

DETERMINING SPECIFIC GRAVITY OF THE
INTACT ANIMAL BY AIR DISPLACEMENT

By

Joseph Anthony Liuzzo

AN ABSTRACT

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of Michigan State University of Agriculture and
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A technique was developed for the determination of body volumes of live animals by air displacement. The method employed was based on the theory that if an animal is placed in a closed chamber, the gas space within the chamber will be reduced in proportion to the body volume of the animal. Measurement of the change in volume is based on the application of Boyles' law which states that the volume of a given mass of gas at a constant temperature is inversely proportional to the pressure to which it is subjected, or $P_1V_1 = P_2V_2$ where P = pressure and V = volume. From the pressure-volume relationships between the chamber in which the animal is placed and a connected chamber of known size, where the two are subjected to varying pressures, the air displaced, and consequently the body volume of the animal can be computed.

The apparatus constructed for this determination consisted of 2 desiccator jars of approximately 2600 ml. capacity, which were connected to each other, to a mercury manometer, to a vacuum pump and to a spirometer by a suitable arrangement of stopcocks. The spirometer was used to saturate incoming air with water vapor to avoid changes in vapor pressure. Temperature changes in the evacuation chamber were measured with a thermistor.

Two experiments were reported in which body volumes were determined at two levels of negative pressure. In the first experiment, specific gravity of 23 adult female guinea pigs was correlated with carcass fat, water, protein and ash, using body volumes determined at a total vacuum of 320 mm. of Hg. The correlation coefficients were -0.70, 0.67, 0.68 and 0.56, respectively. In the second exper-

iment, body volumes of 25 guinea pigs were determined at a total vacuum of 120 mm. of Hg., employing a constant volume method of manometric measurement. Correlation coefficients for air specific gravities thus obtained and carcass fat, water, protein and ash were -0.82, 0.81, 0.72 and 0.72, respectively. In both experiments, the correlation coefficients were significant at the 1% level.

The results of the second experiment indicate that it was advantageous to employ a small negative pressure and the constant volume technique of manometric measurement. It was also shown in this experiment that variations due to the contents of the digestive tract and composition of the viscera did not significantly alter the relationship between air specific gravity and carcass composition.

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Joseph A. Liuzzo
candidate for the degree of
Doctor of Philosophy

Final Examination: May 2, 1958, 9:00 A.M., Room 101, Anthony Hall.

Dissertation: Determining Specific Gravity of the Intact Animal
by Air Displacement.

Outline of Studies:

Major subject: Animal Husbandry--Nutrition.
Minor subjects: Biochemistry, Physiology.

Biographical Items:

Born: December 16, 1926, Tampa, Florida.
Undergraduate studies: University of Florida, 1944-1945.
1947-1950.
Graduate studies: University of Florida, 1954-1955.
Michigan State University, 1955-1958.

Experience:

Laboratory Technician, Nutrition Laboratory, University
of Florida, 1950-1951; Director of Microbiological
Control, 1951-1953, Ass't. to Director of Biological
Research, 1953-1954, Nutrilite Products, Inc., Buena
Park, California; Graduate Research Ass't., University
of Florida, 1954-1955; Graduate Research Ass't., Michigan
State University, 1955-1958.

Member: Sigma Phi Epsilon Social Fraternity, Society of the Sigma
Xi, Phi Sigma Biological Society, American Association
for the Advancement of Science, American Society of
Animal Production.

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INTRODUCTION

One of the major problems in studying growth of domestic animals is to obtain an accurate measure of the composition of body gains. A method of determining body composition would be useful in selecting breeding stock as well as in estimating the composition of animals on nutritional or physiological experiments.

It has been known for many years that the specific gravity values of both inert and active bodies are dependent upon the composition of the body itself. In recent years, this principle has been applied to estimation of the composition of entire eviscerated animal carcasses as well as portions of the carcass in an effort to correlate the body constituents with values determined by specific gravity. The indices thus obtained could save numerous hours of arduous and tedious determinations and calculations in obtaining composition values. Specific gravity methods of determining composition currently used do not apply when knowledge is desired concerning the components of living animals.

Although many methods have been proposed for determination of body constituents in live animals, very little information has been found in the literature relating specific gravity of live animals with body composition.

The primary object of the research project herein reported was to investigate the theory of specific gravity determination by air displacement and to correlate the values thus obtained with major body constituents in living animals.

REVIEW OF LITERATURE

Specific Gravity by Water Displacement

History of specific gravity. - The Greek scientist, Archimedes, who lived in the year 200 B.C., discovered the principle of specific gravity by water displacement when he was confronted with a problem. The records state that Heiron, the King of Syracuse, asked Archimedes to determine whether the goldsmith in making the royal crown had used materials other than the gold that was given to him. The buoyancy that Archimedes experienced while taking a bath gave him an idea how to solve the problem. Archimedes reasoned thus: the loss of weight of a solid of a given mass will depend on the density. No two metals have the same density. The loss of weight of an alloy of gold and another metal will be different from the loss of weight if the given mass consisted of gold only. The loss of weight the crown experienced in water provided ready means for finding out whether the crown was made of pure gold or of an alloy. Since this discovery, the Archimedean principle has been employed in determining the purity of many compounds.

One of the first cases recorded in history in which specific gravity was used to determine body volume of a living being was cited in a review by Boyd (5). He referred to Robertson's (36) publication of 1757 in which the latter reported bribing 10 "middling size men-----for the most part very thin and slim made persons". The men were allowed to submerge in water in his tank measuring 78X30X30 inches. The values for specific gravity calculated from

his data ranged from 0.800 to 1.002. These values were lower than those obtained by subsequent workers, even for the deepest inspiration. Robertson was not satisfied with the results and complained of the uncooperativeness of his subjects, who seemed more interested in the bribe than in the experiment. Robertson's initial experiments were followed by reports of similar investigations throughout the world. In 1915, Spivak (41) critically analyzed the reports preceding his work. He stated that next in importance to weighing the body in air comes the measuring of the body in water. The first measure accounts for the mass and the second for the volume. "One is not complete without the other." He determined the specific gravity of 14 individuals whose weights ranged from 26 to 79 kg. Specific gravity values ranged from 0.976 to 1.049 with an average of 1.005. Spivak accounts for the high and low results obtained by previous investigators by stating that their tank diameters were too large. Supposedly, the smaller diameter accounted for smaller errors in measurements.

Application of specific gravity. - During the first quarter of the 20th century high interest was manifested concerning the chemical composition of animal bodies. In 1922, Murray (23) stated that animal bodies are composed of fat and non-fatty matter. The relative proportions of these two constituents vary within wide limits but can be controlled by food. He observed that the non-fatty matter consists of water, protein and ash. The per cent water varies with the age of the animal in a definite manner. He concluded that the average composition of the whole body at any stage could be calculated when the live weight and per cent fat

were known. Moulton (22) reported that the composition of animals should be compared on a fat-free basis in order to make apparent the effects of age or abnormal development. He observed that mammals showed a rapid decrease in relative water content and an increase in protein and ash content from birth until chemical maturity was reached. After chemical maturity was attained composition remained relatively constant. Moulton further observed that mammals varied in composition at birth. The more mature fetuses had a lower water content than the less mature. The fatness of the individual had no effect on composition on the fat-free basis. Moulton concluded by stating that mammals reached chemical maturity at different ages, but these ages were a fairly constant relative part of the total life cycle. The findings of these and subsequent investigators paved the way for research to correlate specific gravity with body composition.

Boyd (5) gathered much of the information previously published on water displacement specific gravity of humans in an effort to evaluate these findings statistically. The results indicated that: specific gravity in all respiratory phases increased rectilinearly with both structure and age; the increment of specific gravity with each cm. increase of stature was not significantly different in the prenatal and postnatal periods; the rate of increase of specific gravity with age was much higher in the fetal period than in the postnatal; the onset of respiration changed the specific gravity from an average of 1.015 at birth to 1.041 at maturity; and the usual range in specific gravity from deepest inspiration to deepest expiration was approximately 0.065 units or 6.4%.

Welham and Behnke (45) proposed the classification of men as overweight on the basis of specific gravity of the body mass. They suggested a tentative dividing line of 1.060 for the elimination of the obese. Behnke et al. (3) observed that corporeal density could be measured accurately, usually within 0.004 unit by hydrostatic weighing, provided a correction was made for the air in the lungs. Values obtained for specific gravity of healthy men ranging in age between 20 and 40 fell between 1.021 and 1.097. They suggested that low values for specific gravity indicated obesity, and conversely, high values denoted leanness. A loss of weight through exercise and a restricted diet was associated with an increase in specific gravity. Brozek (7), in an effort to associate body composition with specific gravity, placed 34 men whose average age was 25.4 years on a 24 weeks semi-starvation diet. The average control water displacement specific gravity value was 1.071. By mid-starvation the average rose to 1.084 and at the end of starvation to 1.089. The relative amount of body fat was estimated by the formula of Rathbun and Pace (34). Changes of 58.3% and 68.0% of the control fat value as compared with average losses of 16.6% and 22.3% of the total body weight occurred. In 133 pairs of measurements repeated in 3 to 7 days, calculated body fat values as percentages of body weight, agreed within $\pm 1.5\%$ in 90% of the cases.

Messinger and Steele (20) in an attempt to show the relationships of body specific gravity, body fat and water, determined the specific gravity of 9 individuals by the water displacement method. They observed that body fat and water could be calculated with

considerable accuracy from body specific gravity. They noted the inverse relationship between per cent body water and fat and concluded that the proportion of water in the body is highly variable unless it is expressed in terms of fat-free tissue (lean body mass). Osserman et al. (25) in 1950 and Dupertuis et al. (11), one year later, reported the employment of water displacement specific gravity to predict the status of body fat and water in normal human beings. The latter report indicates the specific gravity values to have an average deviation of 0.007. The average error for estimating body fat from specific gravity was only $\pm 3.5\%$ fat. The former investigators stated that the per cent water in lean body mass is a physiological constant for normal men (71.8%) with a standard error of $\pm 0.33\%$. Brozek and Keys (8) reviewed a survey of methods for evaluation of leanness-fatness in man. In addition to specific gravity determinations they emphasized the creatinine coefficient, skinfold thickness, X-ray measurements, and body water measurements. These writers concluded that the estimation of total body fat from specific gravity provides the research worker with an important new tool, even though there are several points on which further work is needed. One such point was listed as consideration of the bony framework in the calculations. The main point was that the limitations of the method are severe for clinical work especially if the volume of residual air at the time of underwater weighing is to be found.

Investigations are numerous in the employment of animals as subjects in experiments conducted to acquire a better understanding of the relationships of body specific gravity and body chemical

composition. One of the more prominent reports is that of Rathbun and Pace (34) who employed guinea pigs as experimental animals. They sacrificed 50 normal male and female guinea pigs with wide variation in weight and fat content. The viscera were removed, leaving only the kidneys and organs with surrounding fat. The hair was clipped from the eviscerated carcass and specific gravity was determined by water displacement. In 21 of the guinea pigs, the specific gravity of the dissected viscera was determined. Fat content was obtained for the viscera and eviscerated carcasses of all 50 guinea pigs. These investigators obtained the following correlation results:

	r_{xy}
Whole animal % fat-carcass % fat	+0.989
Whole animal sp. gr.-carcass sp. gr.	+0.962
% body fat (evisc.)-carcass sp. gr.	-0.972

These results indicated that the fat content of the whole animal is equivalent to that of the eviscerated carcass over the entire range. Therefore, it follows that fat is probably laid down proportionally in the viscera compared with the remainder of the body. Morales et al. (21) investigating with Rathbun and Pace reported that the gross composition of the animal could be determined from a knowledge of (1) weight of the animal, (2) the body density of the animal, (3) the densities of fat, muscle and bone, and (4) the ratios of muscle, skin, and nervous tissue to bone. These assumptions led to the derivation of the following formula for the determination of fat to body weight ratio ($\frac{Mf}{W}$) :

$$\frac{Mf}{W} = \frac{\frac{1 + K}{G} - \frac{Dm + KDb}{DbDm}}{\frac{1 + K}{Df} - \frac{Dm + KDb}{DbDm}}$$

where: K = total constant fractions for muscle, skin and nervous tissue.

