, lighter weight rats than in the older rats but showed no further decreases after rats reached 12 weeks of age (Table 5, Figure 5).

In dramatic contrast, however, to the age-diet study, the absolute and relative volume of erythrocytes, plasma, and blood volume of the grain-fed and high fat-fed rats were not significantly different ($P > 0.05$, Table 7). For examples, when grain and high fat-fed rats averaged approximately 245 g their absolute blood volume was 23 and 24 mls respectively; when their body weight averaged 442 g their absolute blood volume was 26 and 27 mls respectively; at an average of 616 g body weight, both had an absolute blood volume of 36 mls.

Net Gain in Blood Volume

Between 6 and 12 weeks of age, both the grain and the high fat-fed rats gained a small quantity of blood (3.40 mls and 2.60 mls, respectively) for each 100 g gain in body weight compared to the increase in blood per 100 g body weight gain at other age intervals (Table 8). This small increase in blood is probably associated with the

reduction of water in lean body mass occurring during this period of time (Chanutin, 1920).

Although high fat-fed rats had more blood than grain-fed rats of the same age, the high fat-fed rats were actually gaining less blood for each 100 g increase in body weight compared to the grain-fed rats (Table 8). This can probably be attributed to the greater accretion of body fat in the high fat-fed rats. Adipose tissue is reported to contain less blood per kilogram than lean tissue (Allen, et al., 1956). Nevertheless, the total blood volume of the high fat-fed rats increased more than that of the grain-fed rats over the same period of time because of the greater increase in body weight in the high fat-fed rats.

Body Composition

Body composition (total body fat and lean body mass) of both the grain and high fat-fed rats was determined using the body fat prediction equations developed by Grewal, et al. (in press). Body fat and lean body mass in grams and as a percentage of body weight are presented in Table 11.

Body Fat

In both the grain and high fat-fed rats, body fat expressed in total grams as well as a percentage of body weight increased with age. In rats fed grain, body fat ranged from 19 g (10% body weight) in the 6 week old rats

to 122 g (20% body weight) in the oldest grain-fed rats. In the rats fed the high fat diet, body fat ranged from 66 g (27% body weight) in the 6 week old rats to 523 g (50% body weight) in the oldest high fat-fed rats.

In fact, at each age level in the age-diet study, the high fat-fed rats had a higher percentage of body fat compared to the control rats, e.g., 27% compared to 10% body fat at 6 weeks of age, 35% compared to 12% at 12 weeks of age, 39% compared to 15% at 24 weeks of age, and 50% compared to 18% at 48 weeks of age. Even when grain and high fat-fed rats were the same body weight, the high fat-fed rats had a greater percentage of body fat, e.g., 27% compared to 12% body fat at 245 g body weight, 35% compared to 15% at 442 g body weight, and 39% compared to 20% at 616 g body weight.

Lean Body Mass

Since the percentage of body fat in the high fat-fed rats was always greater than the percentage of fat in the grain-fed rats, it follows that the percentage of lean in the high fat-fed rats was always less than that of the grain-fed rats. In contrast to body fat which increased with advancing age both in grams and as a percentage of body weight, grams of lean increased with advancing age but decreased as a percentage of body weight.

As an example, at 24 weeks of age the grain-fed rats had 389 g of lean which was 85% of the body weight, and the high fat-fed rats had 375 g of lean which was only 61% of the body weight. Several weeks later (60 weeks of age) when the grain-fed rats had reached the same body weight as the high fat-fed rats had earlier, these grain-fed rats had 493 g of lean. This was an increase of more than 100 g of lean tissues between 24 and 60 weeks of age. On a body weight basis, however, lean body mass in these older grain-fed rats had decreased from 85% to 80% body weight.

Overall, lean body mass ranged from 175 g or 90% body weight in the youngest grain-fed rats (6 weeks of age) to 493 g or 80% body weight in the oldest grain fed rats (60 weeks of age). In the high fat-fed rats, lean body mass ranged from 179 g or 73% body weight in the youngest high fat-fed rats (6 weeks of age) to 528 g or 50% body weight in the oldest obese rats (48 weeks of age).

Grain and high fat-fed rats of the same age, however, had comparable amounts of lean body mass when expressed in total grams rather than as a percentage of body weight, e.g., 175 and 179 g at 6 weeks of age, 292 and 286 g at 12 weeks of age, 389 and 375 g at 24 weeks, and 465 and 528 g at 48 weeks of age, respectively. Undoubtedly, the increased body weight of high fat-fed rats compared to grain-fed rats of the same age can be attributed to the accretion of body fat since rats of the

same age had equivalent amounts of lean body mass regardless of the ration they were fed. Because of the greater body weight of the high fat-fed rats, however, they had a smaller percentage of lean body mass.

Blood Volume in Terms of Body Composition

Distribution of blood between fat and lean tissue could be calculated using the values of 2% and 9% as the percentage of blood in body fat and lean tissue, respectively (Allen, 1956), and the weight of lean and fat in the rats as determined from the prediction equations. Grain and high fat-fed rats of the same body weight were of particular interest since they were found to have equivalent amounts of blood, yet they had dissimilar body composition due to dietary regimen and age differences.

As an example, both grain and high fat-fed rats weighing 615 g had 36 mls of blood (Table 5). In the grain-fed rats, this body weight was distributed as 122 g body fat and 493 g lean whereas in the high fat-fed rats it was distributed as 241 g body fat and 375 g lean (Table 11). Based on Allen's values for humans which are probably high especially for lean tissue of rats but are the best values presently available, a blood volume of 46 mls would be anticipated for the grain-fed rats (2% of 122 g plus 9% of 493 g). For the high fat-fed rats, expected blood volume would be 40 mls (2% of 241 g plus 9% of 375 g).

Calculations based on Allen's values do not yield equivalent blood volumes (46 mls versus 40 mls) for grain and high fat-fed rats of the same body weight but different body composition. The literature indicates that a more accurate means of estimating blood volume would involve parameters of body composition rather than body weight, but rats of equal weight but dissimilar body composition analyzed in this study had the same absolute quantity of measured blood, 36 mls. After the fat depots from the rats of this study have been analyzed for blood content, it will be possible to make a more definitive statement in regard to the quantity of blood in adipose and lean tissues of rats. Perhaps blood does not exist in a 2:9 ratio in fat and lean tissues in the rat as in humans.

Correlation of Physiological Parameters and Blood Compartments

Body weight (Table 1), body fat, and lean body mass (Table 11) were each correlated with erythrocyte plasma, and blood volume (Tables 2 and 5). Correlation coefficients (r) are presented in Table 12.

Correlations Based on All Rats

When simple correlations were calculated based on all the rats of the study, the better correlations involved blood (rather than erythrocytes or plasma) with body weight, body fat, and lean body mass. These values were body weight

and blood, r=0.75, body fat and blood, r=0.64; lean body mass and blood, r=0.69.

Correlations using only the grain-fed rats were lower than correlations using all the rats. The better correlations were still those that involved blood, but they were only of the magnitude of r=0.50.

On the other hand, correlations based on the high fat-fed rats were higher than correlations based on all the rats. Correlations involving blood again afforded high correlation coefficients; body weight and blood, r=0.90; lean body mass and blood, r=0.87; body fat and blood, r=0.91. Correlations involving plasma were much the same; body weight and plasma, r=0.89; lean body mass and plasma, r=0.84; body fat and plasma, r=0.91. These correlations involving plasma or blood were among the best correlations of all the correlations presented in Table 12.

Correlations Based on Young and Old Rats

Since the younger rats were found to have a higher percentage of blood for their body weight, correlations were also computed separately for the young rats (8 weeks of age and younger) and the older rats (12 weeks of age and older). As suspected, correlations based on the older rats were generally higher than the correlations based on all rats.

For the correlations based on the older rats, the best correlations were again those that involved blood: body weight and blood, r=0.84; lean body mass and blood, r=0.67; and body fat and blood, r = 0.76. Correlations involving plasma were similar to the correlation coefficients for blood.

When correlations were based only on mature grain-fed rats, correlations involving blood were the best. Correlations for lean body mass and blood plus body fat and blood were the same as correlations based on all the mature rats, but the correlation coefficient for body weight and blood decreased to 0.77 compared to 0.84 based on all the mature rats.

Correlations based on mature high fat-fed rats were better than correlations based on all mature rats. The correlations involving blood and those involving plasma were similar to the correlations based on all high fat-fed rats and thus were also among the best correlations in Table 12. Correlations based on the immature rats yielded generally poor results (generally less than 0.50).

Comments Regarding Correlations

As discussed in the Review of Literature, several investigators have sought to identify some parameter which would be superior to body weight for predicting absolute blood quantities. Indicators of body composition, particularly lean body mass, have been suggested as

correlating better with blood volume than does body weight (Muldowney, 1957; Doornenbal, 1962).

When correlations for this study were based on all the rats regardless of age or diet, the coefficient for body weight and blood was actually slightly higher, r=0.75, than the coefficient for lean body mass and blood, r=0.69. When correlations were limited to only mature grain and high fat-fed rats, the coefficient for blood and body weight was improved, r=0.84. The coefficient for blood and lean body mass remained much the same, r=0.67.

The higher r value associated with the correlation of blood and body weight is understandable in view of the findings that grain and high fat-fed rats of equal body weight had equivalent amounts of blood but unequal amounts of lean body mass.

Hematocrits

Regardless of the age or diet of the rats, venous hematocrits (Hv) were always greater than body hematocrits (Hb). The larger values for venous hematocrit were expected since the blood samples for these hematocrits were taken from the heart, whereas body hematocrits represented the hematocrit throughout the entire circulatory system. The percentage of erythrocytes in small vessels and capillaries is lower than in large vessels; consequently body hematocrit is lower than venous hematocrit (Gregerson and Rawson, 1959).

