SPECIFIC GRAVITY OF HUMAN SUBJECTS AS DETERMINED BY AIR DISPLACEMENT AND HELIUM DILUTION PROCEDURES

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ABSTRACT

SPECIFIC GRAVITY OF HUMAN SUBJECTS AS DETERMINED BY AIR DISPLACEMENT AND HELIUM DILUTION PROCEDURES

By Veldon Max Hix

Specific gravity was determined for 24 men and 24 women using the air displacement and helium dilution techniques of measuring body volume.

The air displacement procedure consisted of enclosing the subjects in an air tight chamber of known volume and subjecting them to a known reduction in pressure. Body volume was then computed from the resulting pressure changes according to a rearrangement of the gas laws of Boyle and Charles. The specific gravity values for men ranged from 1.0611 to 1.2192 with a mean of 1.1311 and from 1.0232 to 1.2452 with a mean of 1.1233 for women. The major errors associated with the air displacement method occurred as a result of changes in temperature and relative humidity due to the differential between ambient and body temperatures.

The helium dilution procedure consisted of enclosing the subject in a chamber of known volume and injecting and mixing a known amount of helium in the air around the subject. The resulting helium concentration in the chamber was proportional to the body volume of the subject. Helium concentration was determined by means of a thermal conductivity cell and attached potentiometer.

The specific gravity values obtained by helium dilution ranged from 1.0323 to 1.2370 with a mean of 1.1223 for men and from 1.0439 to 1.2535 with a mean of 1.1238 for women. The major sources of error in the helium dilution method were due to the variation in temperature and relative

humidity and the accumulation of carbon dioxide within the subject chamber. These conditions resulted in difficulty in calculating the helium concentration. Another source of error was the difficulty of obtaining consistent, day to day adjustments of the power supply.

The correlations between specific gravity values by the two methods were 0.964 for men and 0.912 for women. Both of these correlations were highly significant and indicated that there was excellent agreement between the values obtained by each method. Although the actual specific gravity values were rather high, in most cases they appeared to be closely related to the physical stature of the subjects.

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INTRODUCTION

The *in vivo* measurement of human body composition is becoming increasingly important in both clinical studies and biological research. While many of the techniques and procedures for determining body composition have been known for many years, it is only in recent years that they have been successfully applied to human beings. One of the most important of these methods has been the determination of composition from the specific gravity or density of the body. Thus, a great deal of research has been done in an attempt to find a simple, rapid and accurate means of determining the specific gravity of the human body.

Behnke and others (1942) used water displacement or underwater weighing, which they reported to be fairly indicative of composition. The main disadvantage with this method is that it requires the subject to be immersed in water, which is not practical in all cases. In addition, error is introduced by the presence of air in the lungs and body cavities.

In an attempt to find a more accurate procedure, Siri (1955) developed an apparatus to measure body volume by helium dilution. This apparatus appeared to work quite well and good results were obtained using it with human subjects.

In 1962, Gnaedinger used a modification of the Siri apparatus in combination with an air displacement technique to measure the body volume of hogs. He reported poor results due to the activity of the hogs, but suggested that the apparatus might work well for human subjects, where the level of activity could be controlled.

This study was undertaken to determine the usefulness of the air displacement and helium dilution techniques for determining the specific gravity of human subjects and to correlate the specific gravity values obtained by the two methods.

REVIEW OF LITERATURE

Specific Gravity by Air Displacement

An early review of specific gravity measurements by Spivak (1915) indicates that the measurement of body volume by air displacement had its beginning about 80 years ago. According to Spivak (1915), Jaeger was the first to use this principle to determine body volume. He used a Kopp volumeter which was constructed so that its volume could be changed by a known amount. A volume determination was made by placing a subject of unknown volume (Vo) in a chamber of known initial volume (V) at atmospheric pressure (P). The volume of the chamber was then decreased by a known amount (ΔV) and Vo was computed from the resulting pressure change (ΔP) using the following equation:

$$Vo = \frac{(V - \Delta V)\ VP}{(P + \Delta P)} \qquad (1)$$

The procedure was reported to be quite accurate for inert objects, but resulted in a number of errors if live subjects were used. These errors occurred as a result of changes in pressure due to gaseous exchange or increases in air temperature.

Pfaundler (1916) constructed a felt insulated, brass chamber (25 cm. wide and 55 cm. long) with a removable door, which could be closed to form an air tight seal. The apparatus was fitted with a thermometer and a sulfuric acid manometer, which was accurate to 0.1 mm. Pfaundler (1916) subsequently used this device to measure the volume of child cadavers. The body was placed in the chamber immediately after death and allowed to remain until temperature equilibrium was reached. After reading the manometer and thermometer, a positive pressure was introduced into the chamber and the manometer was read again. A negative pressure was then introduced and the manometer reading was made a third time. The net body volume was reported to be the average of the volumes determined by utilizing positive and negative pressures. Small temperature differences were noted, but were regarded as insignificant. One of the major disadvantages of this procedure was that it required over 1 1/2 hours to complete a single determination. This feature alone would severely limit its usefulness with live subjects.

In 1929, Pfleiderer constructed a large chamber of known volume, into which a measured amount of compressed air could be introduced. He reasoned that the increase in chamber pressure would be inversely proportional to the amount of free air in the chamber, which in turn would be inversely proportional to the volume of the subject. This procedure required less time than that of Pfaundler (1916), but was still too time consuming. Some difficulty was also experienced with pressure fluctuations caused by gaseous exchange. Pfleiderer reported a mean error of 1-2% in determining body volume by this method. He suggested that the error in reading the manometer could be reduced by using higher pressures. He also suggested that a smaller subject to chamber volume ratio would reduce the total error in computed volume.

Kohlrausch (1929) used the method of Pfleiderer (1929) to find the body volume of dogs. He modified the procedure slightly by injecting air into the chamber at atmospheric pressure instead of using compressed air. Specific gravity values of 1.046 to 1.074 were reported for the dogs, although only four animals were used. The precision of the measurements was not reported; however, the method was reported to be highly accurate for determining the volume of inert objects.

Bohnenkamp and Schmäh (1931) measured the body volume of human subjects by a procedure similar to that of Kohlrausch (1929). The method was modified by using pure oxygen instead of air. The oxygen was injected into the chamber and body volume was calculated from pressure differences determined with and without the subject in the chamber. These workers reported that the gas present in the lungs and intestines was not included as a part of the body volume by this method. The chamber was kept saturated to minimize the effects of vapor pressure, and corrections were made for temperature changes. Average density values for human beings were 1.096 for males and 1.070 for females.

Noyons and Jongbloed (1938) criticized the procedure of Bohnenkamp and Schmäh (1931) because exact temperatures, water vapor content, and oxygen consumption were not recorded. They also stated that the procedure was impractical to carry out due to excessive calculations. In a subsequent investigation, these authors calculated body volume from the difference between two weighings at different pressures. They applied this technique to cats, which were weighed at atmospheric pressure, and again under negative pressure. The difference in weight was corrected for losses due to insensible perspiration and was then equal to the increase in weight due to the increased pressure of air. The weight increase gave a value for body volume and density.