G = average density of body.

Dm = density of muscle.

Db = density of bone.

Df = density of fat.

This formula gave rise to the following regression equation for predicting % body fat of the eviscerated guinea pig:

$$\% \text{ Fat} = 100 \left(\frac{5.362}{\text{Sp. Gr.}} - 4.880 \right)$$

To correct for the slight discrepancy which exists between specific gravity of the eviscerated animal and that of the whole animal, the following theoretical regression equation for the whole guinea pig was derived by Rathbun and Pace (34) :

$$\% \text{ Fat} = 100 \left(\frac{5.501}{\text{Sp. Gr.}} - 5.031 \right)$$

When the relationship of Mf/W to G in hairless, eviscerated carcasses was plotted together with the true relationship performed experimentally, the regression lines ran parallel. The consistent error in the direction of yielding low values of G was accredited to the adherence of air bubbles to the animal's surface and within its ears. It was observed that a volume of air of about 2 c.c. would cause the experimental curve to coincide with the theoretical. Pace and Rathbun (26) also reported on the body water and chemically combined nitrogen content in relation to fat content of guinea pigs.

The average water content of the fat-free body mass was observed to be 72.4% in the 50 guinea pigs used in the previous experiment. A standard deviation of 2.11% was found. Chemically combined nitrogen constituted $3.52\% \pm 0.72\%$ of the lean body mass. The experimental evidence obtained in this investigation supported the concept that lean body mass is relatively constant in gross chemical composition and that body fat may be considered to act as a diluent.

Pitts (32) recently studied body fat accumulation in the guinea pig. In addition to determining specific gravity of the eviscerated carcass, he removed the organs and chemically determined their composition individually instead of collectively as was done by Rathbun and Pace (34). The organs tested were bone, gut, liver, kidneys, heart, subcutaneous tissue, internal fat depots, spleen, gut content, brain and spinal cord, and muscle. The correlation coefficient of the specific gravities of eviscerated carcasses and of the % fat of the organs plus carcasses was -0.949. This value compares closely with the -0.972 obtained by Rathbun and Pace (34). Pitts states that his procedure was more cumbersome and allowed more opportunities for a loss of fat. He observed 96% of the fat stored in subcutaneous and internal adipose tissue; approximately 3% in the organs; and no more than 1% in the remaining tissues.

Da Costa and Clayton (10) using the albino rat as the experimental animal reported that an inverse relationship exists between carcass fat and water and between carcass fat and specific gravity. They further observed that a direct relationship exists between carcass water and specific gravity. They concluded, as had previous

investigators, that specific gravity is as good an index of the whole animal water content as it is of the fat content.

The last seven years have seen the employment of water specific gravity methods in livestock investigations. This development has occurred in a quest for indices to more easily determine the body composition of these animals. Brown et al. (6) observed the average specific gravity of 66 hogs to be 1.027. The hogs were slaughtered at a weight range of 202 to 230 lbs. The individual specific gravity values were correlated with average backfat thickness, % ether extract, % moisture, % protein, and % protein and ash. The correlation coefficients were -0.49, -0.75, +0.68, +0.65, and +0.72, respectively. All values were significant at the 1% level. The correlations indicated that the fat or lean content of the carcass may be estimated as accurately by specific gravity as by percentage fat cuts or percentage lean cuts. Whiteman et al. (46) continued these investigations and later reported correlation coefficients for half-carcass specific gravities and specific gravities of ham, fat and skin, lean, bone, and % lean, % bone, % fat and skin. The coefficients were +0.95, +0.65, +0.77, +0.55, +0.76, +0.41, and -0.83, respectively. It was further observed that the specific gravity of the hams correlated as well with the same constituents.

Kraybill et al. (17) reported that the specific gravity range of 15 steers and 15 heifers was from 1.017 to 1.070 with a mean value of 1.045. The fat content varied from 13.6 to 39.5% of the body weight with an average fat content of 25.1%. Correlation coefficients between carcass specific gravity and % fat, % water and whole animal specific gravity were as follows: -0.96, +0.98,

and +0.99, respectively. Kraybill et al. (18) continued this work with swine. They attempted to determine the accuracy of body water, body density and backfat thickness measurements on the live animal and carcass for estimating the body fat content of swine. The body density of the eviscerated hog carcass and the measurement of the backfat thickness on the live animal or carcass were found to be closely correlated with the body water and fat contents. These were established as useful indices in the appraisal of fatness. These investigators, following the pattern of Rathbun and Pace (34) with guinea pigs, established theoretical equations which permitted the calculation of percentage of body water and/or fat from the specific gravity of the eviscerated hog. The equations are as follows:

$$\% \text{ Body water} = 100 \left(4.400 - \frac{4.021}{\text{Sp.Gr.}} \right)$$

$$\% \text{ Body fat} = 100 \left(\frac{5.405}{\text{Sp.Gr.}} - 4.914 \right)$$

Clawson et al. (9) also observed a high correlation between specific gravity of the hog carcass and water content of the carcass ($r_{xy} = +0.89$). However, they reported that a lower correlation was found between the carcass specific gravity and the total body water content ($r_{xy} = +0.63$) determined by the antipyrine method. These observations suggested to them that the variable water content of the viscera found between pigs makes the prediction of the water content of the whole body from the carcass specific gravity questionable.

Pearson et al. (27) in determining the suitability of the specific gravity of three untrimmed prok cuts, e.g., the ham, loin, and shoulder, as measures of carcass leanness, found that the specific gravity of each of the cuts was closely associated with the

specific gravity of the entire carcass. The correlation coefficients were +0.94, +0.96, and +0.92 for the respective cuts when both cuts from the same carcass were used. When single cuts were used, the coefficients were less. These investigators concluded that specific gravity of either the entire carcass or a single ham was superior to backfat thickness as a measure of carcass leanness. In a later report, Pearson et al. (28) employing 102 hog carcasses showed a significant relationship between hog carcass specific gravity and fat/lean ratio ($r_{xy} = -0.59$). The specific gravity of the left ham was also correlated significantly with the ratio ($r_{xy} = -0.54$). Price et al. (33) recently observed that the specific gravity of the untrimmed right ham was closely associated with the specific gravity of the entire carcass ($r_{xy} = +0.86$). However, other measurements as cut-out percentages, chemical composition of the ham, loin lean areas, and fat thickness were not as closely predicted by ham specific gravity as by carcass specific gravity. All coefficients were highly significant.

Specific Gravity by Gaseous Displacement

History.- Several investigators (41, 43) have indicated that the history of specific gravity determinations by gaseous displacement had its beginning approximately 75 years ago when Jaeger suggested a relationship between corporeal density and health. He commented that body volume could be measured best by air displacement, in a Kopp volumeter. In this method a body is enclosed in an airtight chamber. The volume of the chamber is changed by a known amount and the volume of the air around and in the body calculated

from the resultant pressure changes. This procedure has been reported as accurate for inert objects but is complicated in the living organism by the pressure changes resulting from gaseous exchange, vaporization and increasing air temperature.

The application of gaseous displacement specific gravity. - During the first third of the 20th century, several reports emerged from German investigators attempting to further Jaeger's theory. Pfaundler (30) constructed a cylinder of strong brass (25 cm. wide and 55 cm. long) in an effort to measure net body volume which he defined as the total body volume minus the volumes of gas in the respiratory and digestive tracts. One side of the chamber was permanently sealed while the other side contained a removable door. The cylinder was insulated with a thick felt casing. A manometer and thermometer were connected to the apparatus. Sulfuric acid served as the manometer measuring fluid. The accuracy of the manometer reading was to 0.1 mm. The procedure used by Pfaundler for measuring body volume was as follows: the body of a child was placed in the chamber immediately after death and allowed to remain there until the environmental and body temperatures were approximately equal; after obtaining temperature and barometer readings, positive pressure was introduced into the chamber; the manometer level was recorded and then negative pressure was introduced; this level was also recorded. Net body volume was an average of the volumes determined by both methods (negative and positive pressures). Temperature differences of up to 1°C. were observed but were regarded as insignificant. If the cadaver was not allowed to cool before operations began, temperature differences

were larger which made accurate determinations impossible. This procedure was repeated several times until constant manometer readings were obtained (± 0.1 cm.). It is therefore evident that this would result in obtaining gas free volumes. A major fault of this procedure was the duration of the operation. The author states that by "procuring practice and by faultless functioning of the apparatus and with all possible care, a reading may be obtained in 1½ hours". This, of course, is not feasible when using a living organism as the experimental subject! It would also be impossible to obtain a constant level of gaseous deprivation in a living object, at least for any length of time. Pfaundler also determined water displacement body volumes by submerging the entire body. Specific gravity values calculated by both procedures proved to be highly variable. In 16 observations, average values of 0.9875 (water displacement) and 1.143 (air displacement) were obtained.

Pfleiderer (31) constructed a large tank chamber into which could be introduced a small amount of compressed air. He observed that the smaller the chamber, the more the gas would be compressed. The resulting gas volume yielded the volume of the space in the receptacle. When a firm body was placed in the chamber, the remaining space was smaller. Thus, by subtraction, the volume of the body volume could be determined. Positive pressure for the determination was created by in-flow of water in a connecting cylinder. He found that positive pressures of about 200 mm. of water were easily tolerated by humans. About 2½ minutes were allowed for equalization of the thermodynamical expansion of gases. This requires

considerably less time than Pfaundler's (30) procedure but it is still too time consuming for the comfort and co-operation of live animals. Pfeleiderer stated that the accuracy of the results depended upon the air-tightness of the apparatus, on the application of good apparatus temperature correction by ventilation and on the comprehension of the limited errors by humidity changes and gaseous exchange. He also observed that the higher the pressure, the smaller the effect of differing error sources. The proportion of the apparatus volume to the body volume should be as small as possible. His results showed a mean error in body volumes of 1 to 2%.