Venous hematocrit values were in a limited range from 40.75 to 47.08 mls erythrocytes per 100 mls blood except for weanling rats which had a venous hematocrit of 29.00 (Table 9). Reports in the literature (Brunner, 1938; Martin, 1972) have indicated that weanling rats typically have venous hematocrits in the lower 30's. The higher body water content of younger rats is probably partially responsible for the lower hematocrit (Chanutin, 1931). Also, fetal hemoglobin is probably still present in the circulatory system of the young rat. Since fetal hemoglobin binds oxygen more tenaciously than adult hemoglobin, a low hematocrit is not detrimental. Body hematocrit values displayed greater variability ranging from 20.86 to 38.83 mls erythrocytes per 100 mls blood (Table 9).

The body to venous hematocrit ratio (Hb/Hv) is referred to as the F cell ratio (Wang, 1959). In the 12 to 24 week old rats the ratio was within a narrow range from 0.807 to 0.838 regardless of diet (Table 9). In the younger rats (less than 8 weeks of age) and in the older rats (48 weeks of age), these values were in a lower range from 0.651 to 0.740. This would infer that these latter two groups of rats must have a larger network of small vessels than the 12 and 24 week old rats. Why the F cell ratio was in the 0.800's in the 60 week old grain-fed rats is difficult to explain.

Frequently, investigators measure only erythrocytes or plasma in blood volume work and base calculations for the unmeasured fraction on the F cell ratio (Gregerson and Rawson, 1959). Theoretically, this should be more accurate than basing calculations for the unmeasured portion on venous hematocrit. Due to the great variability observed in body hematocrits, the calculation of blood volumes from plasma or erythrocyte volume alone may be highly inaccurate. Therefore, if precision is desired, it is necessary to measure both erythrocytes and plasma.

Organ Weights

The fresh weight of the spleens increased with advancing age and increasing body weight from 0.215 g in the weanling rats to 1 g in the 48 week old rats. When expressed in milligrams per 100 g body weight, spleen weights decreased with advancing age and increasing body weight.

At 12, 24, and 48 weeks of age, the high fat-fed rats had smaller relative spleen weights than either of their respective groups of weight or age control grain-fed rats. For example, at 24 weeks of age, the high fat-fed rats had a relative spleen weight of 125 mg compared to the 167 mg relative spleen weight of the grain-fed rats of the same age and the 142 mg relative spleen weight of the grain-fed rats of the same body weight.

The fresh weight of the livers increased with advancing age and increasing body weight from 2.645 g in the weanling rats to 29.328 g in the 48 week old high fat-fed rats. In rats of the same age, livers of the high fat-fed rats were 40%, 37%, 21%, and 58% heavier than grain-fed rats at 6, 12, 24, and 48 weeks of age, respectively.

Liver weights of the older grain-fed rats that were the same body weight as the high fat-fed rats were mid-way between the liver weights of the grain and high fat-fed rats of the same age. Thus, the obese rats had heavier livers than either group of control rats. In the latter case, livers of the high fat-fed rats were 11%, 13%, and 11% heavier than the 250 g, 440 g, and 615 g grain-fed rats, respectively. Liver weights expressed as grams per 100 g body weight decreased with advancing age and increasing body weight from over 5 g per 100 g body weight to less than 3 g per 100 g body weight in the oldest rats.

The enlargement of the liver in the high fat-fed rats may be associated with a slight increase in liver fat (Dobbs, unpublished data), but it seems unlikely that any histological aberrations are associated with it (Sokoloff, L. and O. Mickelsen, unpublished data).

The fresh weight of the hearts increased with advancing age and increasing body weight from 0.318 g in the weanlings to 2.179 g in the 48 week old high fat-fed

rats. The hearts of the high fat-fed rats were 30%, 26%, 11%, and 41% larger than the 6, 12, 24, and 48 week old grain-fed rats, respectively. The hearts of the high fat-fed rats were, however, the same weight as the hearts of grain-fed rats of the same body weight. The grain and high fat-fed rats of the same body weight had also had equal blood volume. Heart weights expressed in milligrams per 100 g body weight decreased with advancing age and increasing body weight.

Whether or not the hypertrophied heart of the high fat-fed rat compared to the grain-fed rat of the same age is associated with an increase in the vascular bed of the heart cannot be ascertained at this time. The eventual evaluation of the radioactivity in the heart will give an indication of the quantity of blood therein.

TABLE 1.--Mean values and standard deviations for ages and body weights of male Osborne-Mendel rats from both the age-diet and the weight-diet study. There were 6 rats per group.

Rat Group[a]	Age (weeks)	Body weight (grams)
W	3± 0	61± 8
G	6± 0	194± 19
HF	6± 0	245± 20[b]
G'	8± 0	250± 16
G	12± 0	333± 29
HF	12± 0	442± 38[b]
G'	24±10	440± 31
G	24± 0	458± 29
HF	24± 0	616± 54[b]
G'	60±24	615± 48
G	48± 0	566± 30
HF	48± 0	1076±209[b]

[a] W=weanling rats; G=grain-fed rats the same age as high fat-fed rats; HF=high fat-fed rats; G'= grain-fed rats the same body weight as high fat-fed rats.

[b] Body weight of high fat-fed rats was significantly greater than that of grain-fed rats of the same age ($P < 0.001$).

TABLE 2.--Mean values and standard deviations of absolute and relative volumes of erythrocytes, plasma, and blood of male Osborne-Mendel rats from the age-diet study. There were 6 rats per group.

Rat Group[a]	Age (weeks)	Erythrocytes		Plasma		Blood	
		mls	ml/kg BW[b]	mls	ml/kg BW[b]	mls	ml/kg BW[b]
W	3	1.04±0.29	17.08±3.00	3.93± 0.68	65.03± 8.15	4.98± 0.92	82.12± 9.70
G	6	4.66±0.81	23.98±3.11	11.86± 3.22	60.63±12.29	16.52± 3.73	84.62±13.23
HF	6	6.53±1.33	26.36±4.36	14.04± 3.25	56.71±11.55	20.57± 4.37	83.08±14.97
G	12	7.89±1.03	23.95±4.32	13.36± 2.52	40.47± 8.74	21.25± 2.91	64.42±11.28
HF	12	9.65±2.39	21.98±5.51	16.04± 3.32	36.12± 5.10	25.70± 4.72	58.09± 8.30
G	24	11.49±0.80	25.17±2.11	19.11± 2.14	41.71± 3.18	30.60± 2.11	66.88± 2.27
HF	24	12.82±1.78	20.89±2.88	22.90± 5.44	37.07± 6.84	35.72± 6.63	57.97± 8.22
G	48	12.59±2.69	22.42±4.44	24.77± 7.15	44.42±13.88	37.33± 7.97	66.84±15.75
HF	48	16.34±5.31[c]	15.09±3.16[c]	39.27±14.08[d]	35.84± 9.26	55.62±18.67[d]	50.94±11.18[d]

[a] W=weanling rats; G=grain-fed rats the same age as high fat-fed rats; HF=high fat-fed rats

[b] mls=milliliters; ml/kg BW=milliliters per kilogram body weight

[c] Value for high fat-fed rats was significantly greater than that for grain-fed rats of the same age ($P < 0.05$).

[d] Value for high fat-fed rats was significantly greater than that for grain-fed rats of the same age ($P < 0.01$).

TABLE 3.--Analysis of variance tables for each of the 6 dependent variables in the age-diet study (split plot design).

Table 3.1
Mls of Erythrocytes as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Age	4	338.986	0.0005
Error	25	6.765	
Diet	1	45.571	0.001
Age X Diet	4	5.438	0.153
Remaining Error	25	2.954	
Total	59		

Table 3.2
MB of Plasma as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Age	4	1302.384	0.0005
Error	25	47.364	
Diet	1	321.692	0.0005
Age X Diet	4	97.010	0.002
Remaining Error	25	17.203	
Total	59		

Table 3.3
MB of Blood Volume as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Age	4	2915.922	0.0005
Error	25	74.278	
Diet	1	610.693	0.0005
Age X Diet	4	144.993	0.006
Remaining Error	25	30.903	
Total	59		

Table 3.4
Ml/kg BW of Erythrocytes as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Age	4	134.944	0.001
Error	25	21.713	
Diet	1	75.174	0.002
Age X Diet	4	42.352	0.001
Remaining Error	25	5.942	
Total	59		

Table 3.5
Ml/kg BW of Plasma as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Age	4	1901.610	0.0005
Error	25	151.076	
Diet	1	276.749	0.001
Age X Diet	4	27.753	0.266
Remaining Error	25	19.952	
Total	59		

Table 3.6
Ml/kg BW of Blood Volume as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Age	4	1786.997	0.0005
Error	25	214.661	
Diet	1	640.855	0.0005
Age X Diet	4	120.697	0.014
Remaining Error	25	31.261	
Total	59		

TABLE 4a.--Tukey's test for comparison of rats of the same age fed different diets in the age-diet study (split plot) for each of the 6 dependent variables. Difference between means of 6 grain and 6 high fat-fed rats of the same age are enclosed in the tables.