In an additional experiment, Noyons and Jongbloed (1938) applied their technique to human subjects. They modified the procedure slightly by using positive pressures, which were reportedly more comfortable for the subjects. Results of twenty determinations on the same subject over a period of 2 weeks showed a mean density of 1.080 ± 0.007.

The major problems encountered with many of the methods mentioned herein include the effects of gradual changes in relative humidity, temperature, and composition of the respiratory gases. To correct for these difficulties, Wedgewood and Newman (1953) proposed a means for imposing a sine wave of changing volume on these variables. Unfortunately, details of the apparatus and procedure are not available and results have not been published.

Liuzzo (1958) constructed an apparatus to measure the body volume of guinea pigs by employing negative pressures. It consisted of two desiccator jars of known volume, each of which could be interconnected or separately connected to the atmosphere. One jar was used as a standard and was hooked to a vacuum pump, so that it could be partially evacuated. A U-tube mercury manometer was used to record pressure. The animal was placed in one jar and a measured negative pressure was drawn on the standard jar. The jars were then interconnected and when equilibrium was reached, the pressure was recorded. The differences in the two pressure readings was proportional to the free air space in the animal chamber. Body volume was calculated from the following equation:

$$V_o = V_2 - \frac{(P_1 - P_2)}{P_2} V_1 \frac{273}{273 + \Delta T} \qquad (2)$$

where: P_1 = initial pressure of standard chamber

P_2 = pressure of system with jars interconnected

V_1 = volume of standard chamber

V_2 = volume of animal chamber

ΔT = temperature change in standard chamber

Using this method, specific gravity values calculated from body volume were significantly correlated with percentages of various body components (fat, water, protein, ash) as determined by chemical analysis.

In an attempt to apply the method of Liuzzo (1958) to larger animals, Gnaedinger (1960) determined the specific gravity of 26 male human beings by air displacement. He compared these results with those obtained by underwater weighing. In this study a standard chamber of 178 liter capacity was used with a subject chamber of 460 liter capacity. The procedure was similar to that of Liuzzo (1958) except that the air in the evacuation chamber was kept dry by the use of calcium chloride. Also, corrections were made for changes in the temperature of both chambers. The vapor pressure of water was calculated from temperature and relative humidity readings and served to correct for the amount of water vapor expired by the subject while inside the chamber. Specific gravity values ranged from 1.045 to 1.167. Although these values were not significantly correlated with similar values obtained by underwater weighing, they appeared to be generally related to the fatness of the subject. The major problem with the air displacement method was the difficulty in obtaining precise measurements of volume from day to day. Also, the uncertainty of obtaining representative measurements of temperature caused some difficulty.

Gnaedinger (1962) later used the same apparatus to determine the body volume of 24 market weight hogs. To improve the accuracy of making pressure readings in this study, a cistern-type, rising-stem mercury manometer was used. No corrections were made for relative humidity or vapor pressure due to the inaccuracy of the sensing device (± 2% of full scale). The following equation was used to calculate body volume:

$$V_o = V_2 - V_1 \frac{\frac{P_s^1}{T_s^1} - \frac{P_s^o}{T_s^o}}{\frac{B\,P}{T_a^o} - \frac{P_a^1}{T_a^1}} \qquad (3)$$

where: V_1 = volume of standard chamber

V_2 = volume of empty animal chamber

T_a^o = temperature of animal chamber before equilization

T_a^1 = temperature of animal chamber after equalization

T_s^o = temperature of standard chamber before equalization

T_s^1 = temperature of standard chamber after equalization

P_s^o = pressure of standard chamber before equalization

$P_s^1 = P_a^1$ = pressure of system after equalization

BP = barometric pressure (740 mm)

Three measurements (Gnaedinger, 1962) were made on each animal and the average was taken as apparent volume. In order to minimize the day to day variations in measuring volumes, the volume of the empty animal chamber was calculated each day. The specific gravity values obtained on hogs by this procedure ranged from 0.975 to 1.222, with a mean value of 1.075 and were non-significantly correlated with percentages of carcass components (fat, water, protein and ash). An estimation of errors was performed on the equation used and the maximum error for a single determination was found to be 0.72%. The major source of error in this procedure was thought to be the activity of the animals in the chamber. Such activity produced greater variations in temperature and relative humidity than would normally be expected. Also, some error was undoubtedly caused by the inconsistency of day to day volume determinations.

Specific Gravity by Helium Dilution

The inherent difficulties and the lack of conclusive results with the air and water displacement procedures prompted a study of other methods. The first of these was the helium dilution technique. This method is based on the fact that when pure helium is injected into a chamber, it will be diluted in proportion to the amount of free air in the chamber. Thus, body volume can be calculated from the following relationship:

$$\text{Volume of subject} = \text{Volume of empty chamber} - \frac{\text{Volume of gas added}}{\text{Final concentration of gas}} \qquad (4)$$

Walser and Stein (1953) first used this procedure to determine the body volume of 10 cats. They also determined volume by underwater weighing and compared the results. They injected a known quantity of helium into a dessicator jar containing the animal. After allowing time for the helium and air to come to equilibrium, a sample of air was removed and analyzed for helium on a Cambridge Analyzer. Body volume was then calculated from the above equation (equation 4). No corrections were made for changes in temperature and relative humidity in the animal chamber; however, sufficient time was allowed for thermal equilibrium to be reached before the helium was injected. Results obtained by this method compared favorably with those obtained by underwater weighing of the carcasses after removal of the lungs. The results showed a mean difference of 0.013 in specific gravity between the two methods.

Helium was used as the diluent gas because it is inert, diffuses rapidly through the air, and is the least soluble of the gases. Walser and Stein (1953) also reported that 98% equilibration of helium with alveolar air takes place in 4 minutes.

The most significant contribution to the determination of body volume by helium dilution was made by Siri (1955). He constructed a detailed apparatus, which he subsequently used to determine the body volume of human subjects. This apparatus consisted of a chamber with a capacity of approximately 413 liters, a helium metering system, and a thermal conductivity cell (used to measure helium concentration). With this apparatus, Siri was able to measure the volume of inert objects with a standard deviation of ± 0.028 liters. The specific gravity values obtained by this method on a heterogeneous group of men and women ranged from 0.990 to 1.076. Siri also performed a detailed estimation of the magnitude of error which could be tolerated in each detail of design, in order to measure body volume of live subjects with a standard deviation of ± 0.1 liter. He stated that the probable error for a single measurement of body volume would be ± 0.13 liters.