Kohlrausch (15) attempted to show a relationship between specific gravity determined by air displacement and fat content in dogs. His investigation was based on the fact that a relationship exists between basal metabolism and the amount of protein. He suggested that by knowing the value for protein, it should be possible to determine fat and ash by a specific gravity measurement. In order to show this fat-specific gravity relationship in short-haired terriers, he employed the following techniques: (a) determinations of the basal metabolism and protein content; (b) showing a relationship between these values; (c) a determination of body volumes; (d) a quantitative determination of fat, water and ash; (e) showing a definite relationship between fat and specific gravity. To determine body volume, he constructed a chamber of boiler plate with dimensions of 80 x 30 x 25 cm. with a capacity of 60 liters. This chamber was surrounded by an insulating sleeve which was 10 cm. deep. A lid with a rubber gasket was used for the hermetic seal. There were two major inlets and one outlet in the system through which saturated air and a known volume of gas could be introduced

and evacuation of the chamber could occur. The chamber was connected to a glass reservoir of about 10 liters capacity. This reservoir was placed in a rubber bladder also of about 10 liters. The bladder was connected to a Niveau reservoir which was filled with 5.43 liters of water at 4°C. Raising the Niveau filled the bladder thus forcing the air into the first reservoir and then into the chamber. When the valves which controlled the inflow of saturated air and the evacuation of the chamber were closed, a water manometer was used to indicate the rise in pressure. In this manner, the volume of the chamber could be calculated according to the formula $PV = P_1 V_1$. Accurate results were observed for the chamber volume over a period of several days (59.30, 59.30, 59.31, 59.30 and 59.30 liters). Kohlrausch reported comparable volume results when objects of known volumes were placed in the chamber. In order to compensate for the change in vapor pressure due to temperature rise and reduction of gas through breathing, about 120 liters of saturated air was introduced into the chamber for a period of about 6 minutes. In four dogs, the specific gravity values varied from 1.046 to 1.074 and the fat, estimated post mortem by direct analysis, varied from 12.2 to 6.2% of body weight. There were no correlation values reported for these measurements. Only the averages over a period of time with indications of the range of values from which the averages were taken are shown. True mathematical relationships between specific gravity as determined by this technique and body composition of the dogs are not shown. Finally, the reliability of this technique is open to question since it is based on the results obtained from only four dogs.

In a second publication, Kohlrausch (16) reported results of a dog (from the previous experiment) which was subjected to several months of training involving heavy work on a motor-driven treadmill. The specific gravity increased from 1.054 to 1.074. It was estimated that the fat content decreased from 1217 grams to 609 grams while the active muscle mass, calculated from basal metabolic rate, showed an estimated increase from 1676 to 1750 grams. There was a total weight decrease from 10,805 to 9,060 grams.

Bohnenkamp and Schmah["] (4) determined the volume of individuals by placing them in an airtight chamber and observing the increase of pressure resulting from the addition of a known volume (by weight) of oxygen. This determination was based on the principle that the increase in pressure on the addition of the gas is a function of the volume of gas to which it is added, which is the volume of the chamber less that of the subject. These investigators suggested that by this method the gas contained in the subjects, in the lungs and intestines, was not counted as part of its volume, but the volume obtained was that of its solid and liquid tissues. These investigators, as previous workers, took precautions against vapor pressure by completely saturating the air with water. Good mixing of air and temperature change corrections were also observed. Specific gravity values averaged 1.095 for males and 1.07 for females. Extreme values observed were 0.98 and 1.13. Noyons and Jongbloed (24) stated that the preceding method of Bohnenkamp and Schmah["] (4) is theoretically satisfactory but its practical execution appears very difficult. In criticizing the procedure they observed that one must always know the exact temperature, the water vapor content,

the oxygen consumption and the carbonic acid production, as well as the air pressure changes. This requires a relatively complicated apparatus and a number of assistants for the operations and simultaneous readings of the recording apparatus. Finally, the calculations are quite tedious. The principle employed by Noyons and Jongbloed (24) was to determine body volumes from the difference of two weighings at different atmospheric pressures. The procedure was briefly as follows: the subjects (cats) were weighed first in normal atmospheric pressure and then under negative pressure. The difference between these weights gave, after corrections for loss of weight due to insensible perspiration, the increase in weight which resulted from the small increased pressure of air. This method could be applied either to positive or negative pressures. Three years later, these investigators (14) reported results obtained when their procedure was applied to the measurement of body volume in humans. In 20 determinations made within several weeks on the same person, a mean specific gravity of 1.080 ± 0.007 was obtained. These measurements confirmed the beliefs of Bohnenkamp and Schmah, that the physiological variations of specific gravities of humans amounts to about $\pm 1\%$. Jongbloed and Noyons (14) observed that the use of positive pressure was better for the subject's comfort than the use of negative pressure. The entire process consumed about $\frac{1}{2}$ hour.

Wedgwood and Newman (44) and Wedgwood et al. (43) in 1953 reported the construction of an apparatus designed to overcome many of the problems involved in previous investigations. They proposed

imposing a sine wave of changing volume on the gradual changes due to heat, vapor and gas exchanges. Their suggestion was that this procedure allowed measurements to be made rapidly, even on the young, uncooperative and infirm. Unfortunately, details of the operation of this apparatus did not appear in the literature. Recent correspondence with this team of workers reveals that they had temporarily abandoned the project.

Several investigators have recently reported the use of helium as a displacement gas for measuring body volume. The principle purpose for the use of this gas is its inert property. Walser and Stein (42) employed this principle in determining body volume of 10 cats. The average difference in specific gravity obtained by this technique and by the method of underwater weighing of the lung-free carcass was 0.013. In this investigation as in previous studies reviewed, the number of samples used was too small to ascertain the reliability of the technique for a large population. Perhaps the most noted contributor to this method of determining body volume of living objects is Siri (37, 38, 39). He designed a complex apparatus which is essentially a closed-circuit system consisting of two chambers. The subject was placed in one chamber and helium in the other. The gases in the system were mixed. The helium concentration, measured by thermal conductivity, was related to the tissue volume displacement of the subject. Data were obtained for 34 subjects whose individual weight variations did not exceed ± 1 kg. of their average weights. The interval between runs varied from two days to six months. The average difference in density between

the first and second runs was $0.0006 \text{ g. cm.}^{-3}$, while the standard deviation in the differences about the mean difference for the group was $\pm 0.0016 \text{ g. cm.}^{-3}$. The group as a whole was highly heterogeneous, consisting of both men and women with densities varying from 0.990 to 1.076, and weights ranging from 55 to 97 kg. The standard deviation in a single measurement of volume appeared to be no greater than about ± 0.12 liter. Since the error was little affected by absolute volume, the corresponding error in density ranged from $\pm 0.0024 \text{ g. cm.}^{-3}$ for a 50 kg. subject to $\pm 0.0012 \text{ g. cm.}^{-3}$ for a person who weighed 100 kg. This instrument has not yet been adapted for use in animal investigations.

Although numerous investigators have devised various methods for determining the body volume of living objects by gaseous displacement, no reports have appeared in the literature, until recently, concerning these methods and their reliability in predicting body composition. In a preliminary report of the technique discussed in this dissertation, Liuzzo et al. (19) employed an apparatus which was designed to measure body volume of live guinea pigs by air displacement. Negative pressure was used to obtain their measurements. Results showed that the specific gravity values thus obtained were significantly related to body composition. The technique of operation and results are reported in detail in Experiment 1 of this thesis.

Recent Indices for Determining Fatness in Livestock

Numerous indices have been reported in the literature over the past fifty years which were primarily designed to predict the fat-lean composition of living animals. Only a few of the most reliable

techniques will be reviewed, particularly those which are most prevalent and applicable today in livestock investigations. In 1934, Hankins and Ellis (12) reported the correlation coefficient of the average backfat thickness and percent fat of edible portion of 60 hog carcasses to be +0.84. The hogs varied in respect to feed consumption, rate of gain, age, breed, sex, type, total gain and market grade. Aunan and Winters (2) also found that the average backfat thickness was associated with the fat and lean content of the carcass. They further reported a correlation between percentage primal cuts and dressing percentage. Hazel and Kline (13) reported a correlation coefficient of +0.81 between backfat measurements of carcasses and live probe of hogs. They observed that the most accurate locations for the live measurements were just behind the shoulder and at the middle of the loin about 1½ inches off the midline of the body.

Reid (35) reported at the Cornell Nutrition Conference for Feed Manufacturers that studies made of the data published on the body composition of cattle and swine revealed that the entire proximate composition could be resolved from a knowledge of the water content of the whole, empty (ingesta-free) body. This is made possible by the highly predictable inverse relationship existing between the concentrations of water and fat and the constant proportions of protein and ash in the fat-free dry substance of the body. He stated that the fat content of the whole, empty body of cattle and swine, respectively, may be computed by use of the equations: $Y = 355.88 + 0.3550X - 202.91 \log X$, and $Y = 178.88 -$

$0.6325X - 66.62 \log X$; where Y = fat content (%) and X = water content (%). Reid concluded that the key to the resolution of the chemical composition and energy value of the live, intact animal is the accuracy with which either the water or fat content can be determined.

Pearson et al. (29) showed results which indicated that there was little difference in the usefulness of the live probe or lean meter in regard to estimating backfat thickness and percentage of either lean or primal cuts in swine. They observed, however, that the higher relationship for the live probe with both loin lean areas and with fat trim indicated live probe to be a more reliable measure of estimating carcass leanness.

EXPERIMENTAL PROCEDURE

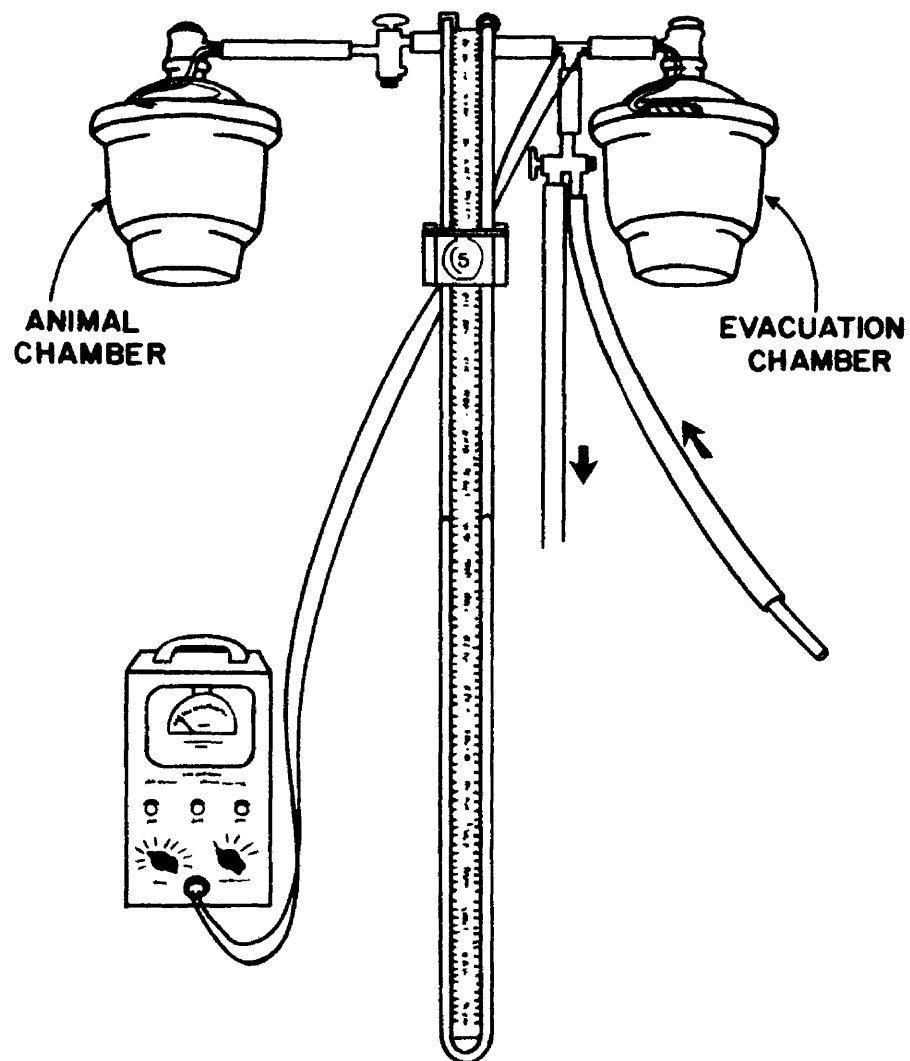
Experiment 1

The Employment of Negative Pressures at 320 mm. of Mercury

The object of this experiment was to observe the accuracy with which body volume could be determined employing a relatively high negative pressure. Pfleiderer (31) observed in his investigations that the higher the pressure, the smaller the effect of various sources of error. It is logical that this theory holds true for negative as well as positive pressures.