Absolute Volumes (ml/kg)
Table 4.1a

Erythrocytes

	16.34	12.82	12.59	11.49	9.65	7.89	6.53	4.66
4.66	11.68**	8.16**	7.93**	6.83**	4.99**	3.23	[1.87]	-
6.53	9.81**	6.59**	6.36**	4.96**	3.12	1.36	-	
7.89	8.45**	4.93**	4.70**	3.60*	[1.76]	-		
9.65	6.69**	3.17	2.94	1.84	-			
11.49	4.85**	[1.33]	1.10	-				
12.59	[3.75]*	0.23	-					
12.82	3.52*	-						
16.34	-							

*LSR.05 = 3.44
**LSR.01 = 4.14

Relative Volumes (ml/kg body weight)
Table 4.4a

Erythrocytes

	26.36	25.17	23.98	23.95	22.42	21.98	20.89	15.09
15.09	11.27**	10.08**	8.89**	8.86**	[7.33]**	6.89**	5.80*	-
20.89	5.47*	[4.28]	3.09	3.06	1.53	1.09	-	
21.98	4.38	3.19	2.00	[1.97]	0.44	-		
22.42	3.94	2.75	1.56	1.53	-			
23.95	2.41	1.22	0.03	-				
23.98	[2.38]	1.19	-					
25.17	1.19	-						
26.36	-							

*LSR.05 = 4.88
**LSR.01 = 5.86

Table 4.2a

Plasma

	39.27	24.77	22.90	19.11	16.04	14.04	13.36	11.86
11.86	27.41**	12.91**	11.04**	7.25	4.18	[2.18]	1.50	-
13.36	25.91**	11.41**	9.54*	5.75	[2.68]	0.68	-	
14.04	25.23**	10.73**	8.86*	5.07	2.00	-		
16.04	23.23**	8.73*	6.86	3.07	-			
19.11	20.16**	5.66	[3.79]	-				
22.90	16.37**	1.87	-					
24.77	[14.50]**	-						
39.27	-							

*LSR.05 = 8.30
**LSR.01 = 9.98

Table 4.5a

Plasma

	60.63	56.71	44.42	41.71	40.47	37.07	36.12	35.84
35.84	24.79**	20.87**	[8.58]	5.87	4.63	1.23	0.28	-
36.12	24.51**	20.59**	8.30	5.59	[4.35]	0.95	-	
37.07	23.56**	19.64**	7.35	[4.64]	3.40	-		
40.47	20.16**	16.24**	3.95	1.24	-			
41.71	18.92**	15.00**	2.71	-				
44.42	16.21**	12.29**	-					
56.71	[3.92]	-						
60.63	-							

*LSR.05 = 8.94
**LSR.01 = 10.74

Table 4.3a

Blood

	55.62	37.33	35.72	30.60	25.70	21.25	20.57	16.52
16.52	39.10**	20.81**	19.20**	14.08**	9.18	4.73	[4.05]	-
20.57	35.05**	16.76**	15.15**	10.03	5.13	0.68	-	
21.25	34.37**	16.08**	14.47**	9.35	[4.45]	-		
25.70	29.92**	11.63*	10.02	4.90	-			
30.60	25.02**	6.73	[5.12]	-				
35.72	19.90**	1.61	-					
37.33	[18.29]**	-						
55.62	-							

*LSR.05 = 11.12
**LSR.01 = 13.37

Table 4.6a

Blood

	84.62	83.08	66.88	66.84	64.42	58.09	57.97	50.94
50.94	33.68**	32.14**	15.94**	[15.90]**	13.48**	7.15	7.03	-
57.97	26.65**	25.11**	[8.91]	8.87	6.45	0.12	-	
58.09	26.53**	24.99**	8.79	8.75	[6.33]	-		
64.42	20.20**	18.66**	2.46	2.42	-			
66.84	17.78**	16.24**	0.04	-				
66.88	17.74**	16.20**	-					
83.08	[1.24]	-						
84.62	-							

*LSR.05 = 11.18
**LSR.01 = 13.44

TABLE 4b.--Tukey's test for comparison of rats of different ages fed the same or different diets in the age-diet study (split plot) for each of the 6 dependent variables. Difference between means of 2 adjacent age groups of grain-fed rats are enclosed in the tables; difference between 2 adjacent age groups of high fat-fed rats are double enclosed. Differences between 2 age groups on different diets have not been denoted.

Absolute Volumes (mls)

Table 4.1b

Erythrocytes

	16.34	12.82	12.59	11.49	9.65	7.89	6.53	4.66	1.04
1.04	15.30**	11.78**	11.55**	10.45**	8.61**	6.85**	[5.49**]	[3.62]	-
4.66	11.68**	8.16**	7.93**	6.83**	4.99*	[3.23]	1.87	-	
6.53	9.81**	6.29**	6.06**	4.96*	[3.12]	1.36	-		
7.89	8.45**	4.93*	4.70*	[3.60]	1.76	-			
9.65	6.69**	[3.17]	2.94	1.84	-				
11.49	4.85*	1.33	[1.10]	-					
12.59	3.75	0.23	-						
12.82	[3.52]	-							
16.34	-								

*LSR.05 = 4.41

**LSR.01 = 5.30

Relative Volumes (ml/kg body weight)

Table 4.4b

Erythrocytes

	26.36	25.17	23.98	23.95	22.42	21.98	20.89	17.08	15.09
15.09	11.27**	10.08**	8.89*	8.86*	7.33	6.89	[5.80]	1.99	-
17.08	[9.28**]	8.09**	[6.90]	6.87	5.34	4.90	3.81	-	
20.89	5.47	4.28	3.09	3.06	1.53	[1.09]	-		
21.98	[4.38]	3.19	2.00	1.97	0.44	-			
22.42	3.94	[2.75]	1.56	1.53	-				
23.95	2.41	[1.22]	[0.03]	-					
23.98	2.38	1.19	-						
25.17	1.19	-							
26.36	-								

*LSR.05 = 7.44

**LSR.01 = 8.94

Table 4.2b

Plasma

	39.27	24.77	22.90	19.11	16.04	14.04	13.36	11.86	3.93
3.93	35.34**	20.84**	18.97**	15.18**	12.11*	[10.11]	9.43	[7.93]	-
11.86	27.41**	12.91*	11.04	7.25	4.18	2.18	[1.50]	-	
13.36	25.91**	11.41*	9.54	[5.75]	2.68	0.68	-		
14.04	25.23**	10.73	8.86	5.07	[2.00]	-			
16.04	23.23**	8.73	[6.86]	3.07	-				
19.11	20.16**	[5.66]	3.79	-					
22.90	[16.27**]	1.87	-						
24.77	14.50**	-							
39.27	-								

*LSR.05 = 11.37

**LSR.01 = 13.67

Table 4.5b

Plasma

	65.03	60.63	56.71	44.42	41.71	40.47	37.07	36.12	35.84
35.84	29.19**	24.79**	20.87*	8.58	5.87	4.63	[1.23]	0.28	-
36.12	28.91**	24.51**	[20.35**]	8.30	5.59	4.35	[0.95]	-	
37.07	27.96**	23.56**	19.64*	7.35	4.64	3.40	-		
40.47	24.56**	[20.16*]	16.24	3.95	[1.24]	-			
41.71	23.32**	18.92*	15.00	[2.71]	-				
44.42	20.61*	16.21	12.29	-					
56.71	[8.32]	3.92	-						
60.63	[4.40]	-							
65.03	-								

*LSR.05 = 18.50

**LSR.01 = 22.24

Table 4.3b

Blood

	55.62	37.33	35.72	30.60	25.70	21.25	20.57	16.52	4.98
4.98	50.64**	32.35**	30.74**	25.62**	20.72**	16.27*	[15.59**]	[11.54]	-
16.52	39.10**	20.81**	19.20**	14.08*	9.18	[4.73]	4.05	-	
20.57	35.05**	16.76*	15.15*	10.03	[5.13]	0.68	-		
21.25	34.37**	16.08*	14.47	[9.35]	4.45	-			
25.70	29.92**	11.63	[10.02]	4.90	-				
30.60	25.02**	[6.73]	5.12	-					
35.72	[19.90**]	1.61	-						
37.33	18.29**	-							
55.62	-								

*LSR.05 = 14.50

**LSR.01 = 17.44

Table 4.6b

Blood

	84.62	83.08	82.12	66.88	66.84	64.42	58.09	57.97	50.94
50.94	33.68**	32.14**	31.18**	15.94	15.90	13.48	7.15	[7.03**]	-
57.97	26.65*	25.11*	24.15*	8.91	8.87	6.45	[0.12]	-	
58.09	26.53*	[24.99**]	24.03*	8.79	8.75	6.33	-		
64.42	[20.20]	18.66	17.70	[2.46]	2.42	-			
66.84	17.78	16.24	15.28	[0.04]	-				
66.88	17.74	16.20	15.24	-					
82.12	[2.50]	[0.96]	-						
83.08	1.59	-							
84.62	-								

*LSR.05 = 22.18

**LSR.01 = 26.67

TABLE 5.--Mean values and standard deviations of absolute and relative volumes of erythrocytes, plasma, and blood of male Osborne-Mendel rats from the weight-diet study. There were 6 rats per group.

Rat Group[a]	Weight (grams)	Erythrocytes		Plasma		Blood	
		mls	ml/kg BW[b]	mls	ml/kg BW[b]	mls	ml/kg BW[b]
W	61	1.04±0.29	17.08±3.00	3.93±0.68	65.03± 8.15	4.98±0.92	82.12± 9.70
G'	250	6.08±1.39	24.47±6.09	16.75±3.38	66.96±12.42	22.83±4.26	91.44±16.53
HF	245	6.53±1.33	26.36±4.36	14.04±3.25	56.71±11.55	20.57±4.37	83.08±14.97
G'	440	9.99±2.64	22.77±6.41	16.49±5.38	37.02±10.90	26.49±5.24	59.79±10.07
HF	442	9.65±2.39	21.98±5.51	16.04±3.32	36.12± 5.10	25.70±4.72	58.09± 8.30
G'	615	13.85±2.88	22.57±3.95	22.15±5.57	36.08± 8.05	35.95±6.88	58.66± 9.05
HF	616	12.82±1.78	20.89±2.88	22.90±5.44	37.07± 6.84	35.72±6.63	57.97± 8.22

[a] W=weanling rats; G'=grain-fed rats the same body weight as high fat-fed rats; HF=high fat-fed rats

[b] mls=milliliters; ml/kg BW=milliliters per kilogram body weight

TABLE 6.--Analysis of variance tables for each of the 6 dependent variables in the weight-diet study (randomized block design).