Gnaedinger (1962) used an apparatus modeled after that of Siri and adapted it to the measurement of body volume of pigs. The apparatus consisted of an animal chamber of 460 liter capacity, a helium metering system, and a helium analyzer. The helium analyzer was made up of a thermal conductivity cell, a power supply and a recording potentiometer. Thermistors were used to record temperatures. The method required the calibration of the apparatus between two reference volumes, which covered the expected range in volume for all subjects. The deflection obtained on the recorder was calculated for each reference volume, and the volume of the animal was calculated from the following equation:

$$V_o = \frac{V_2 R_2 (R_o-R_1) - V_1 R_1 (R_o-R_2) + v(S_o-S_1)(R_2-R_1) - v(S_2-S_1)(R_o-R_1)}{R_o (R_2-R_1)} \quad (5)$$

where: V_o = volume of subject

V_1 = volume of reference 1

V_2 = volume of reference 2

v = volume of helium used

R_o = observed deflection with animal in chamber

R_1 = computed deflection with V_1

R_2 = computed deflection with V_2

$S_o = R_o/\gamma$

$S_1 = R_1/\gamma$

$S_2 = R_2/\gamma$

The value for γ was calculated from the temperature and relative humidity of the animal chamber and the temperature of the helium. A plot of deflection versus helium concentration was assumed to be linear, and the following regression equation was used for computing the deflection from helium concentration at each reference volume:

$$R_i = 245.2\, C_i + 89.1 \quad (6)$$

Specific gravity values obtained (Gnaedinger, 1962) were related, but non-significantly correlated to various carcass components determined by chemical analysis. It was reported, however, that the helium dilution method was more predictive of the body composition of hogs than was the air displacement procedure used in conjunction with it. The major problem associated with the helium dilution method was reported to be the varied activity of the animals in the chamber. This activity caused marked changes in temperature, relative humidity and respiratory rates. The increased respiration rate of the more active animals resulted in an abnormal accumulation of carbon dioxide in the chamber. This lowered the thermal conductivity of the gas mixture passing through the thermal conductivity cell, and resulted in a lower value for body volume.

Gnaedinger (1962) suggested that subsequent work be done using anesthesized animals, or perhaps human beings, so that the level of activity could be controlled. He also recommended that the air in the subject chamber be kept saturated to minimize differences in relative humidity. He further suggested that the ambient temperature should be maintained near the body temperature of the subjects to eliminate changes in temperature within the chamber.

Fomon et al. (1962) also used a modification of the Siri (1955) apparatus to determine the body volume of infants. They used a steel chamber with a lucite hood, which had a capacity of 30,395 ml. A gas buret was used to meter exactly 350 ml. of helium into the chamber. Two identical pumps circulated the sample and reference gases through a thermal conductivity cell at a rate of 100 ml. per minute. Carbon dioxide and water were removed from the gases entering the cell by the use of soda lime and magnesium perchlorate. A voltmeter was used to measure the imbalance of a Wheatstone bridge hookup. A spirometer was used to measure the change in volume of the system due to alterations in temperature and the partial pressure of gases. The apparatus was calibrated with aluminum blocks of known volume and the following equation was derived:

$$K = \frac{(He)}{R - R_Z} \qquad (7)$$

where: K = increase in concentration of helium in the chamber per unit of response of the recorder

R = observed response of recorder

R_Z = recorder response at zero partial pressure of helium

(He) = partial pressure of helium at time of measurement

With a subject in the chamber, the helium concentration was equal to K x R. Body volume was then calculated by using equation 4. The mean error of 12 determinations on aluminum blocks of known volume was 0.42%. Specific gravity determinations on two infants made serially ranged from 1.031 at 31 days to 1.061 at 55 days of age.

Estimation of Body Composition from Specific Gravity

The specific gravity values of human subjects as determined by the above procedures have little meaning unless they are related to body composition. In a good many of the studies reported herein, the specific gravity has been determined for the purpose of predicting the fat or water content of the body. A number of studies of this kind have been carried out on various animals, but the first significant work on human subjects was done by Behnke *et al.* (1942) and Welham *et al.* (1942). They determined the specific gravity of a large number of navy men and professional athletes by water displacement. They concluded that low values for specific gravity indicate obesity, whereas, high values denote leanness. They set a specific gravity value of 1.060 as the borderline between leanness and obesity.

Behnke *et al.* (1942) also stated that the human body can be divided into a fat-free portion of more or less constant composition, and a fatty portion of variable quantity. They concluded that the fatty portion is the main factor affecting the specific gravity of the whole body.

This work has been corroborated by several researchers. Brozek (1949) determined the specific gravity of 34 young men in various stages of starvation. He computed fat content from the following equation, which is based on the relative densities of fat (0.92) and lean tissue (1.10):

$$\% \text{ fat} = 100 \left(\frac{5.548}{\text{Sp.Gr.}} - 5.044\right) \qquad (8)$$

Osserman *et al.* (1950) determined the specific gravity of 81 navy men by underwater weighing. He also determined body water by the antipyrine dilution method. He then used the equation of Brozek (equation 8) to compute body fat and derived the following equation for determing body water:

$$\% \text{ water} = 100 \left(4.317 - \frac{3.960}{\text{Sp.Gr.}}\right) \qquad (9)$$

Dupertuis (1951) also used the equation of Brozek (equation 8) and found a high relationship (r = -0.85) between the computed values and values estimated from somatotype photographs.

Siri (1953) measured the total body water and body volume of 100 normal human subjects. He derived the following relationship between body water, fat, volume, and weight based on the densities of water, fat, and lean tissue:

$$\text{Fat} = 2.66 \times \text{volume} - 0.78 \times \text{water} - 1.9 \times \text{weight} \qquad (10)$$

He stated that the empirical formulas now being used for fat and water estimation are fairly accurate over limited ranges in body composition, but are of little value for individual subjects under abnormal conditions.