The apparatus constructed for the body volume determinations is shown in Figure 1. It consists of 2 desiccator jars of approximately 2600 ml. capacity which are connected to each other, to a mercury manometer, to a vacuum pump and to a spirometer by a suitable arrangement of stopcocks. The spirometer was used to saturate incoming air with water vapor to avoid changes in vapor pressure. Temperature changes in the evacuation chamber were measured with a Sargent Thermistor Thermometer.

The experimental animal selected for this investigation was the guinea pig. Rats had been used initially, but results were too erratic, thus confirming Pfleiderer's (31) theory that the proportion of the apparatus volume to the body volume should be as small as possible. Rathbun and Pace (34) previously reported that female guinea pigs averaged 4.7% more fat than males and the difference was greater with larger animals. Therefore, the guinea pigs used in this study were adult females.



**Figure 1. The apparatus designed to measure body volume by
air displacement.**

The determination of the guinea pig body volumes by air displacement was performed as follows: the mercury levels of the manometer were read while the instrument was "at rest" (levels read on both sides to minimize errors); a vacuum of approximately 320 mm. of Hg. was drawn on the evacuation chamber; after recording the temperature in this chamber and reading the mercury level, equalization between the two chambers was allowed to occur by turning the stopcock closest to the animal chamber; thirty seconds were allowed for equalization after which the temperature was recorded and the mercury level re-read. The initial manometer reading was designated as P_0 , the reading before equalization as P_1 , and the reading after equalization as P_2 . The values obtained were incorporated into formula (1).

$$(1) \quad V_2 = \frac{P_1 - P_2}{P_2} \left(V_1 \frac{273}{273 + \Delta T} \right)$$

where V_2 = volume of the empty animal chamber.

V_1 = known volume of the evacuation chamber.

ΔT = temperature change in the evacuation chamber.

The same procedure was repeated with the animal in the chamber to determine the volume of the animal chamber while occupied (V_3). Replicate determinations were made for V_2 and V_3 with each animal. The average of these values was incorporated into formula (2) for determining the volume occupied by the animal.

$$(2) \quad \text{Volume occupied by the animal} = V_2 - V_3.$$

The specific gravity of the animal was determined by formula (3).

$$(3) \text{ Specific gravity} = \frac{\text{weight of animal in air}}{\text{volume of animal}}$$

In this experiment, 23 female guinea pigs ranging in weight from 507 to 1,064 gms. were used. They were deprived of food and water for 24 and 12 hours, respectively, prior to the determination. The hair was clipped from the animals as short as possible to reduce weighing errors in the subsequent water specific gravity determination. Their specific gravity was determined by the air displacement procedure after which they were sacrificed and eviscerated of all organs except the kidneys and surrounding fatty tissues. In order to check the validity of the air displacement method, specific gravity of the eviscerated carcass was determined by the water displacement method of Rathbun and Pace (34). Their method has been accepted as an accurate measure of specific gravity. The carcasses were then ground by passing them twice through 3/8, 11/64, and 3/32 inch grinder plates. After thorough mixing, the ground carcasses were stored in glass jars at -20°F.

Body fat, water, protein and ash of the ground carcasses were determined by A.O.A.C. methods (1). The fat determination was modified by employing the Goldfish Ether Extractor. In this modification, 2 to 4 gms. of tissue (on the wet basis) were dried in a hot air oven at 105°C. for 24 hours. The dried tissue was placed in the extraction thimbles and extracted with anhydrous ethyl ether for 3 to 4 hours. The per cent fat was determined by gravimetric means. The body composition values were correlated with specific gravity obtained by air and water displacement. Results were analyzed statistically according to the methods of Snedecor (40).

Experiment 2

The Reduction of Negative Pressure

The objectives of this experiment were to ascertain the feasibility of using smaller negative pressures for the body volume determinations and to investigate the influence of visceral composition upon specific gravity of the intact animal. The effects of a smaller negative pressure on the first of the four V_3 replicates were of particular interest since it was concluded in Experiment 1 that this high value was possibly due to the partial removal of gases from the animal's blood and body fluids by the vacuum.

Several manometer fluids were tested to attain this reduction. Kerosene was suspected of losing gases under the subatmospheric conditions, for the measurements would not reach constancy. Ethylene glycol and water did not move down the manometer tube fast enough for rapid measurements after equalization between the chambers. The failure of these fluids to give the desired results led to the continued employment of mercury at low negative pressures.

In order to validate the vacuum reduction, the body volumes of six female guinea pigs, determined at negative pressures of 120 and 220 mm. of Hg., were compared. In addition to body volumes, the following results were also compared: the gases removed in the first V_3 trial, the replication of V_3 values and the standard deviations of V_2 values.

The results for these comparisons are shown and discussed under the section Results and Discussion. However, it must be brought to attention here that the standard deviation of the mean

of V_2 values was considerably larger when 120 mm. of Hg. were used (± 13.3 ml.) than when 220 mm. of Hg. were employed (± 1.8 ml.).

In contrast to these results, the V_3 values obtained from six guinea pigs showed little variation under both negative pressures. This led to the conclusion that at 120 mm. of Hg., the vacuum was not high enough to record a substantial difference in chamber pressures when the animal chamber was empty. It is possible that this was caused by changes in the "dead-space" in the manometer tube which resulted from the lowered negative pressure. In order to eliminate this as a possible source of error, a "side-arm" was fused to the base of the manometer and connected to a leveling bulb with rubber tubing. This mercury reservoir was raised or lowered to obtain all readings at the same point on the inner arm of the manometer.

In order to obtain a measure of V_2 with the constant volume modification, the following techniques were employed: the mercury levels of the manometer were read while the instrument was "at rest" (for a vacuum of 320 mm. of Hg., P_0 = the 480 mm. mark on the manometer scale; for 220 mm. of Hg., P_0 = 580 mm. mark; for 120 mm. of Hg., P_0 = 680 mm. mark); the P_0 levels were adjusted at the desired marks with the mercury bulb; a vacuum was drawn in the evacuation chamber to the 800 mm. scale mark which resulted in the desired negative pressure; after recording the temperature in this chamber and reading the mercury level, equalization between the two chambers was allowed to occur; after the 30 seconds equalization period, the temperature was recorded and the mercury level on the right side of the tube was brought back to the 800 mm. mark with the mercury

reservoir. The latter action yielded the P_2 reading and eliminated changes in "dead-space" during equalization. The P_1 and P_2 readings were employed in formula (1), shown in Experiment 1.

In order to determine the value of the new constant volume technique, several pressure comparison determinations were made with V_2 values obtained at 120 and 220 mm. of Hg. with and without this technique. The results justified the use of the constant volume technique, employing a negative pressure of 120 mm. of Hg.

In order to observe the effects of the lowered vacuum and constant volume technique on the correlation of air specific gravity and body composition, another guinea pig study was undertaken. In this study 34 adult female guinea pigs were used for body volume determinations by air displacement. Thereafter, they were sacrificed and eviscerated. The water displacement measurements and carcass composition studies were conducted as in Experiment 1. The removed organs of each animal were ground to determine their per cent body water and fat. These organs consisted of: the gastrointestinal tract, heart, lungs, liver, spleen, reproductive and genital organs and the contents of the G.I. tract. The chemical determinations of these organs and of the contents of the digestive tract were made to observe their effects in the correlation between air specific gravity and body composition. In this study, the effects of two levels of saturated air were also investigated. The body volumes of guinea pigs #29 through #36 were determined after drawing saturated air into the evacuation chamber with a P_1 of 120 mm. of Hg., while with the remaining guinea pigs, the chamber was saturated at a P_1 of 320 mm. of Hg.

RESULTS AND DISCUSSION

Experiment 1

The specific gravities of the guinea pigs were calculated from the body volumes shown in Appendix Table A. The correlation coefficient for the water and air specific gravities was 0.74, significant at the 1% level. The regression equation for the two specific gravities (Figure 2) was $Y = 1.1295X - 0.0857$. The standard error of the estimate was 0.0227. Appendix Table B shows the chemical composition of the eviscerated carcasses. The correlation coefficient for water specific gravity (Appendix Table A) and % body fat was -0.99. The regression equation for these values (Figure 3) was $Y = 100 (4.6038 - 4.1871X)$. The standard error of the estimate was 1.35% fat. The correlation coefficient for air specific gravity and % body fat was -0.70, significant at the 1% level. This regression equation (Figure 4) was $Y = 100(2.3027 - 1.9245X)$ with a standard error of the estimate of 7.0% fat. A summary of the correlation coefficients of the 4 major body components and air and water specific gravities is given in Table 1.

TABLE 1

SUMMARY OF CORRELATION COEFFICIENTS**		
Body component	Water Sp. Gr.	Air Sp. Gr.
% fat	-0.99	-0.70
% water	0.97	0.67
% protein	0.96	0.68
% ash	0.65	0.56

**All values significant at the 1% level.

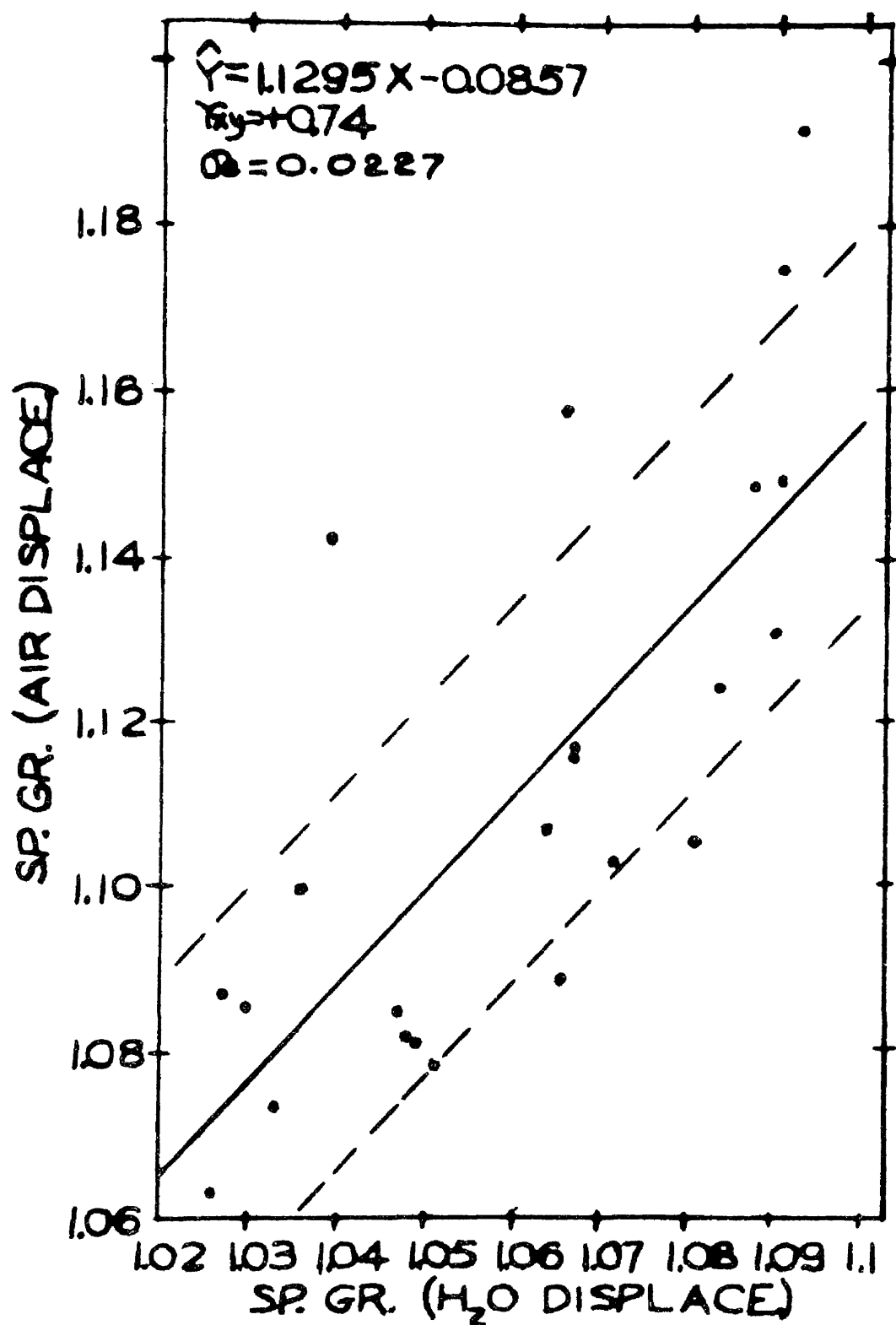


Figure 2. Regression line of specific gravities as determined by water and air displacements - Experiment 1.