Table 6.1
Mls of Erythrocytes as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Weight	3	2284.269	0.0005
Diet	1	23.9.771	0.0005
Weight X Diet	3	2170.085	0.0005
Error	38	28.9319	
Total	45		

Table 6.2
Mlt of Plasma as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Weight	3	1009.041	0.0005
Diet	1	296.361	0.113
Weight X Diet	3	308.938	0.056
Error	38	118.757	
Total	45		

Table 6.3
Mb of Blood Volume as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Weight	3	1990.450	0.0005
Diet	1	19.533	0.707
Weight X Diet	3	10.818	0.971
Error	38	143.811	
Total	45		

Table 6.4
Ml/kg BW of Erythrocytes as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Weight	3	731.393	0.007
Diet	1	309.118	0.170
Weight X Diet	3	218.426	0.263
Error	38	166.654	
Total	45		

Table 6.5
Ml/kg BW of Plasma as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Weight	3	2160.953	0.0005
Diet	1	314.163	0.188
Weight X Diet	3	312.407	0.166
Error	38	184.266	
Total	45		

Table 6.6
Ml/kg BW of Blood Volume as Dependent Variable

Source of Variance	Degrees of Freedom	Mean Square	Approx. Sig. Level
Weight	3	1526.449	0.004
Diet	1	382.562	0.265
Weight X Diet	3	499.511	0.190
Error	38	315.555	
Total	45		

TABLE 7.--Tukey's test for comparison of means in the weight-diet study for each of the 6 dependent variables (randomized block design).

Absolute Volumes (mls)
Table 7.1

Erythrocytes

	13.85	12.82	9.99	9.65	6.53	6.08	1.04
1.04	12.81**	11.78*	8.95	8.61	5.49	5.04	-
6.08	7.77	6.74	3.91	3.57	0.45	-	
6.53	7.32	6.29	3.46	3.12	-		
9.65	4.20	3.17	0.34	-			
9.99	3.86	2.83	-		*LSR.05 = 9.96		
12.82	1.03	-			**LSR.01 = 11.904		
13.85	-						

Relative Volumes (ml/kg body weight)
Table 7.4

Erythrocytes

	26.36	24.47	22.77	22.57	21.98	20.89	17.08
17.08	9.28	7.39	5.69	5.49	4.90	3.81	-
20.89	5.47	3.58	1.88	1.68	1.09	-	
21.98	4.38	2.49	0.79	0.59	-		
22.57	3.79	1.90	0.20	-			
22.77	3.59	1.70	-		*LSR.05 = 23.91		
24.47	1.89	-					
26.36	-						

Table 7.2

Plasma

	22.90	22.15	16.75	16.49	16.04	14.04	3.93
3.93	18.97	18.22	12.82	12.56	12.11	10.11	-
14.04	8.86	8.11	2.71	2.45	2.00	-	
16.04	6.86	6.11	0.71	0.45	-		
16.49	6.41	5.66	0.26	-			
16.75	6.15	5.40	-		*LSR.05 = 20.18		
22.15	0.75	-					
22.90	-						

Table 7.5

Plasma

	66.96	65.03	56.71	37.07	37.02	36.12	36.08
36.08	30.88**	28.95*	20.63	0.99	0.94	0.04	-
36.12	30.84**	28.91*	20.59	0.95	0.90	-	
37.02	29.94*	28.01*	19.69	0.05	-		
37.07	29.89*	27.96*	19.64	-			
56.71	10.25	8.32	-		*LSR.05 = 25.14		
65.03	1.93	-			**LSR.01 = 30.04		
66.96	-						

Table 7.3

Blood

	35.95	35.72	26.49	25.70	22.83	20.57	4.98
4.98	30.97**	30.74**	21.51	20.72	17.85	15.59	-
20.57	15.38	15.15	5.92	5.13	2.26	-	
22.83	13.12	12.89	3.66	2.87	-		
25.70	10.25	10.02	0.79	-			
26.49	9.46	9.23	-		*LSR.05 = 22.21		
35.72	0.23	-			**LSR.01 = 26.54		
35.95	-						

Table 7.6

Blood

	91.44	83.08	82.12	59.79	58.66	58.09	57.97
57.97	33.47*	25.11	24.15	1.82	0.69	0.12	-
58.09	33.35*	24.99	24.03	1.70	0.57	-	
58.66	32.78*	24.42	23.46	1.13	-		
59.79	31.65	23.29	22.33	-			
82.12	9.32	0.96	-		*LSR.05 = 32.90		
83.08	8.36	-			**LSR.01 = 39.31		
91.44	-						

TABLE 8.--Net gain in blood per 100 gram increment in body weight over a specified age span. There were 6 male Osborne-Mendel rats per group.

Rat group[a]	Net blood gain (milliliters/100 g body weight increment)			
Age span (weeks):	3-6	6-12	12-24	24-48
G	8.18	3.40	7.48	6.84
HF	8.12	2.60	5.76	4.33
Age span (weeks):	3-8	8-24	24-60	
G'	9.06	1.93	5.40	

[a] G=grain-fed rats the same age as high fat-fed rats; HF=high fat-fed rats; G'=grain-fed rats the same body weight as high fat-fed rats

TABLE 9.--Mean values and standard deviations for hematocrits of male Osborne-Mendel rats from both the age-diet and the weight-diet study. There were 6 rats per group.

Rat group[a]	Mls erythrocytes/100 mls blood		
	Hv [b]	Hb [c]	F cells [d]
W	29.00± 4.40	20.86± 2.90	0.724±0.065
G-6	42.08± 2.52	28.78± 4.36	0.681±0.075
HF-6	43.25± 3.14	31.88± 2.65	0.738±0.049
G'	41.00± 6.80	26.68± 4.69	0.651±0.044
G-12	46.42± 2.13	37.39± 4.52	0.807±0.107
HF-12	44.83± 2.06	37.52± 5.87	0.838±0.134
G'	46.17± 1.83	38.70±12.37	0.834±0.252
G-24	47.08± 2.13	37.68± 3.43	0.800±0.063
HF-24	44.75± 1.70	36.30± 4.25	0.812±0.082
G'	46.79± 1.45	38.83± 6.46	0.826±0.129
G-48	46.21± 1.78	34.31± 7.37	0.740±0.136
HF-48	40.75± 5.14	30.05± 6.00	0.740±0.142

[a] W=weanling rats; G=grain-fed rats the same age as high fat-fed rats (number indicates age in weeks); HF=high fat-fed rats (number indicates age in weeks); G'=grain-fed rats the same body weight as high fat-fed rats

[b] Hv=venous hematocrit

[c] Hb=body hematocrit

[d] F cells=body hematocrit ÷ venous hematocrit

TABLE 10.--Mean values and standard deviations of absolute and relative fresh weights of spleen, liver, and heart of male Osborne-Mendel rats from both the age-diet and the weight-diet study. There were 6 rats per group.

Rat group[a]	Spleen		Liver		Heart	
	grams	mg/100 gm BW[b]	grams	gm/100 gm BW[b]	grams	mg/100 gm BW[b]
W	0.215±0.066	358±107	2.645±0.388	4.372±0.441	0.318±0.016	531±65
G-6	0.628±0.182	322± 80	9.202±1.631	4.728±0.546	0.709±0.082	366±22
HF-6	0.658±0.128	270± 56	12.929±1.907	5.268±0.573	0.925±0.098	379±42
G'	0.641±0.153	258± 70	11.600±0.560	4.379±0.483	0.952±0.185	380±53
G-12	0.750±0.072	226± 28	12.824±1.708	3.850±0.374	1.061±0.072	320±16
HF-12	0.738±0.078	167± 7	17.548±2.030	3.984±0.419	1.334±0.224	303±48
G'	0.751±0.063	171± 16	15.508±0.775	3.540±0.331	1.361±0.077	310±21
G-24	0.763±0.133	167± 27	15.688±2.494	3.412±0.338	1.399±0.155	306±26
HF-24	0.764±0.101	125± 18	19.042±1.653	3.111±0.353	1.538±0.101	251±21
G'	0.869±0.212	142± 34	17.112±2.299	2.801±0.466	1.542±0.098	252±17
G-48	0.909±0.169	160± 26	18.522±2.939	3.278±0.516	1.540±0.122	272±13
HF-48	1.001±0.341	92± 16	29.328±7.836	2.702±0.371	2.179±0.434	204±25

[a] W=weanling rats; G=grain-fed rats the same age as high fat-fed rats (number indicates age in weeks); HF=high fat-fed rats (number indicates age in weeks); G'=grain-fed rats the same body weight as high fat-fed rats

[b] mg/100 gm BW=milligrams per 100 grams body weight; gm/100 gm BW=grams per 100 grams body weight

TABLE 11.--Mean values and standard deviations for body fat and lean body mass of male Osborne-Mendel rats from both the age-diet and the weight-diet study. Grams of body fat and lean body mass were determined from prediction equations.