EXPERIMENTAL PROCEDURE

Experimental Subjects

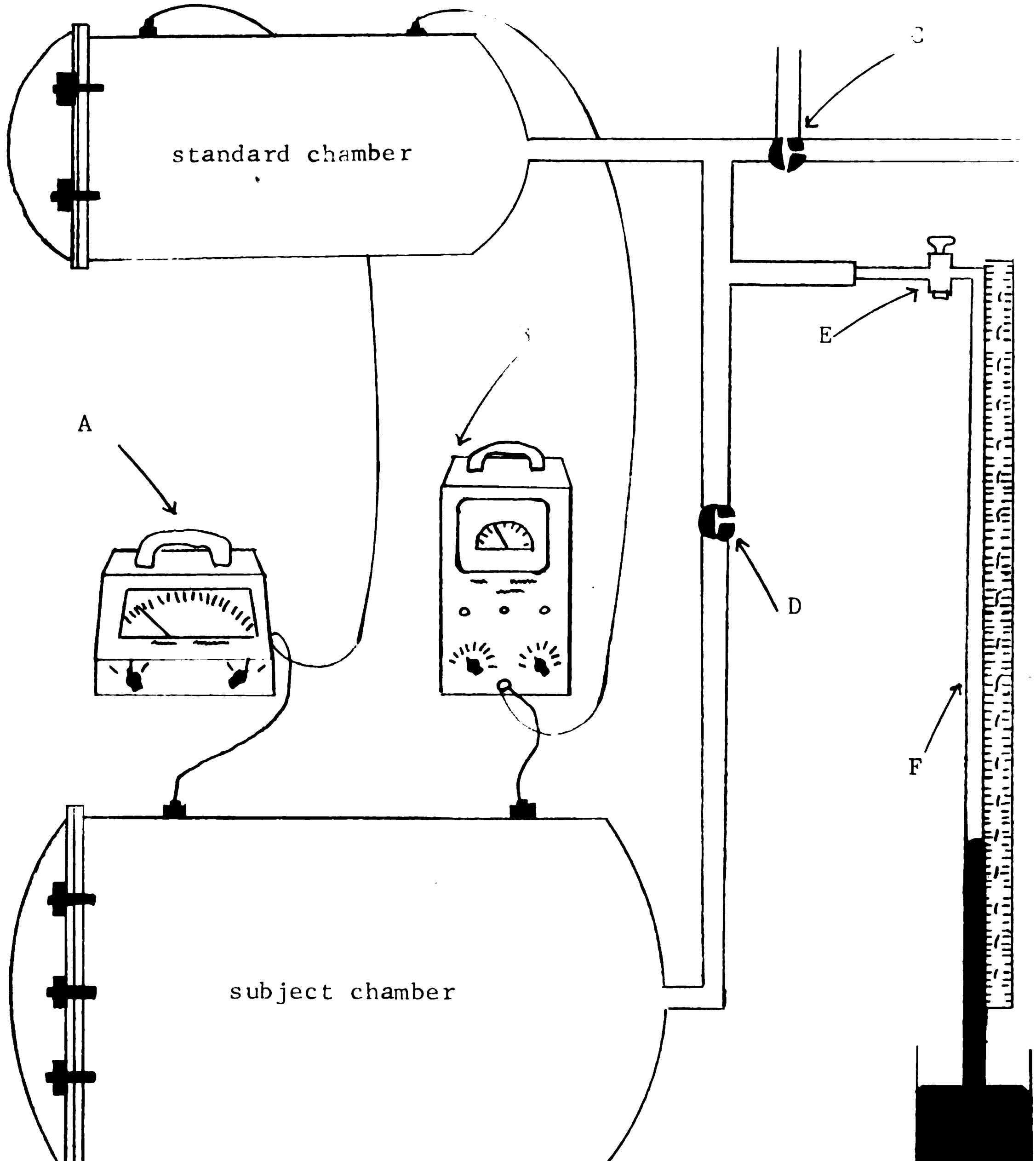
In the first part of this study, twenty-four men varying from 21 to 47 years of age were used. These subjects varied in weight with extremes ranging from 130 to 225 pounds. Each subject was clad only in a pair of light trunks for both measurements in order to minimize any differences due to wearing apparel.

In the second phase of this experiment, twenty-four women from 19 to 35 years of age were used. These subjects showed less variation in weight than the men with a range of 110 to 160 pounds. Although the subjects were asked to wear either Bermuda shorts or slacks, there was more variation in the amount of clothing worn than was true for the men.

Since the object of the experiment was to compare the two methods of determining specific gravity, no attempt was made to standardize the digestive contents of the subjects. Prior to making the determinations, the height and weight of each subject was recorded. Body volume was determined first by helium dilution and then by air displacement without opening the chamber between measurements. Specific gravity values were obtained for each subject by dividing weight by volume as determined by each method.

Determination of Body Volume by Helium Dilution

The apparatus used was essentially the same as that used by Gnaedinger (1962) for determining the body volume of live pigs. A chamber of approximately 460 liter capacity (Figure 1) was used to confine the subjects. The chamber was constructed so that it could be sealed by bolting the door



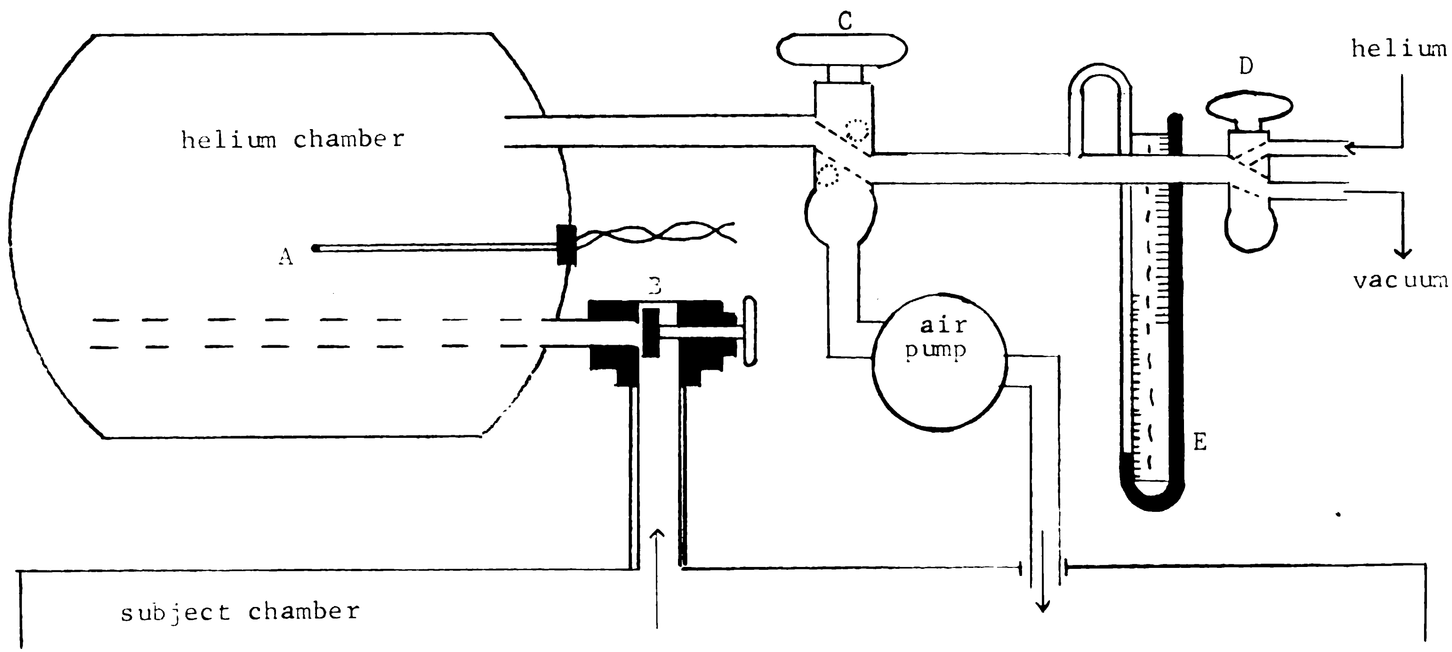
A = hygrometer
B = thermistor
C&D = 3-way valves
E = glass stopcock
F = mercury manometer, cistern type

Figure 1. Schematic of chamber arrangement.

against a rubber-covered flange. The chamber contained: (a) a squirrel cage fan for circulating the air, (b) a thermistor for recording temperature, and (c) an electric hygrometer element for measuring relative humidity.