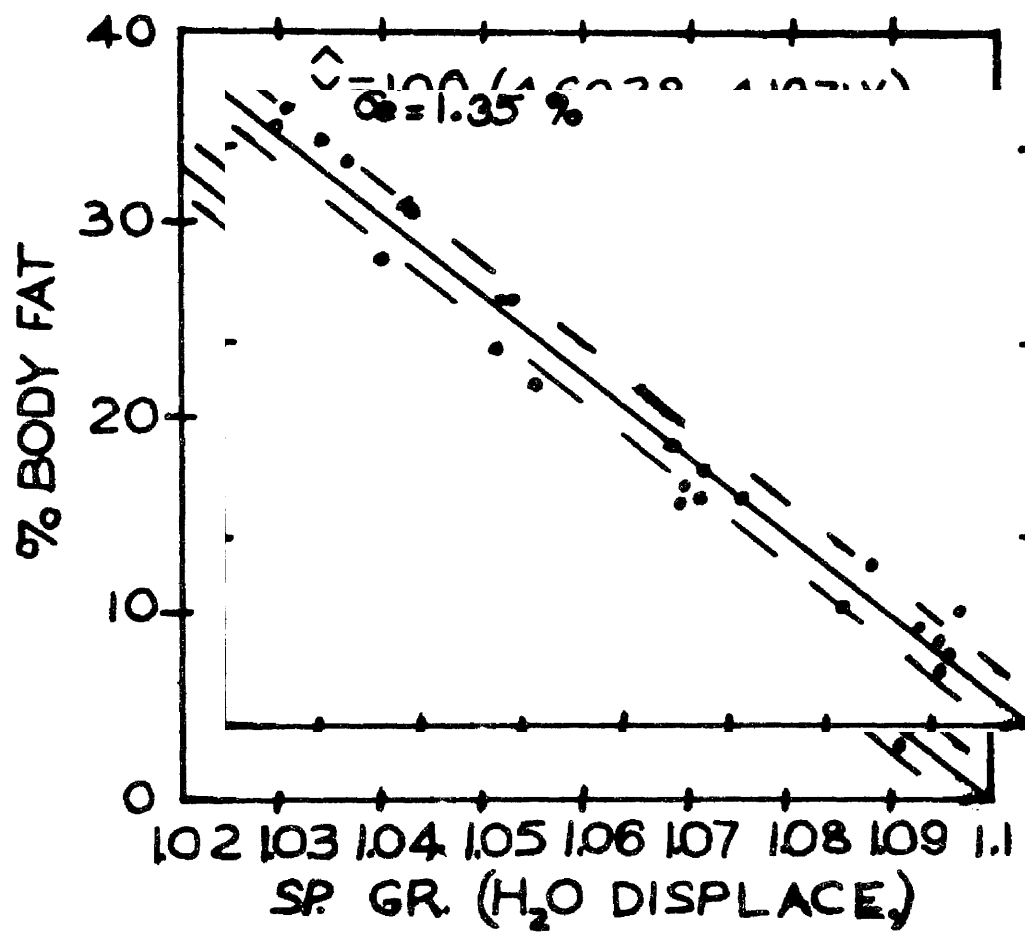


Figure 3. Regression line of water specific gravity and % body fat - Experiment 1.

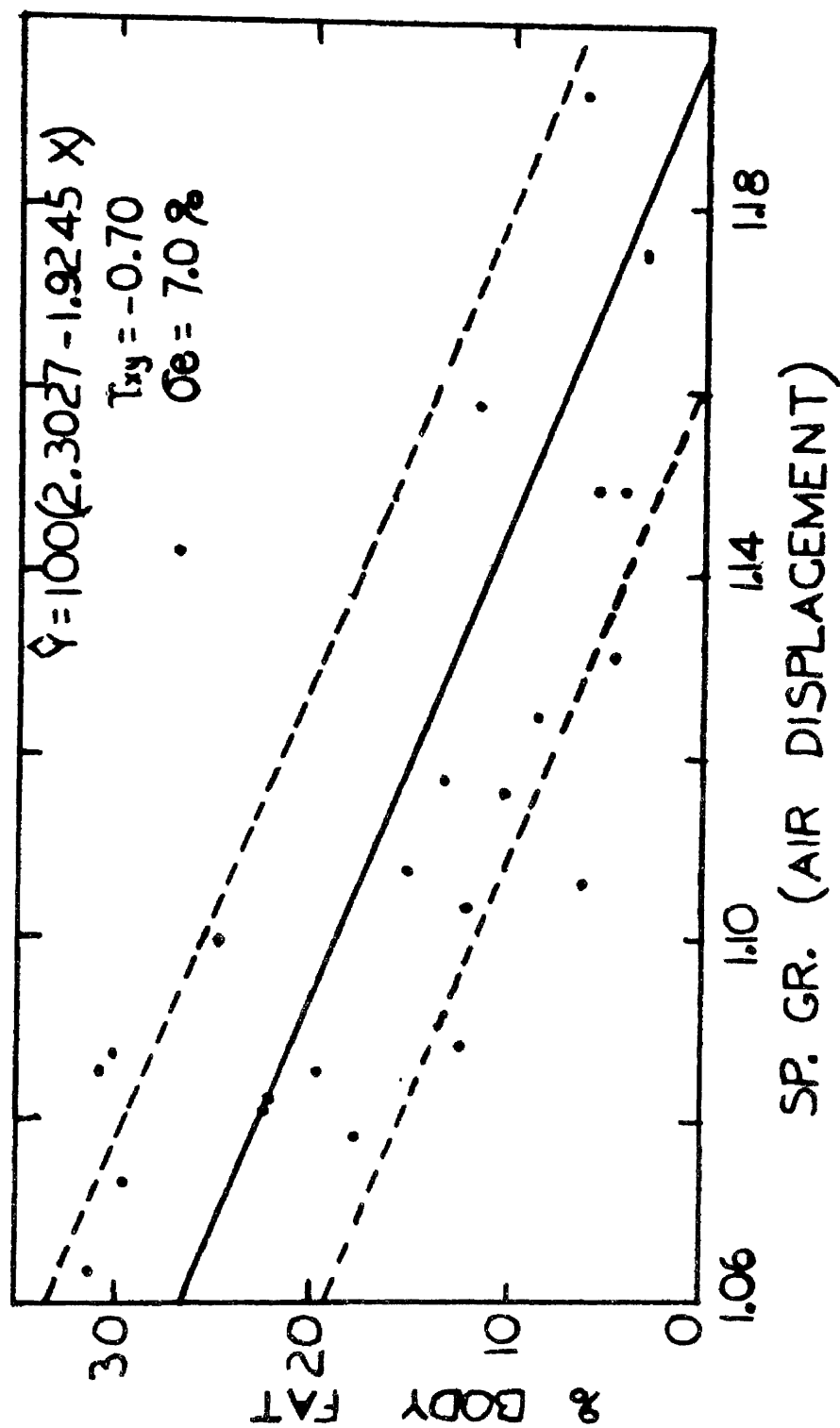


Figure 4. Regression line of air specific gravity and % body fat - Experiment 1.

It is evident from these results that body water, protein, and ash as well as fat bear a significant relationship to specific gravity as determined by both methods. The variations in air specific gravity accounted for approximately 49, 45, 46 and 31 per cent of the variation in fat, water, protein and ash, respectively.

A comparison of the relationships of specific gravity obtained by the water displacement method with those obtained by air specific gravity thus show the former method was more closely correlated with each chemical component. It is apparent that the air displacement method was less accurate. However, in the interpretation of these results, exact agreement should not be expected between the air and water methods since the air method is based on the intact animal, which includes all viscera and contents of the digestive tract, whereas the water displacement method must necessarily be made on the eviscerated carcass. In the air displacement method, pressure changes would be distributed uniformly throughout the chamber and respiratory tract of the animal, thereby correcting for the air spaces of the lungs, trachea and nasal passages automatically. Since it is impossible to make such corrections with the water displacement method, the eviscerated carcass was used. Although the work of Behnke et al. (3) has verified the relationship between water specific gravity and chemical components, only a limited amount of work using a small number of animals has been reported on the air displacement method and body components (15, 16).

Another possible source of variation would arise from differences in the amount of both solids and gases in the digestive tract

of the living animal. An attempt was made to limit this variation by depriving the experimental animal of food and water prior to the determination.

The influx of warm air from the animal chamber into the evacuation chamber before recording P_2 , caused a measurable temperature change in the latter chamber. However, temperature variations in the animal chamber are of little importance since this chamber is left open to the atmosphere except for 30 seconds required for equalization. In order to estimate improvement in accuracy obtained by correcting V_2 and V_3 for temperature, correlation coefficients were calculated for water and air specific gravities with volumes corrected and uncorrected for temperature. The correlation coefficient for the specific gravity calculated from volumes corrected for temperature was significant at the 1% level (0.74); however, when not corrected the correlation (0.43) was significant at only the 5% level. Therefore, it is apparent that correcting volumes for temperature resulted in greater accuracy.

A further source of variation would enter into the measurement, if the degree of saturation of air in the chamber were not controlled. This variable was eliminated by keeping the air saturated in both chambers. It might be questioned whether changes in gas volume due to respiratory gas exchange would introduce significant errors. Inasmuch as the animal chamber was connected into the closed system for only about 30 seconds to obtain P_2 , the calculated error due to this source would not exceed 0.8 ml. in animals with normal metabolic rate and R.Q. in the usual range.

Table 2 shows a comparison of three methods of measuring per cent fat: determined ether extract and values calculated from the regression equations of water specific gravity-per cent carcass fat and air specific gravity-per cent carcass fat. The mean fat values were 16.13, 16.11 and 16.13 per cent, respectively. An analysis of variance indicated no significant difference between methods but as expected, a large variation occurred within methods. The correlation coefficients for these comparisons were the same as for the per cent fat and specific gravity values determined by both methods.