Rat group[a]	Number[b]	Body fat		Lean body mass	
		grams	% body weight	grams	% body weight
W	6	6± 1	10	55± 7	90
G-6	6	19± 5	10	175±16	90
HF-6	6	66±10[c]	27	179±15	73
G'	6	30± 5	12	220±12[d]	88
G-12	6	41± 9	12	292±26	88
HF-12	6	156±44[c]	35	286±26	65
G'	6	66±18	15	374±19[d]	85
G-24	6	69±21	15	389±18	85
HF-24	6	241±34[c]	39	375±30	61
G'	6	122±49	20	493±31[d]	80
G-48	6	101±36	18	465±33	82
HF-48	4	523±18[c]	50	528±31	50

[a] W=weanling rats; G=grain-fed rats the same age as high fat-fed rats (number indicates age in weeks); HF=high fat-fed rats (number indicates age in weeks); G'=grain-fed rats the same body weight as high fat-fed rats

[b] Number refers to number of rats per group.

[c] High fat-fed rats had significantly more body fat than grain-fed rats of the same age or body fat ($P < 0.001$)

[d] G' rats had significantly more lean body mass than grain and high fat-fed rats of the same age ($P < 0.001$)

TABLE 12.--Correlation coefficients for simple correlations of blood compartments (erythrocytes, plasma, blood) and physiological parameters (body weight, lean body mass, body fat) for male Osborne-Mendel rats.

Rat group	n [a]	Blood compartments (milliliters)	Body weight (grams)	LBM [b] (grams)	Body fat (grams)
Grain & high fat-fed rats [c]	70	Erythrocytes	0.01	0.04	-0.02
		Plasma	0.50	0.43	0.47
		Blood	0.75	0.69	0.64
Grain-fed rats	42	Erythrocytes	-0.27	-0.29	-0.20
		Plasma	0.08	0.04	0.17
		Blood	0.50	0.47	0.50
High fat-fed rats	22	Erythrocytes	0.80	0.81	0.77
		Plasma	0.89	0.84	0.91
		Blood	0.90	0.87	0.91
Older grain & high fat-fed rats [d]	46	Erythrocytes	0.64	0.64	0.49
		Plasma	0.83	0.61	0.77
		Blood	0.84	0.67	0.76
Older grain-fed rats	30	Erythrocytes	0.73	0.72	0.57
		Plasma	0.65	0.52	0.72
		Blood	0.77	0.67	0.77
Older high fat-fed rats	16	Erythrocytes	0.64	0.63	0.63
		Plasma	0.90	0.83	0.91
		Blood	0.88	0.83	0.89
Younger grain & high fat-fed rats [e]	24	Erythrocytes	0.51	0.61	0.04
		Plasma	0.44	0.49	0.13
		Blood	0.44	0.45	0.27

[a] n refers to number of rats per group

[b] LBM=lean body mass

[c] All rats from age-diet and weight-diet studies

[d] Older rats (12 weeks of age and older)

[e] Younger rats (less than 12 weeks of age)

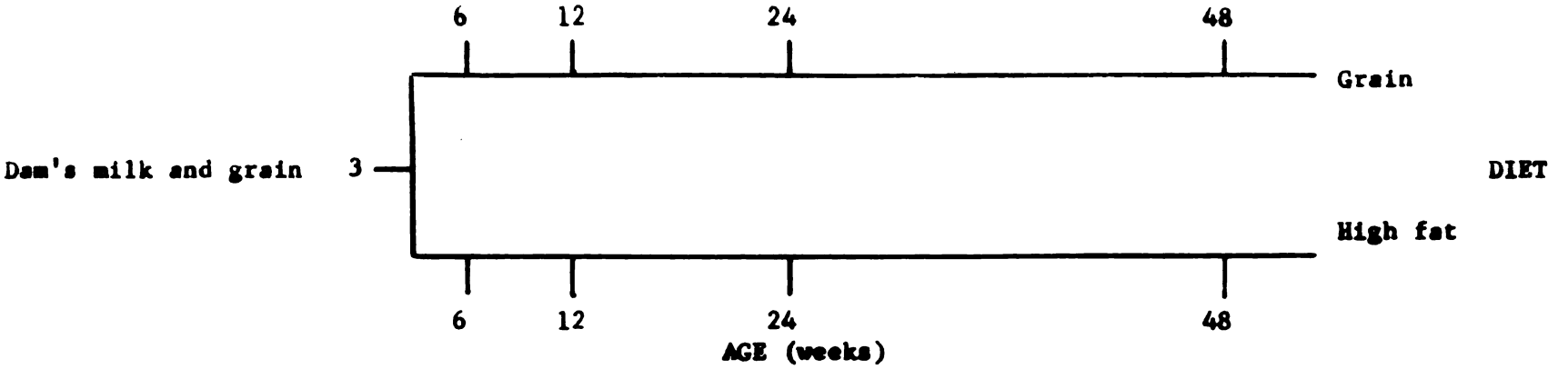


FIGURE 1a.--Experimental design of age-diet study. There were 6 male Osborne-Mendel rats in each group for a total or 54 rats.

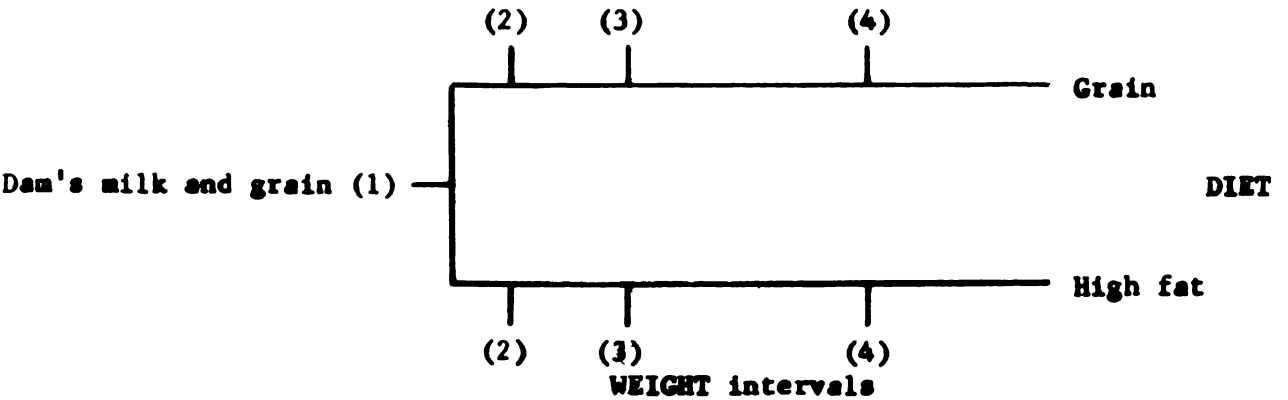
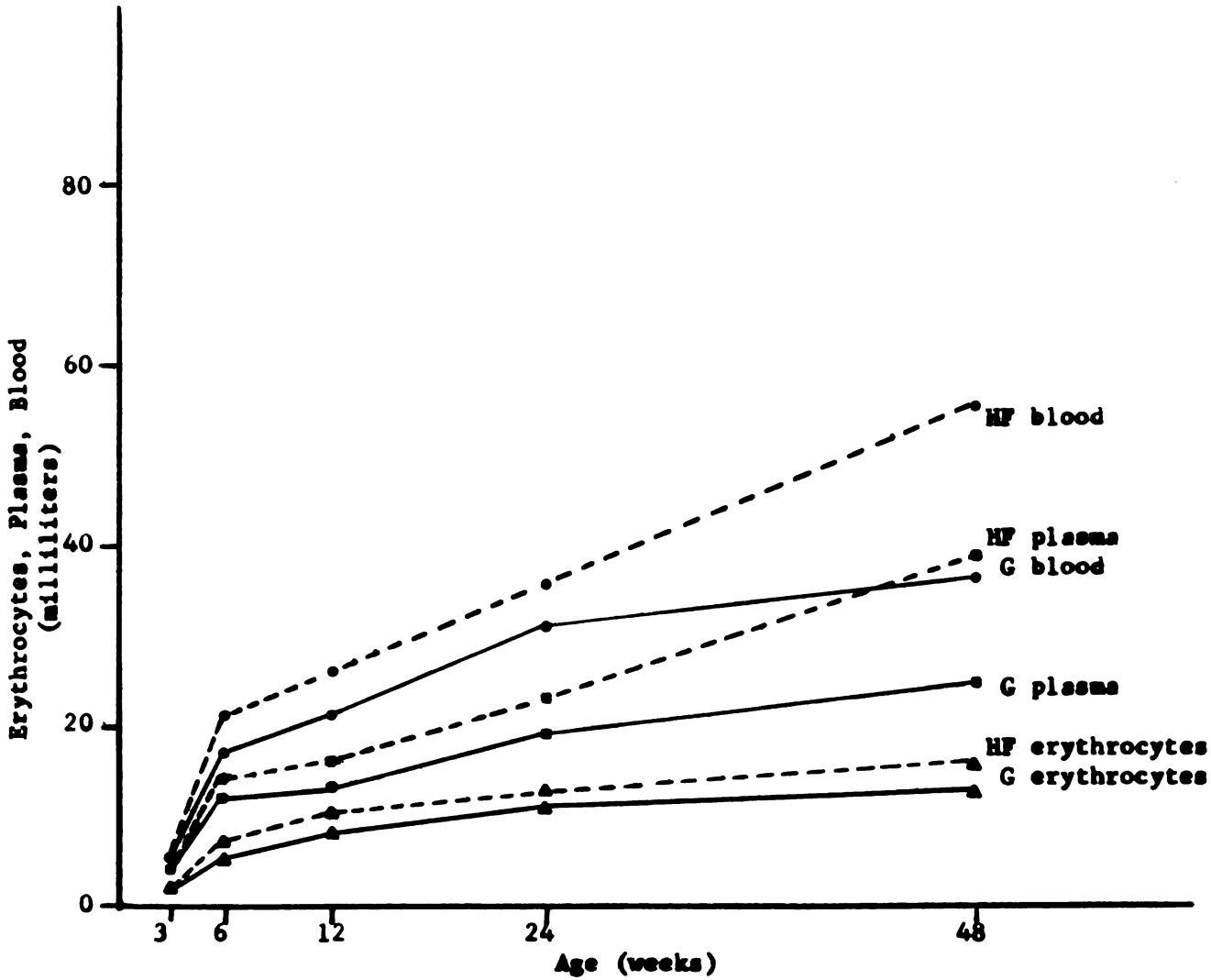


FIGURE 1b.--Experimental design of weight-diet study. There were 6 male Osborne-Mendel rats in each group for a total of 42 rats.