Figure 2 shows the helium metering system, which included a propane gas tank of 22 liter capacity. The tank was arranged so that it could be connected to the subject chamber or to a vacuum pump. Suitable fittings and stopcocks were arranged so that: (a) the tank could be evacuated completely, (b) helium could be introduced into the tank, (c) the helium-filled tank could be maintained at atmospheric pressure, and (d) the helium could be injected into the subject chamber without altering its pressure. A Cenco, Hy-vac model vacuum pump was used to evacuate the chamber. A dry ice-acetone trap was utilized to remove water vapor from the air entering the pump. Helium was injected into the subject chamber and the helium-air mixture was circulated between the two chambers at a rate of 1.5 cubic feet per minute by means of an Eberbach air pump. Temperature of the helium was measured before injection by means of a thermistor mounted inside the tank.

The helium-air mixture in the subject chamber was analyzed by means of a thermal conductivity cell with an attached power supply and potentiometer. The thermal conductivity cell was a Gow-Mac Model 9737, 30-S containing 8 tungsten, type 9225, resistance filaments mounted in a brass, 2-pass T/C cell. The current to the cell was supplied by a Gow-Mac power supply, model 9999-C-1:1. The potentiometer for recording the signal from the cell was a Sargent model SR recorder, 2.5 mv.



A = thermistor
B = steam valve
E = closed end manometer
C&D = vacuum stopcocks

Figure 2. Schematic of helium metering system

Since the signal from the cell greatly exceeded the range of the recorder, an attenuating resistance circuit (Figure 3) was wired between the power supply and the cell. This served to attenuate most of the signal, leaving only the residual to be shown on the recorder. The circuit consisted of two resistance decades wired in series to give a total resistance of 10 ohms in 0.1 ohm steps. The total signal attenuated was calculated by calibrating the decades with reference to the deflection obtained on the recorder for each 0.1 ohm change in resistance. Thus, by multiplying the number of ohms required by the deflection per ohm, the total signal from the cell could be calculated in units of deflection. In this study the instrument was calibrated so that a 0.1 ohm change in resistance was equal to 65 units of deflection on a full scale of 100 units.

The thermal conductivity cell was maintained in an oil bath (Figure 4) at a constant temperature of 47 ± .01°C by means of a thermistor-actuated Sargent Thermonitor. This unit consisted of two heaters. One was a 250 watt knife-type heater, and the other a cycling 60 watt light bulb. A Lightning stirrer was used to circulate the oil in the bath. This stirrer was mounted independently of the oil bath so as to minimize vibration of the thermal conductivity cell.

Figure 5 shows the apparatus used for drawing air through the cell. The water cans (5 gallon metal reagent containers) were interconnected at the bottom so that they would have a common drainage point. Tygon tubing was used to connect the cell to the cans and to the subject chamber. In this way, a closed system was formed and air was drawn through both

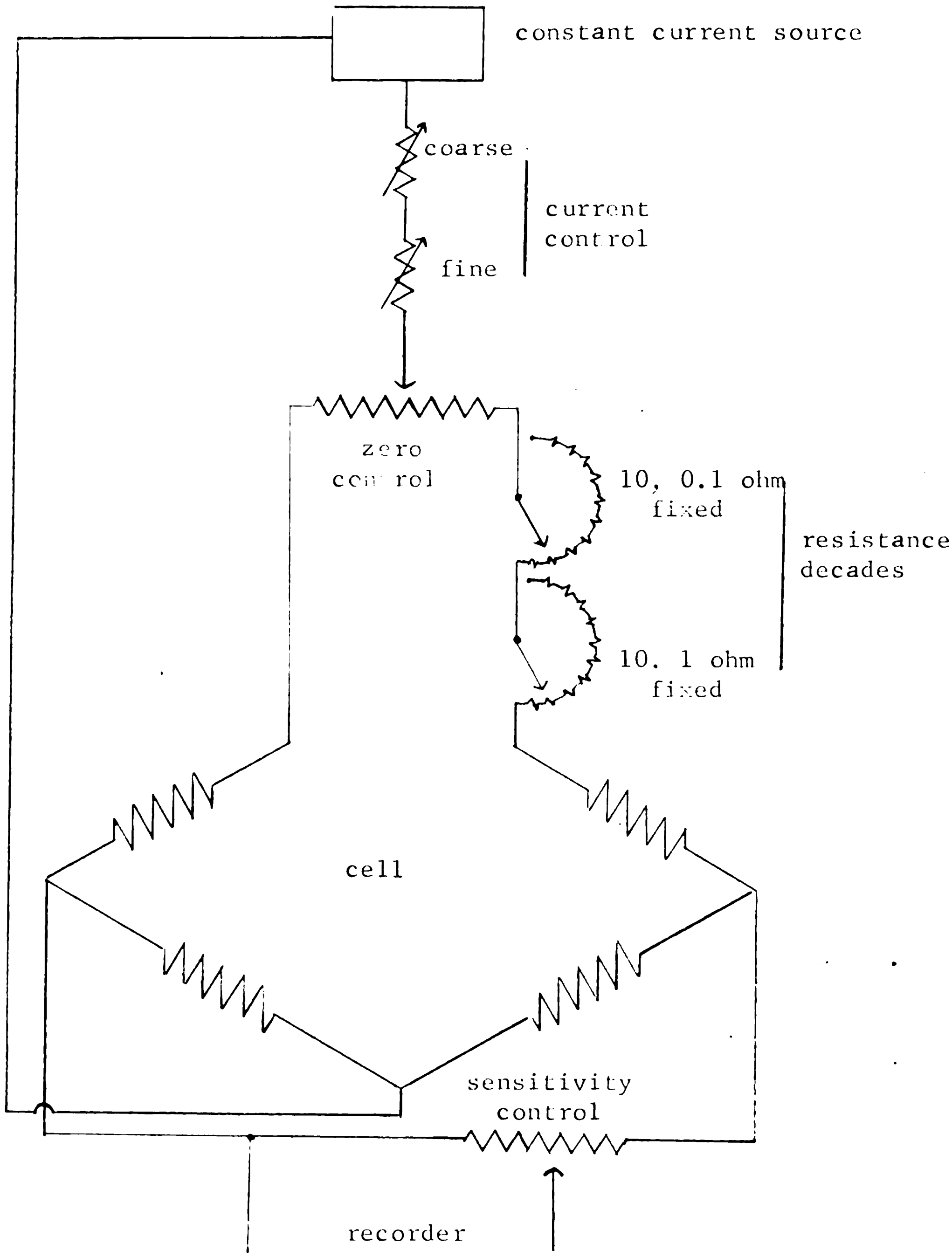
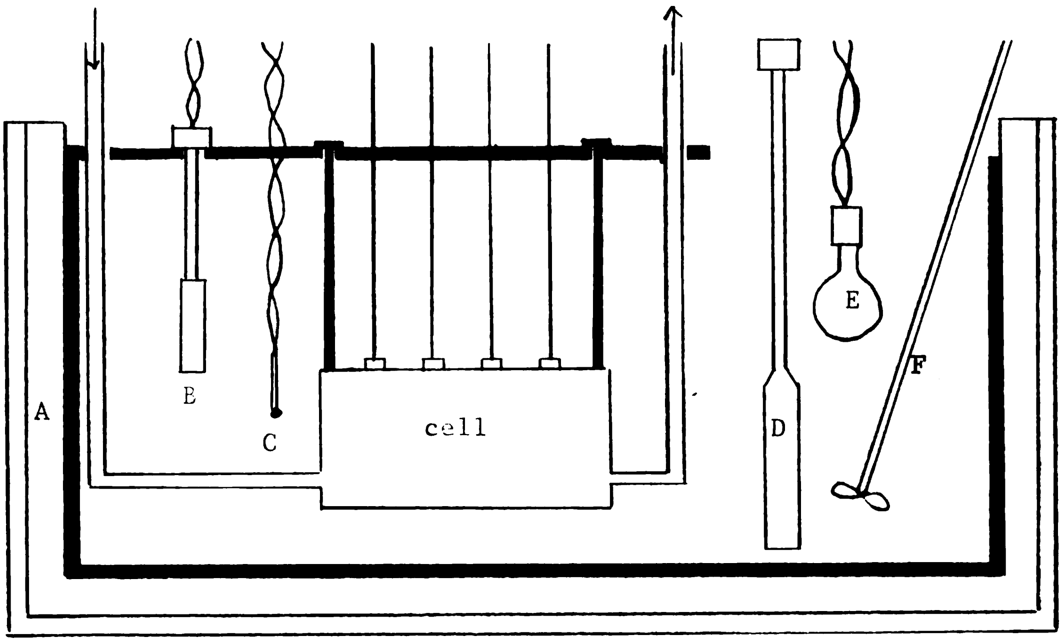