The body volumes of the guinea pigs were calculated from the true volume of the empty animal chamber (2613.5 ml.) in an effort to more accurately correlate the two specific gravity methods. Normally, V_2 values used for these calculations were determined by air displacement prior to each set of V_3 determinations. The true volume of the empty animal chamber was determined by filling the chamber with water and measuring its amount. Appendix Table C shows the values thus obtained for air displacement specific gravities. A correlation coefficient of 0.53 was obtained between these air specific gravity values and those obtained by water displacement. This coefficient is significant only at the 5% level whereas the coefficient obtained when the individually determined V_2 values were employed was significant at the 1% level (0.74). Therefore, indications are that the volume of the empty animal chamber should be determined by air displacement prior to each set of V_3 determinations.

TABLE 2
COMPARISON OF % FAT DETERMINED BY CHEMICAL MEANS AND
FROM REGRESSION EQUATIONS

G.P. No.	Ether extract of eviscerated carcass	% Fat from:	
		Water Sp. Gr. of eviscerated carcass ¹	Air Sp. Gr. of live animal ²
1	31.24	30.74	25.66
2	24.94	26.64	18.59
3	30.83	29.32	21.44
5	29.72	27.94	23.75
6	17.71	20.40	22.89
7	15.17	15.09	17.18
8	30.22	30.41	21.06
9	11.79	14.20	7.39
11	27.05	25.47	10.53
14	6.35	7.92	17.46
15	12.50	13.95	20.92
16	13.47	13.53	15.34
17	3.45	3.36	4.08
18	4.20	3.53	8.99
19	10.23	13.70	15.40
20	8.70	6.67	13.96
21	22.47	21.32	22.31
22	19.92	21.87	21.39
23	4.79	3.78	12.51
24	5.49	4.70	9.22
25	22.43	21.41	22.04
26	12.02	11.65	17.92
27	6.39	2.90	0.91
Means	16.13	16.11	16.13

Summary of Analysis of Variance				
	DF	SS	MS	F
Total	68	5002.55		
Between fats	2	.01	0.005	< 1
Within fats	66	5002.54	75.790	

¹% fat calculated from the regression expression--

% fat = 100 (4.6038-4.1871X).

²% fat calculated from the regression expression--

% fat = 100 (2.3027-1.9245X).

The first of 4 replicates of V_3 for each animal was consistently higher than the following 3 volumes in this experiment. The average of the 23 first V_3 values was 1948.3 ml. as compared to 1921.4, 1921.5 and 1921.3 ml., respectively, for the succeeding three. A "t value" of 4.87 indicates the difference between the first and subsequent values to be significant at the 1% level. It is possible that this may be due to the partial removal of gases from the animal's blood, body fluids or digestive tract as a result of exposure to negative pressure. Once this increment of gas was removed, it apparently was not restored appreciably during the time between measurements, as evidenced by the close agreement between the 3 succeeding replicates. Therefore, the first value was discarded in determining the average volume of the chamber. In 37 average volumes obtained for the unoccupied animal chamber over a period of 4½ months, the standard error was ± 3.14 ml. or 0.12% of the chamber volume.

Experiment 2

A summary of results obtained when comparisons were made between negative pressures of 120 and 220 mm. of Hg. is shown in Table 3. The gases removed from the animal during its initial exposure to vacuum were calculated by subtracting from the first V_3 value an average of the subsequent three values. The total gases removed for the 12 determinations under 120 mm. was 223.7 ml. while under a vacuum of 220 mm. of Hg. a total of 247.6 ml. were removed. The average amount of gas removed per determination was 18.6 and 20.6 ml., respectively. These values indicate that the increase in negative

TABLE 3
COMPARISON BETWEEN VACUUMS OF
120 AND 220 MM. OF MERCURY

Gases Removed ¹		Animal Volumes ¹	
At 120 MM	At 220 MM	At 120 MM	At 220 MM
10.0	27.6	649.0	640.6
9.1	38.8	685.2	673.0
-9.4	7.4	731.5	703.0
112.3	12.1	629.4	639.6
62.7	15.5	780.1	787.4
28.4	6.0	851.4	830.2
-3.7	23.1	570.5	561.3
9.0	2.7	702.3	663.7
-4.2	16.4	967.4	748.3
5.6	9.0	712.1	685.0
-0.1	97.5	796.4	792.2
4.0	-3.1	873.9	861.4
Total=	223.7		**
Av. =	18.6		

$$r_{xy} = +0.84$$

V ₂ Results ¹		
	At 120 MM	At 220 MM
$\bar{x}$ =	2648.0	2599.2
S $\bar{x}$ =	± 13.3	± 1.8
t =	3.63**	

V ₃ Results ¹					
Guinea pig no.	$\bar{x}$	At 120 MM S $\bar{x}$	$\bar{x}$	At 220 MM S $\bar{x}$	
28	2009.6	± 13.6	2006.4	± 12.7	
29	1953.7	5.9	1945.9	5.3	
30	1878.9	8.8	1875.5	7.1	
31	1819.5	27.8	1937.5	9.9	
32	1819.5	10.0	1816.7	11.9	
33	1744.0	9.7	1750.6	7.8	

¹ reported as milliliters.

**significant at the 1% level.

pressure resulted in an increase of gases removed from the animals on the first V_3 determination, however, this average increase of 2.0 ml. per animal is not significant. The correlation coefficient of 0.84 indicates that there is a significant relationship in the animal volumes obtained by both negative pressures. The average V_2 values were 2648.0 and 2599.2 ml. for 120 and 220 mm. of Hg., respectively. There was a significant difference at the 1% level. The standard deviations of the means were ± 13.3 and ± 1.8 ml., respectively. It is apparent from these results that the lower vacuum was responsible for a greater volume variation for V_2 . As was suggested previously, the level of vacuum was thought to be too low to cause a substantial difference in pressures between the two chambers. Table 3 also shows the relationship of V_3 averages in 6 guinea pigs when subjected to both negative pressures. It can be seen that the average values differed little for the same animals at the two pressures. This close relationship between the two pressures is also observed in the standard deviation of the means. The close relationship between V_3 values at the two pressure levels and the large variation of the V_2 values under these conditions support the theory that a vacuum of 120 mm. of Hg. was not high enough to accurately measure the volume of the empty animal chamber. However, when part of this volume was occupied by the animal, the smaller pressure value measured the volume of the chamber as well as the higher negative pressure.

The comparisons of V_2 values obtained under a vacuum of 120 and 220 mm. of Hg., employing the constant volume technique, are summarized below in Table 4.

TABLE 4
COMPARISON OF V_2 VALUES USING
CONSTANT VOLUME TECHNIQUE

Test	Condition	Vacuum mm. of Hg.	Av. V_2 ml.	$S\bar{x}$	"t" value
1	variable vol.	120	2645.1	3.39	7.82**
	variable vol.	220	2613.4	2.21	
2	variable vol.	120	2648.0	13.31	3.63**
	variable vol.	220	2599.2	1.82	
3	variable vol.	120	2639.7	1.77	0.67
	constant vol.	120	2637.6	2.58	
4	constant vol.	120	2642.7	2.01	0.93
	constant vol.	220	2640.4	1.43	

**significant at the 1% level.

Tests 1 and 2 indicate a significant difference between V_2 values determined at 120 and 220 mm. of Hg. when the constant volume technique was not employed. In slight contrast to these results, no difference was observed in test 3 when V_2 values were determined at a vacuum of 120 mm. of Hg. with and without constant volume. Theoretically, to support the results of tests 1 and 2, a significant difference should have been evident. No explanation is offered for these contradictory results. When both negative pressures were employed at constant volume, no significant difference was observed in V_2 values as is revealed by the results in test 4. The results obtained in tests 1, 2, and 4 justified the continued use of the constant volume technique in an effort to overcome the "dead-space" change in the manometer tube.

The results obtained for air and water specific gravities are shown in Appendix Table D. The specific gravity ranges were from 1.028 to 1.1071 for water displacement and 1.021 to 1.1439 for air displacement. The correlation coefficient for these two measurements was 0.64, which is lower than the 0.74 value obtained in Experiment 1. This decline in r_{xy} value was attributed to the fact that the air body volumes of the first seven animals were determined in an environment of minimum air saturation. A vacuum of only 120 mm. of Hg. had been used to draw the saturated air into the system. The remaining animals, however, were determined under a maximum saturation, for a vacuum of 320 mm. of Hg. had been employed to draw the saturated air into the chambers. When the specific gravities of animals 37 through 62 were correlated, the r_{xy} value was equivalent to that obtained in Experiment 1 (0.74). These results indicated the necessity for maximum saturation of the air in the evacuation chamber to compensate for the vapor tension in the animal chamber.

It had been suspected that body volumes would be more accurate if the V_2 used was a constant figure rather than an average of four V_2 values determined at each trial. To justify these assumptions, all body volumes determined by air displacement were calculated from a constant V_2 value, 2642.7 ml. This value was the V_2 mean obtained from 32 air displacement determinations over a period of one week. When the air specific gravity, determined from these body volumes, was correlated with the water specific gravity, the r_{xy} value rose from 0.74 to 0.83, as is shown in Table 5. To verify

the accuracy of this standard V_2 value, 52 additional V_2 values were determined. The mean of these values was 2645.2 ml. or 2.5 ml. more than the 2642.7 ml. used for the determinations of body volumes. The "t value" between these means was not significant ($t = 0.98$). Thus, it may be concluded that a V_2 value of 2642.7 ml. was an accurate estimate of the volume of the empty animal chamber when employing a vacuum of 120 mm. of Hg. under constant volume conditions. Subsequently, all air specific gravity values were derived from body volumes which were calculated from this standard V_2 value. The regression equation plotted in Figure 5 for the two specific gravity methods was $Y = 1.3462X - 0.3733$ with a standard error of the estimate of 0.015.

Appendix Table F shows the percent body fat, water, protein and ash for animals 37 through 62. The regression equation for water specific gravity and percent body fat was $Y = 100 (4.4702 - 4.0540X)$. The standard error of the estimate was 1.52% fat and the r_{xy} value was -0.97. On Figure 6 is plotted the regression equation for air specific gravity and percent body fat. This expression is $Y = 100 (2.3679 - 2.0956X)$. The standard error of the estimate was 3.77% fat and the correlation coefficient was -0.82.

Table 5 shows a summary of correlation coefficients for Experiments 1 and 2. It is evident from these results that it was advantageous to employ the constant volume technique of manometric measurements at reduced negative pressures. All of the r_{xy} values in Experiment 2 were considerably higher than those of Experiment 1 and approach the accuracy of the various methods now employed in determining fatness of live animals.