GURE 2.--Absolute volumes of erythrocytes (▲), plasma (●), and blood (●) of grain (G, ——) and gh fat-fed (HF, -----) male Osborne-Mendel rats from the age-diet study. There were 6 rats in each group.

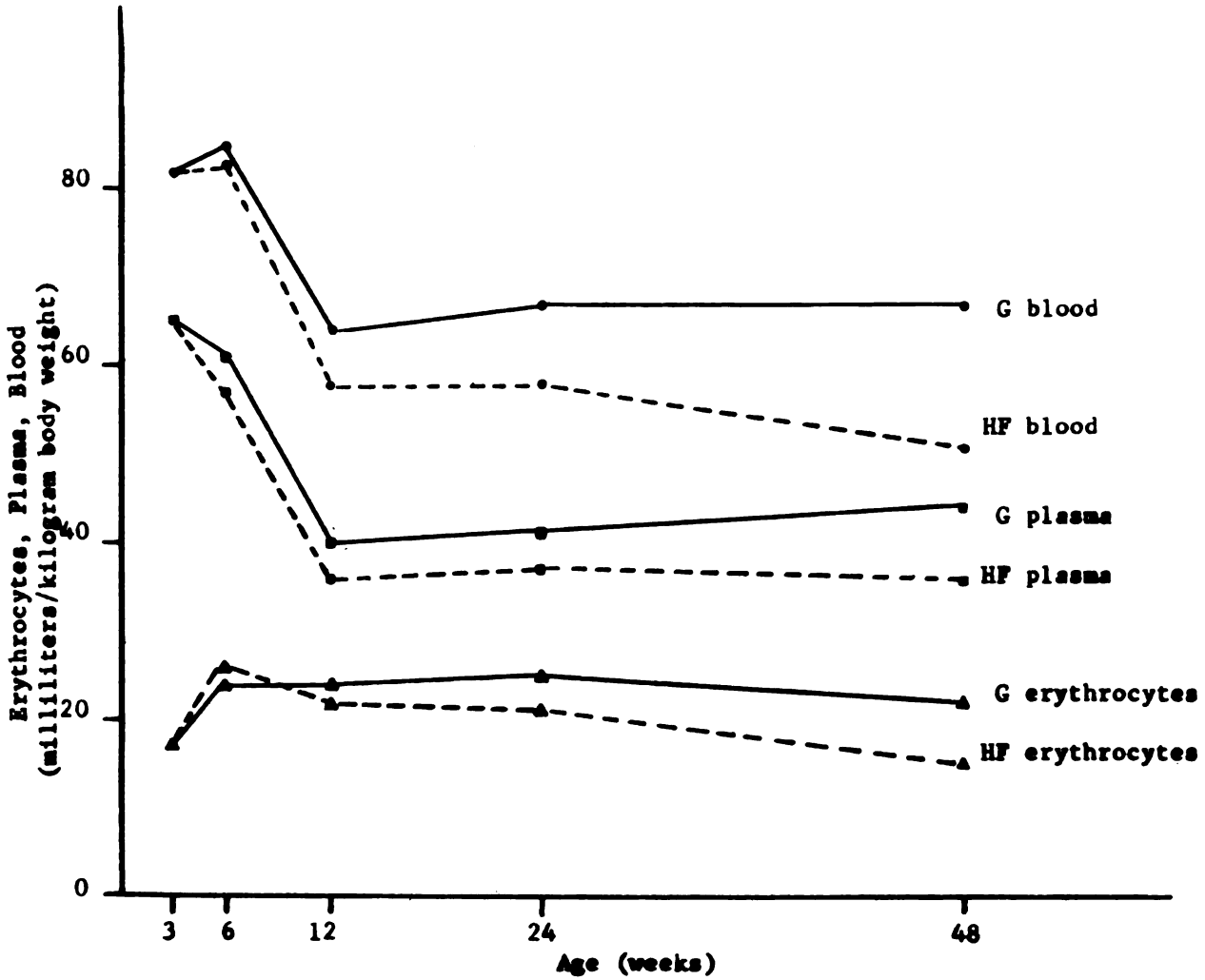


FIGURE 3.--Relative volumes of erythrocytes (▲), plasma (●), and blood (●) of grain (G,———) and high fat-fed (HF,-----) male Osborne-Mendel rats from the age-diet study. There were 6 rats in each group.

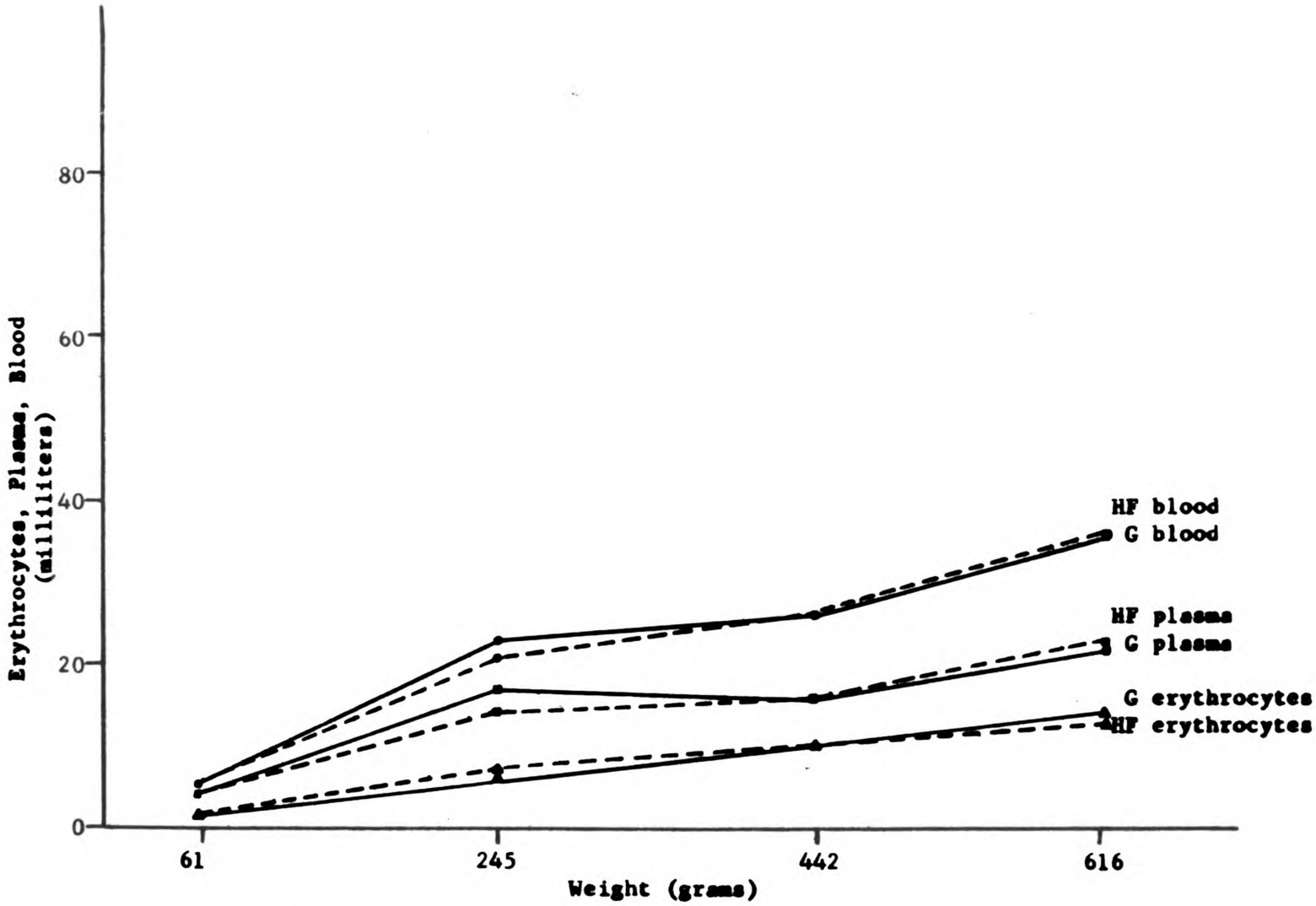


FIGURE 4.--Absolute volumes of erythrocytes (▲), plasma (■), and blood (●) of grain (G,———) and high fat-fed (HF,-----) male Osborne-Mendel rats from the weight-diet study. There were 6 rats in each group.