Figure 3. Schematic of helium analyzer showing location of resistance decades



A = styrofoam insulation, 1 inch
B = thermistor thermoregulator
C = thermistor
D = heater, 250 watts
E = heater, 60 watt light bulb
F = stirrer

Figure 4. Schematic of oil bath and accessories

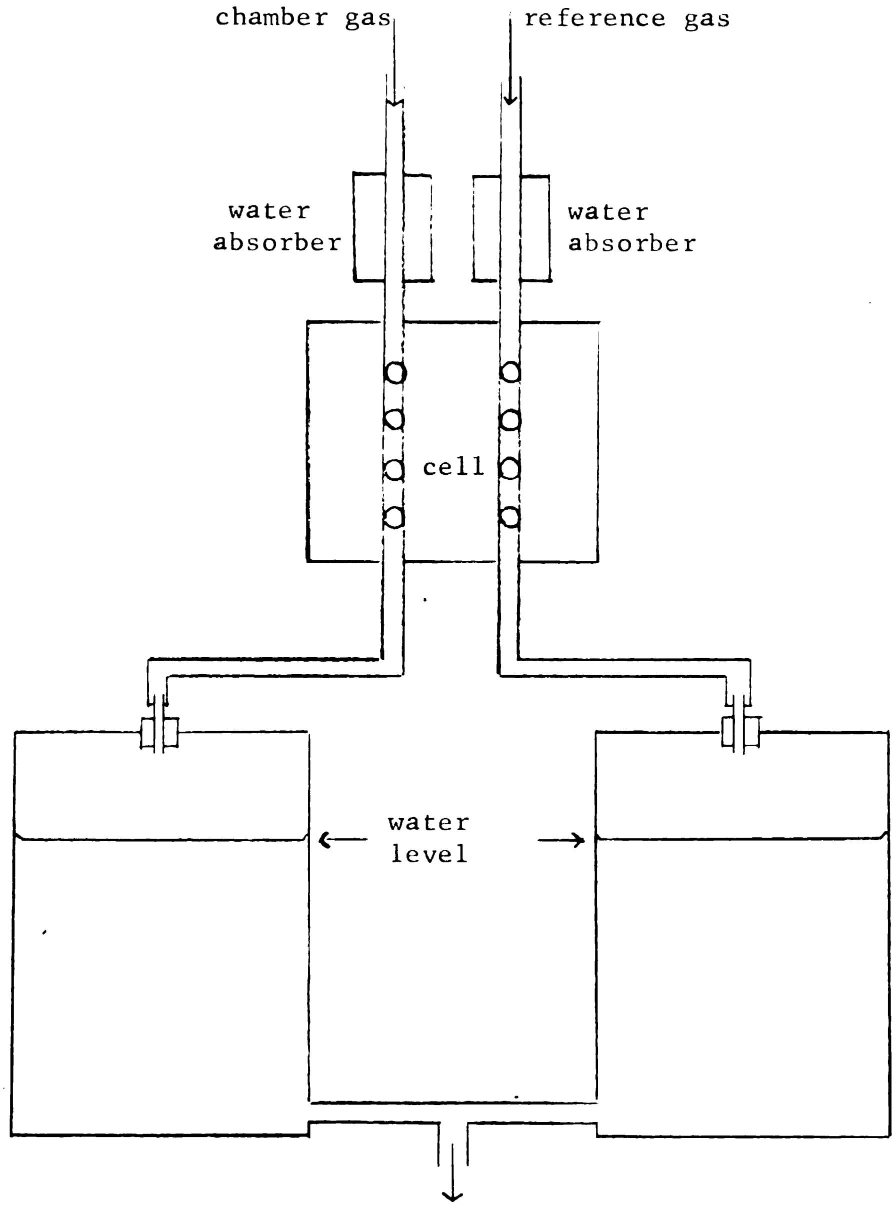


Figure 5. Schematic of gas sampling system

sides of the cell at the same rate. The flow rate through the cell varied from 700 ml. per minute at the beginning of a determination to 550 ml. per minute at the end.

Before the subject entered the chamber, the helium tank was filled by evacuating to 5 mm.of mercury, filling with helium, evacuating to 1 mm. of mercury, and again filling with helium to atmospheric pressure. This procedure was assumed to be adequate to remove all air from the chamber and fill it with pure helium.

At this point the current to the cell was adjusted to approximately 110 ma., and the recorder was zeroed with the reference gas (outside air) flowing through both sides of the cell. The subject was situated in the chamber on his back with his head toward the door. The door was bolted just tight enough to prevent excessive air leakage and still allow the chamber to remain at atmospheric pressure. At this point one side of the cell was connected with the subject chamber. This resulted in a negative deflection on the recorder, which represented the gaseous exchange due to respiration and was mainly due to the presence of carbon dioxide.

The following manipulations and recordings were then made in order: (a) temperature of the helium, (b) temperature and relative humidity of the subject chamber, (c) interconnection of helium and subject chambers with valve B (Figure 2) and injection of the helium, and (d) resistance value of the decade. The exact time when the helium injection was started was marked on the recorder chart. As the air and helium became mixed, a positive deflection was obtained. As this deflection exceeded the width of the chart, it was brought back by increasing the decade resistance.