TABLE 5

SUMMARY OF CORRELATION COEFFICIENTS**

A. Comparison of Specific Gravities-% Body Composition of Eviscerated Animals.

Body component	Water Sp. Gr.		Air Sp. Gr.	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
% Fat	-0.99	-0.97	-0.70	-0.82
% Water	0.97	0.95	0.67	0.81
% Protein	0.96	0.87	0.68	0.72
% Ash	0.65	0.69	0.56	0.72

B. Comparison of Water and Air Specific Gravities.

Expt. 1	$V_2 = 2642.7$	Expt. 2	
		$V_2 = \text{Individual means}$	
		#29-62	#37-62
0.74	0.83	0.64	0.74

C. Comparison of Eviscerated and Whole Animal with Air Specific Gravity.

	Eviscerated animal	Whole animal
% Fat	-0.79	-0.73
% Water	0.78	0.61

**All values significant at the 1% level.

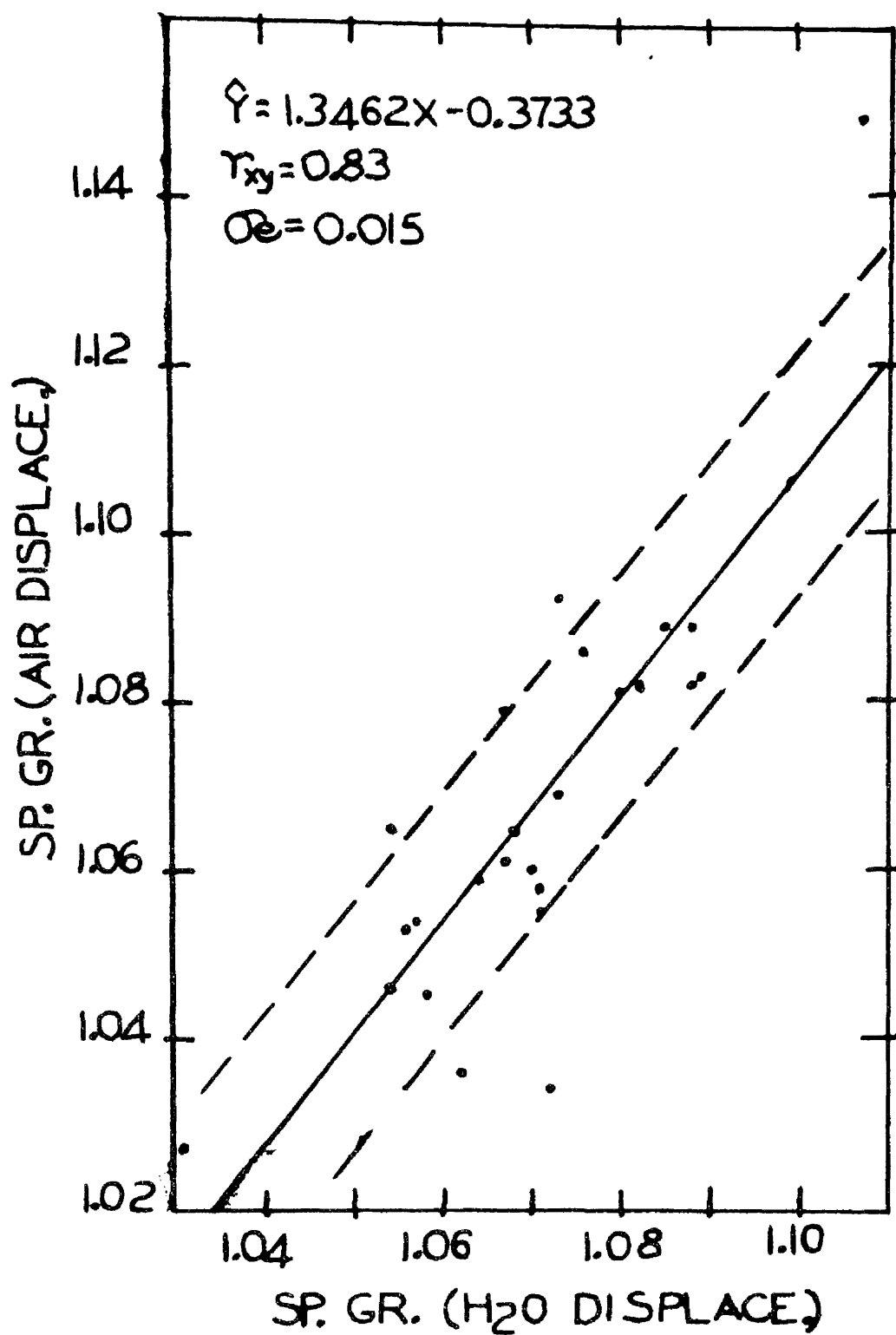


Figure 5. Regression line of specific gravities as determined by water and air displacements - Experiment 2.

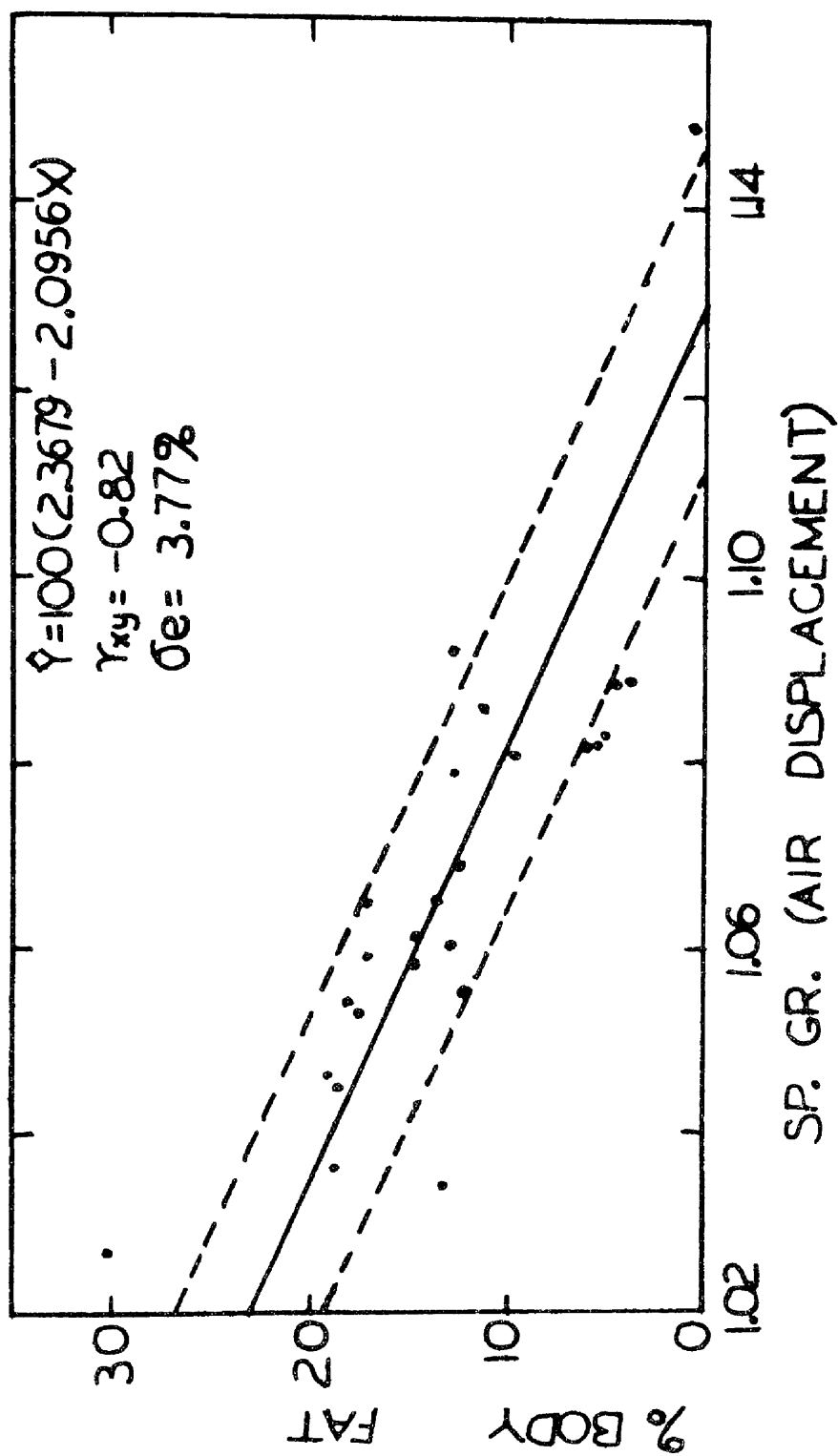


Figure 6. Regression line of air specific gravity and % body fat - Experiment 2.

Table 5 also shows the r_{xy} values obtained when air specific gravity values of 23 guinea pigs were correlated with the percent body fat and water of the carcasses on the eviscerated and whole animal basis (Appendix Table G). It was observed that the r_{xy} values on the whole animal basis for percent body fat and water (-0.73 and 0.61, respectively) were slightly lower than those values obtained with the eviscerated carcass (-0.79 and 0.78, respectively). Thus, the composition of the viscera did not appear to be an additional source of error in the air displacement method, otherwise the relationship of specific gravity and percent fat on the whole body basis would have been higher than that for specific gravity and percent fat of the eviscerated carcass. This is in accordance with the results of Rathbun and Pace (34) using the water specific gravity method. The correlation coefficient of the percent fat of the whole animal and percent fat of the eviscerated carcass was 0.95. This r_{xy} value is only slightly lower than Rathbun and Pace's (34) value of 0.99. A contributing factor for this small difference may be that in this experiment the uneliminated fecal material was analyzed with the viscera, whereas Rathbun and Pace used the clean empty viscera. The inclusion of fecal material was necessary in this experiment because body volume determined by air displacement could be influenced by the contents in the gastro-intestinal tract.

In reviewing the preceding results, we may conclude the following:

- (1) satisfactory measurement of the body volume of living animals can be obtained by the air displacement technique;

- (2) there is an advantage in using the constant volume technique of manometric measurements;
- (3) a reduced negative pressure of 120 mm. of Hg. may be drawn on the evacuation chamber when this constant volume technique is employed;
- (4) it is more accurate to use a constant V_2 value obtained as a mean of many determinations than employing the mean of four individual volumes;
- (5) a maximum saturated air inflow in the evacuation chamber is desirable;
- (6) the viscera and gastro-intestinal contents do not significantly influence the specific gravity values determined by air displacement under the conditions of this experiment;
- (7) factors yet undetermined prevent a higher correlation between air specific gravity and chemical composition of the eviscerated carcass;
- (8) the method reported here is much faster and less tedious than the techniques reported by previous investigators (4, 14, 15, 24, 30, 31);
- (9) the specific gravity values obtained have been found to be closely correlated with body composition.

SUMMARY

A technique was developed for the determination of body volumes of live animals by air displacement. The apparatus constructed for this determination consisted of 2 desiccator jars of approximately 2600 ml. capacity, which were connected to each other, to a mercury manometer, to a vacuum pump and to a spirometer by a suitable arrangement of stopcocks. The spirometer was used to saturate incoming air with water vapor to avoid changes in vapor pressure. Temperature changes in the evacuation chamber were measured with a thermistor.

Two experiments were reported in which body volumes were determined at two levels of negative pressure. In the first experiment, specific gravity of 23 adult female guinea pigs, calculated from body volumes determined at a total vacuum of 320 mm. of Hg., was correlated with carcass fat, water, protein and ash. The correlation coefficients were -0.70, 0.67, 0.68 and 0.56, respectively. In the second experiment, body volumes of 25 female guinea pigs were determined at a total vacuum of 120 mm. of Hg., employing a constant volume method of manometric measurement. Correlation coefficients for air specific gravities thus obtained and carcass fat, water, protein and ash were -0.82, 0.81, 0.72 and 0.72, respectively. Variations due to the contents of the digestive tract and composition of the viscera did not significantly alter the relationship between air specific gravity and carcass composition. Two additional modifications in the technique which increased the

accuracy of the method were the employment of a constant V_2 value for calculation of body volumes and a maximum inflow of saturated air in the evacuation chamber.