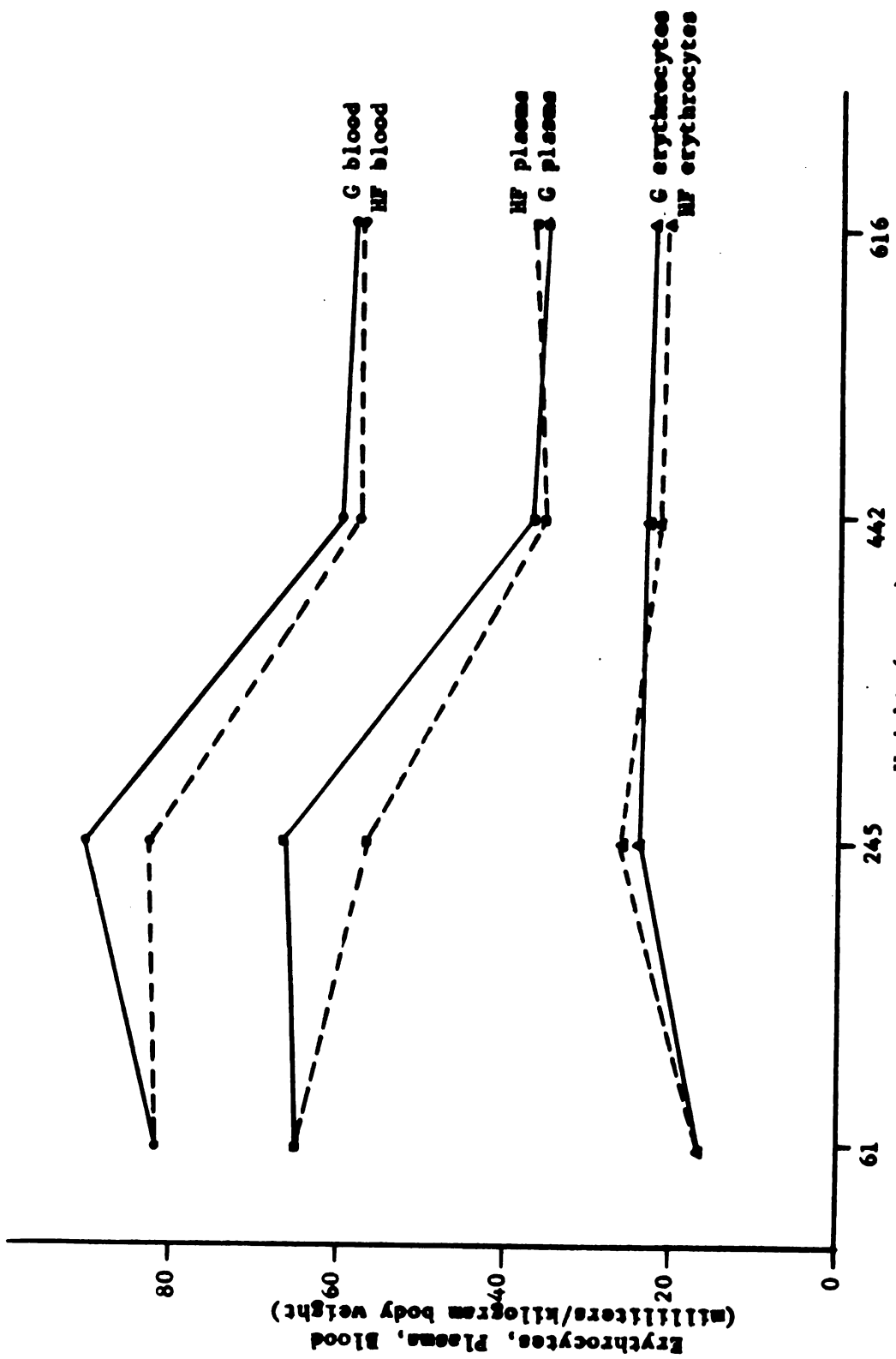


FIGURE 5.--Relative volumes of erythrocytes (▲), plasma (●), and blood (●) of grain (G, ——) and high fat-fed (HF, - - - -) male Osborne-Mendel rats from the weight-diet study. There were 6 rats in each group.

ADVANTAGES OF STUDY COMPARED TO PREVIOUS WORK

Methodology

Although the accuracy of the double radioisotope dilution method has been well-documented in the literature (Schreiber, 1954), most blood volume studies involving obese subjects have measured either erythrocyte or plasma volume. From a methodology view point, the results of measured erythrocyte and measured plasma volume to yield blood volume allows for more definitive statements in regard to changes observed in each of the blood compartments.

Controlled Experiment

The rat experiment was far more expedient in presenting a controlled experimental situation for study than the vast majority of obesity studies in the literature in which blood volume was measured. With the animal experiment, a group of obese rats could be compared to a control group of rats. Usually the control groups consisted of litermate rats, thus, eliminating unaccountable variation. Blood volume was measured in the rats at specified ages and weights. Sufficient measurements were made that the

changes in the volume of erythrocytes, plasma, and blood could be analyzed from weanling rats through nearly one year old rats, a considerable portion of the life span of the rat.

It is not that the controlled situation which prevailed in the rat experiment cannot be duplicated in a human experiment, but rather that it is disregarded for simpler but less controlled designs. For example, it would require finding obese and lean subjects at certain ages and also locating lean subjects the same body weight as obese subjects. Blood volume studies in humans, however, have been consistently less structured than this. Frequently, the group of human subjects were composed of subjects from both sexes, ranged in age from teens to geriatrics, and in studies where correlations with blood compartments and physiological parameters were attempted included any number of both lean and obese subjects. Frequently, no control group was employed.

Alexander (1963), for example, concluded that obese subjects have more blood than lean subjects but less blood in milliliters per kilogram body weight. This was the case for the age-diet study, but in light of the results from the weight-diet study in which "normal" weight and obese rats of the same body weight were found to have the same amount of blood, Alexander's statement needs to be

qualified. Without appropriate control subjects, however, Alexander had no indication that he was oversimplifying the situation.

Other studies have concluded that there is no difference in blood in obese and "normal" weight controls of the same age (Mayer and Hagmen, 1953), but they have limited the studies to younger animals. Since in the age-diet study, significant differences in blood volume were not found between "normal" weight and obese rats until they were 48 weeks of age, a narrow age range or employment of only one age group limits interpretation.

SUGGESTIONS FOR FURTHER STUDY

Heart Histology

The weight of the heart in high fat-fed rats was greater than the weight of the heart in grain-fed rats of the same age. In grain and high fat-fed rats of the same body weight, although the high fat-fed rats were considerably younger, heart weights were the same. Apparently the heart enlarged to accommodate the animal's needs. Further examination of the heart by histological, physiological, or chemical procedures would help to identify those factors associated with the increased heart weight as may be present in obesity.

Vascularization

In grain and high fat-fed rats of the same age, absolute blood volume of the high fat-fed rats was elevated. Further study is needed to determine if the increased blood volume is accompanied with increased vascularization. Since the grain and high fat-fed rats of the same age had equivalent amounts of lean body mass, is the increased vascularization, if any, predominantly in the adipose tissue? Since grain and high fat-fed rats of the same body weights but different body composition equivalent

blood volumes, can it be concluded that the blood is distributed differently in the vasculature of these rats?

Hematocrit in Extreme Obesity

The 48 week old high fat-fed rats had low venous and body hematocrits; thus, the hematocrits were more comparable to the younger rats (weanlings and 6 weeks old) than the older rats (12 and 24 weeks old). In extreme obesity is the organism physiologically hypoxic as a result of low hematocrits? If so, is this the cause for the physical "slowness" associated with obesity? Could an experiment be developed to eludicate the hypothesized hypoxic state of obesity?

CONCLUSIONS

With advancing age and increasing body weight, the absolute volume of erythrocytes, plasma, and blood volume increased. In the obese rats the increase was greater than in the "normal" weight rats of the same age such that at 48 weeks of age, the obese rats had 50% more blood and weighed 100% more than the "normal" weight rats. With advancing age, relative volume of each of the blood compartments decreased. In the obese rats the decrease was greater than in the lean rats of the same age such that at 48 weeks of age the obese rats had significantly less blood per kilogram of body weight than the "normal" weight rats. Obese and "normal" weight rats of equal body weights had similar quantities of erythrocytes, plasma and blood.

Correlations between physiological parameters (body weight, body fat, and lean body mass) and blood compartments (erythrocytes, plasma, and blood) were improved when based on older rats (12 weeks of age and older) rather than all the rats of the study. The body weight and plasma volume correlation as well as the body weight and blood volume correlation yielded the best correlation coefficients ($r=0.83$ and 0.84). Correlation coefficients tended to

improve when correlations were limited to the high fat-fed rats.

The weight of spleen, liver, and heart increased with advancing age and increasing body weight. When expressed on the basis of 100 g body weight, the organ weights decreased with advancing and increasing body weight.

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APPENDICES

APPENDIX A

COMPOSITION OF DIETS

Component	Grain[1] %	High Fat[2] %
Protein	23.4	24.5
Fat	3.0	60.0
Carbohydrate (by difference)	53.5	7.5
Fiber	3.8	2.0
Ash	6.3	5.0
Moisture	10.0	1.0
Kilocalories per gram[3]	3.4	6.7

[1]The grain diet contained (in %): ground corn 60.7; soybean meal (50% protein), 28.0; alfalfa meal (17% protein), 2.0; fish meal (62.5% protein), 2.5; dried whey (67% lactose), 2.5; limestone (38% Ca), 1.6; dicalcium phosphate (18.5% P, 23.5% Cal), 1.75; and iodized salt, 0.5. The following were also added: (in mg/kg feed) Mn, 121; Fe, 95; Cu, 7; Zn, 4; I_2, 4; Co, 2; choline chloride, 400; Ca pantothenate, 6; riboflavin, 3; niacin, 33; menadione, 2; DL-methionine, 500; penicillin, 2; streptomycin, 8; and arsanilic acid, 968; (in µg/kg feed) vitamin B_{12}, 7; and (in IU/kg feed) vitamin A, 8010; vitamin D_2, 750; and vitamin E, 5.