About 4 1/2 minutes were required for the helium-air mixture to reach equilibrium throughout the chamber and the subject's respiratory passages. The equilibrium point was noted on the chart as the point where the deflection ceased to increase and started to decrease. The recording was continued for about 5 minutes beyond the equilibrium point and produced a curve of constant negative slope as shown in Figure 6. The slope was then extrapolated back to the point of helium injection, at which time the helium concentration was at a maximum. The total deflection (R_o) at the maximum point was used in computing body volume by the following equation:

$$V_o = \frac{V_2 \; R_2 \; (R_o - R_1) - V_1 \; R_1 \; (R_o - R_2)}{R_o \; (R_2 - R_1)} \qquad (11)$$

where: V_o = volume of subject

V_1 = volume of reference 1 = 62.93 liters

V_2 = volume of reference 2 = 104.80 liters

R_o = observed deflection at zero time with a subject in the chamber

R_1 = computed deflection at conditions of R_o with reference to V_1

R_2 = computed deflection at conditions of R_o with reference to V_2

In order to use this equation, the apparatus had to be calibrated between two references of known volume as shown above. Several runs were made on each reference and a plot of helium concentration versus deflection was made as shown in Figure 7. Helium concentration was computed from equations 12 and 13 below:

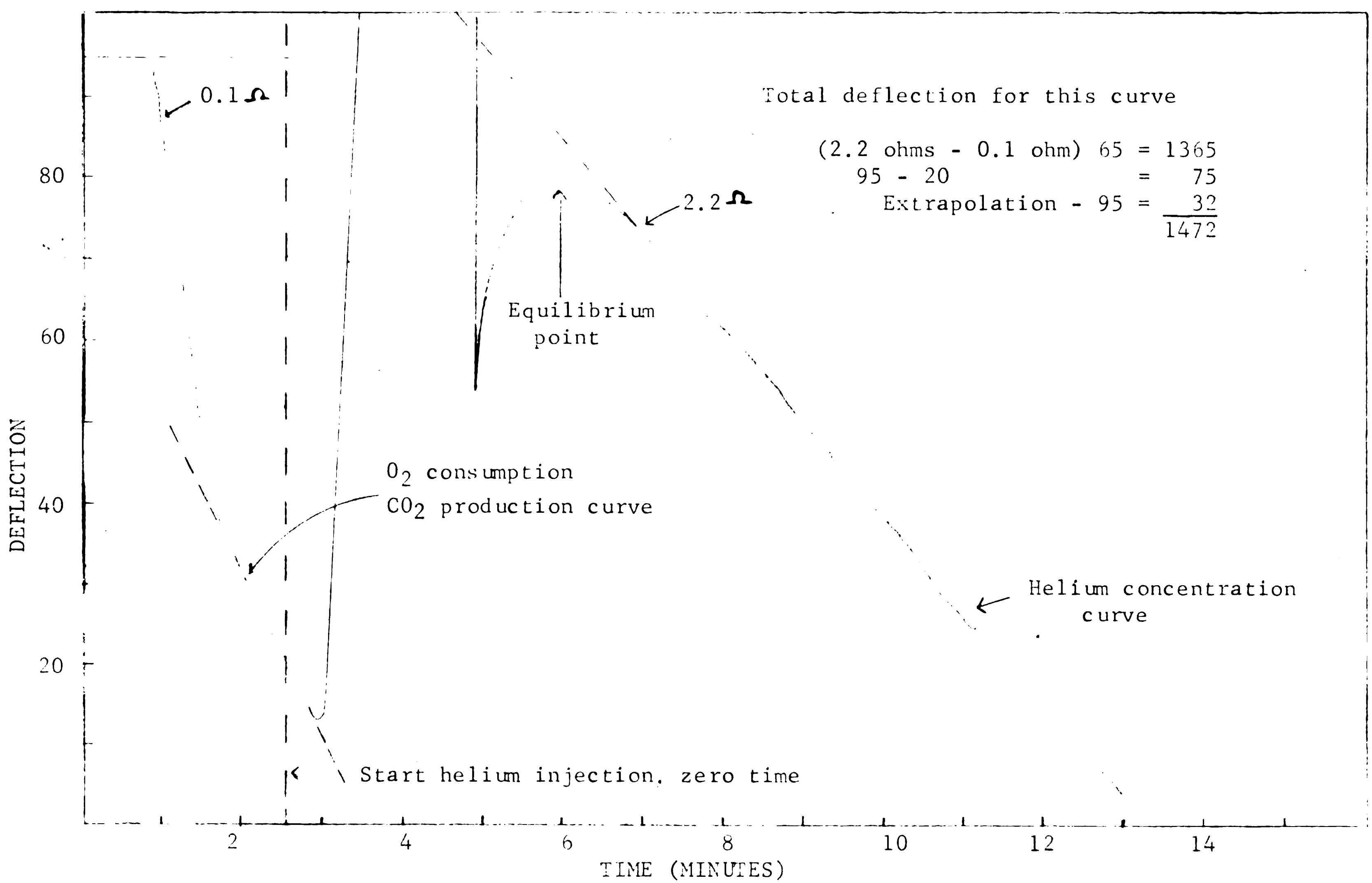


Figure 6. Schematic drawing of a typical helium concentration curve

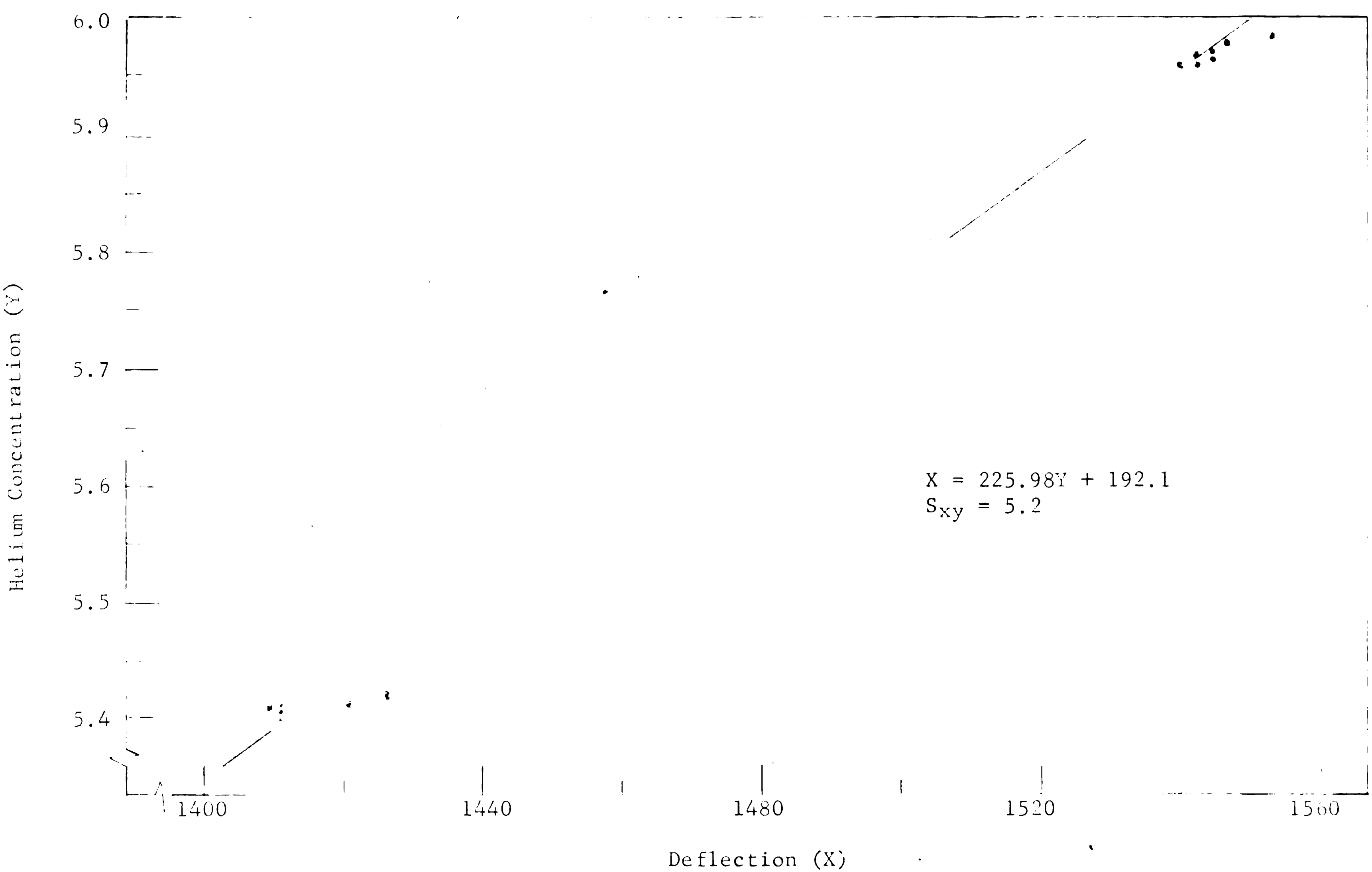


Figure 7. Graph of deflection values versus helium concentration at both reference volumes

$$C = \frac{V}{(V_c - V_i)\gamma + V} \tag{12}$$

where: V = volume of helium chamber

V_c = volume of empty subject chamber

V_i = volume of reference used

$$\gamma = \frac{T_h (P - p)}{T_c (P)} \tag{13}$$

where: T_h = temperature of helium at time of injection

T_c = temperature of subject chamber at time of mixing

P = barometric pressure (740 mm)

p = vapor pressure of water at T_c and P

From the plot of deflection versus helium concentration for the two reference volumes, the following regression equation was computed for use with live subjects:

$$R_i = 225.98\ C_i + 192.1 \tag{14}$$

From equations 12, 13, and 14 the values of R_1 and R_2 were calculated for use in equation 11.

Determination of Body Volume by Air Displacement

As with the helium dilution procedure, the apparatus (Figure 1) was the same as that described by Gnaedinger (1962). The subject chamber was the same as that described for the helium dilution procedure. The standard chamber was similar to the subject chamber except that it had a capacity of 177.8 liters. The chambers were situated so that they could be interconnected or separately connected (valves C and D, Figure 1) to the atmosphere. Each chamber contained a squirrel cage fan for circulating the air and a thermistor for measuring temperature. Pressure readings were made with a cistern-type, rising stem, mercury manometer which was calibrated to read in absolute pressure. A value of 740 mm. of mercury was used as barometric pressure.