APPENDIX

APPENDIX TABLE A
SUMMARY OF BODY VOLUME DATA¹

G.P. no.	Live wt.	Air Displacement		Water Displacement		Animal volume	Animal Sp. Gr.
		Animal volume	Animal Sp. Gr.	Carcass air wt.	Carcass H ₂ O wt.		
1	914.9	860.5	1.0632	722.2	18.14	703.80	1.0261
2	792.9	720.9	1.0999	629.1	21.80	607.30	1.0359
3	904.2	833.3	1.0851	708.8	20.36	688.44	1.0295
5	927.5	864.3	1.0731	738.1	23.48	714.62	1.0328
6	857.0	795.3	1.0776	651.5	31.49	620.01	1.0508
7	756.4	683.1	1.1073	598.0	35.69	562.31	1.0635
8	1064.1	978.8	1.0871	835.1	21.92	813.18	1.0269
9	674.6	582.5	1.1581	517.5	31.87	485.63	1.0656
11	774.7	678.5	1.1418	622.1	23.21	598.89	1.0387
14	620.9	561.5	1.1058	479.0	35.72	443.28	1.0806
15	737.1	677.6	1.0878	559.1	34.72	524.38	1.0662
16	682.5	611.1	1.1168	536.5	33.78	502.72	1.0672
17	604.1	514.0	1.1753	456.5	38.28	418.22	1.0915
18	507.4	441.3	1.1498	389.0	32.49	356.51	1.0911
19	799.1	715.8	1.1164	559.0	35.03	523.97	1.0668
20	567.2	504.6	1.1240	427.2	32.95	394.25	1.0836
21	869.8	804.9	1.0806	683.4	31.66	651.74	1.0486
22	850.0	783.1	1.0854	653.7	29.54	624.16	1.0473
23	535.2	473.0	1.1315	409.2	33.95	375.25	1.0905
24	675.4	588.0	1.1486	498.2	40.42	457.78	1.0883
25	908.6	839.7	1.0820	667.1	30.79	636.31	1.0484
26	756.8	685.9	1.1034	500.9	33.52	467.38	1.0717
27	558.7	468.8	1.1918	413.9	35.10	378.80	1.0926

¹All weights expressed in grams and all volumes in milliliters.

APPENDIX TABLE B
CHEMICAL COMPOSITION OF
EVISCERATED CARCASSES

G.P. no.	% Body fat	% Water	% Protein	% Ash	% Total
1	31.24	52.38	14.99	2.11	100.72
2	24.94	54.76	16.16	4.17	100.03
3	30.83	52.15	14.86	2.55	100.39
5	29.72	53.66	14.25	2.40	100.03
6	17.71	63.91	15.48	2.88	99.98
7	15.17	62.99	18.44	3.77	100.37
8	30.22	52.43	14.13	2.38	99.16
9	11.79	67.64	17.26	3.69	100.38
11	27.05	54.24	15.56	2.94	99.78
14	6.35	71.01	19.38	3.80	100.54
15	12.50	65.65	18.83	3.26	100.24
16	13.47	64.32	18.31	3.67	99.77
17	3.45	73.30	19.03	4.24	100.02
18	4.20	68.11	20.59	7.50	100.40
19	10.23	69.45	17.39	3.55	100.62
20	8.70	68.59	19.14	3.19	99.62
21	22.47	58.63	16.57	2.45	100.12
22	19.92	60.51	15.89	3.57	99.89
23	4.79	72.75	19.49	4.22	101.25
24	5.49	71.70	20.07	3.53	100.79
25	22.43	57.98	16.39	3.05	99.85
26	12.02	66.39	18.06	3.76	100.23
27	6.39	70.27	19.97	3.93	100.56
				Average	100.21

APPENDIX TABLE C
ANIMAL SPECIFIC GRAVITY DETERMINED
BY TRUE EMPTY ANIMAL CHAMBER VOLUME¹

G.P. no.	V ₃ value	Body volume	Animal wt.	Air Sp.Gr.
1	1739.5	874.0	914.9	1.0468
2	1883.8	729.7	792.9	1.0866
3	1762.9	850.6	904.2	1.0630
5	1735.8	877.7	927.5	1.0567
6	1811.4	802.1	857.0	1.0684
7	1923.7	689.8	756.4	1.0965
8	1619.5	994.0	1064.1	1.0705
9	2014.1	599.4	674.6	1.1254
11	1910.2	703.3	774.7	1.1015
14	2056.1	557.4	561.5	1.0073
15	1940.0	673.5	677.6	1.0061
16	1991.7	621.8	682.5	1.0976
17	2088.8	524.7	604.1	1.1513
18	2165.2	448.3	507.4	1.1318
19	1890.7	722.8	799.1	1.1056
20	2100.2	513.3	567.2	1.1050
21	1799.9	813.6	869.8	1.0691
22	1826.7	786.8	850.0	1.0803
23	2136.8	476.7	535.2	1.1227
24	2010.6	602.9	675.4	1.1202
25	1758.9	854.6	908.6	1.0632
26	1916.9	696.6	756.8	1.0864
27	2134.0	479.5	558.7	1.1652

¹V₂ = 2613.5 ml.

APPENDIX TABLE D

AIR AND WATER SPECIFIC GRAVITIES¹

G.P. no.	Water Sp. Gr.	Air specific gravity			
		Regular V_2		$V_2 = 2642.7$	
		Sp. Gr.	Body vol.	Sp. Gr.	Body vol.
29	1.0892	1.1368	599.8	1.1385	598.9
30	values discarded - leaking chambers.				
31	1.0509	1.0924	784.4	1.0924	784.4
32	1.0280	1.0423	992.3	1.2797	808.2
33	1.0525	1.0975	763.8	1.0975	763.8
34	1.0879	1.0960	459.1	1.0960	459.1
35	1.0893	1.1000	553.4	1.1000	553.4
36	1.0771	1.0840	783.8	1.0840	783.8
37	1.0545	1.0539	937.0	1.0465	943.6
38	1.0567	1.0621	881.8	1.0542	888.4
39	1.0762	1.0875	612.6	1.0861	613.4
40	1.0558	1.0537	928.6	1.0528	929.4
41	1.1071	1.1439	448.0	1.1486	446.2
42	1.0645	1.0558	640.3	1.0588	638.5
43	1.0803	1.0791	670.1	1.0810	668.9
44	1.0309	1.0265	1251.0	1.0275	1249.8
45	1.0667	1.0584	728.4	1.0610	726.6
46	1.0707	1.0548	697.1	1.0576	684.7
47	1.0582	1.0464	895.6	1.0449	896.8
48	1.0708	1.0569	612.6	1.0548	613.8
49	1.0735	1.0562	842.0	1.0690	831.9
50	1.0622	1.0210	716.6	1.0356	706.5
51	1.0668	1.0842	702.5	1.0793	705.7
52	1.0895	1.0877	670.8	1.0826	674.0
53	1.0885	1.0763	614.2	1.0818	611.1
54	1.0877	1.0823	505.0	1.0890	501.9
55	1.0699	1.0556	763.5	1.0604	760.0
56	1.0733	1.0864	650.6	1.0923	647.1
57	1.0852	1.0865	583.4	1.0893	581.9
58	values discarded - ovarian tumor.				
59	1.0539	1.0766	849.7	1.0653	858.7
60	1.0719	1.0732	692.9	1.0344	718.9
61	1.0683	1.0441	812.1	1.0652	796.0
62	1.0824	1.0534	615.7	1.0817	599.6

¹ Air specific gravities of nos. 29 thru 36 were determined under 120 mm. Hg. of saturated air, whereas 37 thru 62 were determined under 320 mm. Hg. of saturated air.

APPENDIX TABLE E
 BODY VOLUME DATA FOR WATER DISPLACEMENT METHOD¹

G.P. no.	Live wt.	Carcass air wt.	Carcass H ₂ O wt.	Animal volume
29	681.86	504.10	41.30	462.80
31	856.88	629.45	30.50	598.95
32	1034.25	795.42	21.68	773.74
33	838.24	617.30	30.79	586.51
34	503.19	390.95	31.58	359.37
35	608.75	458.00	37.53	420.47
36	849.65	519.85	37.23	482.65
37	987.51	551.95	28.54	523.41
38	936.60	673.85	36.17	637.68
39	666.21	507.78	35.96	471.82
40	978.50	602.80	31.89	570.91
41	512.49	389.39	37.66	351.73
42	676.05	533.16	32.32	500.84
43	723.11	532.52	39.57	492.95
44	1284.16	820.50	24.66	795.84
45	770.93	551.20	34.44	516.76
46	724.15	556.83	36.76	520.07
47	937.15	564.93	31.07	533.86
48	647.44	511.16	33.79	477.37
49	889.34	660.36	45.22	615.14
50	731.67	571.06	33.44	537.62
51	761.69	559.03	35.03	524.00
52	729.65	515.84	42.36	473.48
53	661.09	472.73	38.45	434.28
54	546.57	407.57	32.87	374.70
55	805.93	515.13	33.66	481.47
56	706.82	545.31	37.25	508.06
57	633.85	458.03	35.98	422.05
59	914.75	678.34	34.74	643.60
60	743.66	548.87	36.85	512.02
61	847.94	574.49	36.74	537.75
62	648.57	462.46	35.22	427.24

¹All weights are expressed in grams and all volumes in milliliters.

APPENDIX TABLE F
CHEMICAL COMPOSITION OF EVISCERATED CARCASSES

G.P. no.	% Body fat	% Water	% Protein	% Ash	% Total
37	19.08	61.19	16.40	3.18	99.85
38	18.15	61.92	16.64	3.83	100.54
39	11.39	67.99	17.90	3.54	100.82
40	17.71	61.33	16.71	2.93	98.68
41	0.74	74.61	19.60	5.20	100.15
42	17.00	62.63	17.66	3.25	100.54
43	9.93	65.12	17.68	3.71	96.44
44	30.35	53.97	13.85	2.27	100.44
45	14.69	65.06	17.61	2.95	100.31
46	14.91	63.62	18.34	3.66	100.53
47	18.72	62.30	16.49	2.75	100.26
48	12.18	66.59	17.58	3.23	99.58
49	12.49	68.52	15.89	3.16	100.06
50	18.99	62.02	16.25	2.85	100.11
51	12.99	66.71	17.32	2.79	99.81
52	5.22	72.22	19.52	2.97	99.93
53	5.86	73.02	17.85	3.23	99.96
54	3.83	71.44	20.11	4.09	99.47
55	12.95	66.07	17.32	3.45	99.79
56	12.76	66.76	17.80	3.94	101.26
57	4.70	72.33	18.48	4.87	100.38
59	17.42	63.35	16.50	2.88	100.15
60	13.34	64.89	17.36	3.43	99.02
61	13.73	66.21	17.14	2.99	100.07
62	5.90	71.32	18.95	3.11	99.28
Average =					99.89

APPENDIX TABLE G
PERCENT FAT AND WATER OF EVISCERATED
AND WHOLE CARCASSES

G.P. no.	% Fat		% Water	
	Eviscer.	Whole	Eviscer.	Whole
37	19.08	12.23	61.19	71.88
38	18.15	13.23	61.92	69.86
39	11.39	10.09	67.99	70.04
40	17.71	11.47	61.33	72.35
41	0.74	0.66	74.61	78.11
42	17.00	16.39	62.63	66.09
43	9.93	8.47	65.12	71.36
45	14.69	9.21	65.06	72.68
46	14.91	10.78	63.62	70.21
48	12.18	10.95	66.59	70.50
49	12.49	9.88	68.52	73.60
50	18.99	14.89	62.02	67.85
51	12.99	10.02	66.71	72.50
52	5.22	4.23	72.22	77.81
53	5.86	4.99	73.02	76.56
54	3.83	3.02	71.44	76.03
55	12.95	8.83	66.07	74.14
56	12.76	10.45	66.76	71.23
57	4.70	4.17	72.33	75.97
59	17.42	13.56	63.35	68.77
60	13.34	9.54	64.89	72.17
61	13.73	10.24	66.21	72.34
62	5.90	4.58	71.32	76.77

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