[2]The high fat diet contained: (in %) Crisco, 60.0; high protein casein, 25.0; sucrose, 3.5; Wesson modified Osborne-Mendel salt mix (21), 5.0; nonnutritive fiber, a cellulose type, purchased from General Biochemicals, 2.0; liver powder, 2.0; DL-methionine, 0.25; aureomycin (chlortetracycline·HCl, 972 µg/mg), 0.01; vitamin diet fortification mixture, 2.2. The latter contained: (in g/kg vitamin mixture) vitamin A (200,000 IU/g), 4.5; vitamin D (400,000 IU/g), 0.25; alpha-tocopherol, 5.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.25; ρ-amino-benzoic acid, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine hydrochloride, 1.0; thiamin hydrochloride, 1.0; calcium pantothenate, 3.0; and (in mg/kg vitamin mixture) biotin, 20; folic acid, 90; vitamin B_{12}, 1.35.

[3]Values used for calculating kilocalories were 4, 4, and 9 for 1 g of protein, carbohydrate, and fat, respectively.

APPENDIX B

SUMMARY OF METHOD

1. Bleed rat of 0.3 ml blood (cardiac puncture).
2. Tag cells of whole blood sample with 5 μCi Cr^{51} (incubation time = 30 minutes).
3. Wash cells three times with sterile saline to remove excess Cr^{51}.
4. Add 5 mCi of stock solution I^{125} rat serum albumin and 0.4 ml saline to washed cells to yield approximately 0.5 ml injection solution.

*5. Measure 0.1 ml injection solution and prepare 1:10 dilution with distilled water for counting (0.1 ml injection solution + 0.9 ml distilled water).

6. Determine hematocrit of injection solution.
7. Fill syringe (1 ml capacity) with 0.3 ml injection solution and weigh filled syringe.
8. Inject rat (femoral vein).

*9. Weigh empty syringe and wash ten times with distilled water (total of 10 ml wash); count 1 ml wash.

10. Take post-injection blood samples at 10 and 15 minutes after injection (cardiac puncture).

*11. Measure 0.1 ml of post-injection blood and prepare 1:10 dilution with distilled water for counting (0.1 ml post-injection blood + 0.9 ml distilled water).

12. Determine hematocrit of both post-injection blood samples.

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*Requires counting of radioactivity.

APPENDIX C

SUMMARY OF CONVERTING COUNTS PER MINUTE

TO MILLILITERS BLOOD

Summary of Erythrocyte (Cr^{51}) Calculations

1. To determine radioactivity in injection syringe:

   a. Determine hematocrit (Hct) of injection solution

   b. Measure 0.1 ml injection solution and prepare 1:10 dilution with distilled water (0.1 ml injection solution + 0.9 ml distilled water)

   c. Count this 1 ml of diluted injection sample with counter on Cr^{51} setting

      1.) Determine time for 10,000 counts

      2.) Calculate counts per minute (cpm) for 1 ml diluted injection sample which is actually equivalent to cpm of 0.1 ml of injection sample

   d. Determine total cpm in syringe

$$\text{cpm} \times \frac{\text{volume* of injection solution in syringe}}{0.1} = \text{cpm in syringe}$$

$$\text{*Volume} = \frac{\text{net weight}}{\text{specific gravity}}$$

Net weight = filled syringe weight - empty syringe weight

Specific gravity =

$$1.02 \frac{(100 - \text{Hct of injection sample})}{100} + 1.06 \frac{(\text{Hct of injection sample})}{100}$$

2. To correct for residual radioactivity in syringe after injection:

   a. Weigh syringe (1 ml capacity) before and after injection

   b. Wash ten times with distilled water (total of 10 ml wash)

   c. Count 1 ml of wash with counter on Cr^{51} setting

      1.) Determine time for 10,000 counts

      2.) Calculate cpm for 1 ml wash

      $$\frac{10{,}000 \text{ counts}}{\text{time}} = \text{cpm/ml}$$

   d. Determine total cpm in wash

      $$\text{cpm/ml} \times \frac{\text{total volume of wash}}{1 \text{ ml}} = \text{cpm in wash}$$

3. Total cpm injected = total cpm in syringe - total cpm in wash

4. To determine erythrocyte volume per kilogram (kg) body weight:

   a. Draw post-injection blood samples and determine hematocrit (Hct) of samples

   b. Meausre 0.1 ml of post-injection blood and prepare 1:10 dilution with distilled water (0.1 ml post-injection blood + 0.9 ml distilled water)

   c. Count this 1 ml of diluted post-injection sample with counter on Cr^{51} setting

      1.) Determine time for 10,000 counts

      2.) Calculate cpm for 1 ml diluted post-injection sample

      $$\frac{10{,}000 \text{ counts}}{\text{time}} = \text{cpm in sample}$$

   d. Determine mls of erythrocytes/kg body weight =

      $$\frac{\text{total cpm injected}}{\text{cpm in sample}} \times \frac{\text{Hct of post-injection sample}}{1000} \times \frac{1}{\text{weight in kg}}$$

Summary of Plasma (I^{125}) Calculations

To compute plasma data calculations are as above except:

1. Counter is on I^{125} setting.
2. Plasmacrit (1 - Hct) rather than Hct values are used.

Blood Volume Calculation

Blood volume is then equal to the summation of erythrocyte volume and plasma volume. If calculations must be repeated many times, computations may be computerized. The following headings are suggested for the computer printout: subject #, erythrocyte volume (total mls), erythrocyte volume (ml/kg), plasma volume (total mls), plasma volume (ml/kg), total volume (total mls), total volume (ml/kg), venous hematocrit, body hematocrit, body hematocrit/venous hematocrit ratio.

APPENDIX D

EXPLANATION OF TUKEY'S TEST FOR COMPARISON OF MEANS

Tukey's test is an a posteriori test for the comparison of multiple means. One of the chief advantages of multiple comparison tests is that a single compact presentation of comparisons between a large number of treatment means can be made. Among the multiple comparison tests the Tukey's test offers the advantage of being one of the most "conservative tests" in that type I errors tend to be over-minimized. The experimentwise error rate is maintained at the chosen alpha level. The error rate per comparison is lower than that attained with the Least Significant Difference, Duncan's, or Student-Newman-Keuls procedures, but the rate is not held constant. A disadvantage of Tukey's test is that the power of the test is lower than with the previously mentioned procedures.

To perform a Tukey's test for comparison of means, mean values must be ranked and differences between means computed to complete the Tukey's table. The following example utilizes the mean values for mls of erythrocytes from the weight-diet study. These data were originally presented in Table 5.

Tukey's table:

	13.85	12.82	9.99	9.65	6.53	6.08	1.04 ← Ranked means
1.04	12.81*	11.78*	8.95	8.61	5.49	5.04	-
6.08	7.77	6.74	3.91	3.57	0.45	-	← Differences between means
6.53	7.32	6.29	3.46	3.12	-		
9.65	4.20	3.17	0.34	-			
9.99	3.86	2.83	-				
12.82	1.03	-					
13.85	-						

↑ Ranked means

Next the value for the Tukey's test must be determined. This value is commonly referred to as the LSR value (least significant range). As in other tests of significance, the investigator specifies the significance level at which the test is to be performed. The general formula of Tukey's test for determining the LSR at the .05 level of significance is as follows:

$$LSR_{.05} = Q_{.05}\, s_{\bar{y}}$$

Q values are derived from a table of critical values of the studentized range (Rohlf and Sokal, 1969). To determine the Q value, the investigator must establish a significance level, know the number of means to be compared, and the number of degrees of freedom of the error term.

The Q value is then multiplied by $s_{\bar{y}}$, the standard deviation of the dependent variable. The $s_{\bar{y}}$ value is equivalent to $\sqrt{\frac{\text{MS within}}{r}}$ where "MS within" is the mean square error in an analysis of variance table and "r" is the number of replicates in the experiment.

In the example, the $Q_{.05}$ derived from a table of critical values of the studentized range = 4.537. The $s_{\bar{y}}$ value = $\sqrt{28.932 / 6}$ = 2.196. LSR = (4.537) (2.196) = 9.96. Differences in the above Tukey's table which are greater than 9.96 are declared significantly different at the .05 level by the Tukey's test.

The above example was taken from the weight diet study. Since this was a randomized block there was but one error term. Consequently, determining the mean square error in order to calculate $s_{\bar{y}}$ was rather straight forward.

In the age diet study, a split-plot design, the situation is more involved since there are two error terms. According to Steel and Torrie (1960) the mean square error for the factor of diet is used to determine significant differences between grain and high fat animals of the same age group. Thus, $s_{\bar{y}}$ =

$\sqrt{\frac{E_b}{r}}$ where "E_b" is the mean square error for diet and "r" is the number of replicates in the experiment.

To determine differences between two age groups either on the same or different diets $s_{\bar{y}}$ involves both error terms. That is, $s_{\bar{y}} = \sqrt{\frac{(b-1)\ E_b + E_a}{r_b}}$ where "b" is the number of diets, "E_a" is the mean square error for the factor of age, "E_b" is the mean square error for the factor of diet, and "r" is the number of replicates in the experiment.

Thus, as a result of the split-plot design two sets of Tukey's tests are presented for the age diet study (Tables 4a, 4b). Table 4a is for determining significant differences between grain and high fat animals of the same age group. Table 4b is for determining significant differences between two age groups either on the same or different diets.