The subject was situated in the chamber on his back as described in the helium dilution procedure. The door was then bolted and sealed, but the chamber remained connected to the atmosphere until the chambers were interconnected. A partial vacuum of approximately 345 mm. of mercury was drawn on the standard chamber by means of the vacuum pump, previously described in the helium dilution procedure. Two minutes were allowed for the temperature and pressure to equilibrate. The pressure of the standard chamber (P_s^o) was then read simultaneously with its temperature (T_s^o) by closing stopcock E (Figure 1) at the instant that the temperature was read. The temperature of the subject chamber (T_a^o) was then read simultaneously with the interconnection of the chambers with valve D. Two minutes were allowed for pressure and temperature to establish equilibrium between the chambers. Valve E was then opened momentarily to allow the pressure change to show on the manometer, and then closed simultaneously with the disconnecting of the chambers (valve D) and the reading of the temperature of the subject chamber (T_a^1). The temperature of the standard chamber (T_s^1) was then recorded along with the manometer reading (P_s^1, P_a^1). Since the chambers were allowed to come to equilibrium, the pressure in each chamber at this point was the same. No corrections were made for relative humidity since the sensing device was inaccurate (± 2% of full scale) and due to the fact that the relative humidity changed only as a result of variation in temperature. Using the above data the volume of the subject (V_o) was calculated from the following equation:

$$V_o = V_2 - V_1 \frac{\frac{P_s^1}{T_s^1} - \frac{P_s^o}{T_s}}{\frac{BP}{T_a^o} - \frac{P_a^I}{T_a^I}} \qquad (15)$$

where: V_2 = volume of empty subject chamber

V_1 = volume of standard chamber

BP = barometric pressure (740 mm)

Two measurements were made on each subject, and the average was taken as apparent volume. Since the volume of the empty subject chamber varied somewhat from day to day due to temperature variation, the volume of this chamber was determined each day. Ambient temperature was maintained above 27°C in order to minimize the temperature variation from day to day, and also served to minimize temperature changes within the chamber.

RESULTS AND DISCUSSION

Relationships between Specific Gravity Values

Results of specific gravity determinations for 24 men are shown in Table 1. The values obtained by helium dilution ranged from 1.0323 to 1.2370 with a mean of 1.1223. Air displacement values varied from 1.0611 to 1.2192 with a mean of 1.1309. Figure 8 shows that the specific gravity values obtained by air displacement for the men were higher than the corresponding helium dilution values in all but four cases. Thus, the helium dilution procedure consistently produced higher values for body volume than did air displacement.

A highly significant correlation of 0.997 was obtained between body volume measurements determined by the two methods. Specific gravity values were also significantly correlated with a "r" value of 0.964. Although the relationship between the two methods was highly significant, the differences in actual specific gravity values showed considerable variation among subjects. The differences between specific gravity values ranged from a low of 0.0009 to a high of 0.0412 with a mean difference of 0.0128.

Table 2 shows the results of specific gravity determinations on 24 women. The helium dilution values ranged from 1.0439 to 1.2535 with a mean of 1.1238, while air displacement values varied from 1.0232 to 1.2452 with a mean of 1.1233. Figure 9 shows that there was a great deal more variation in specific gravity values obtained by the two methods with women than with men, since neither method produced consistently higher or lower results.

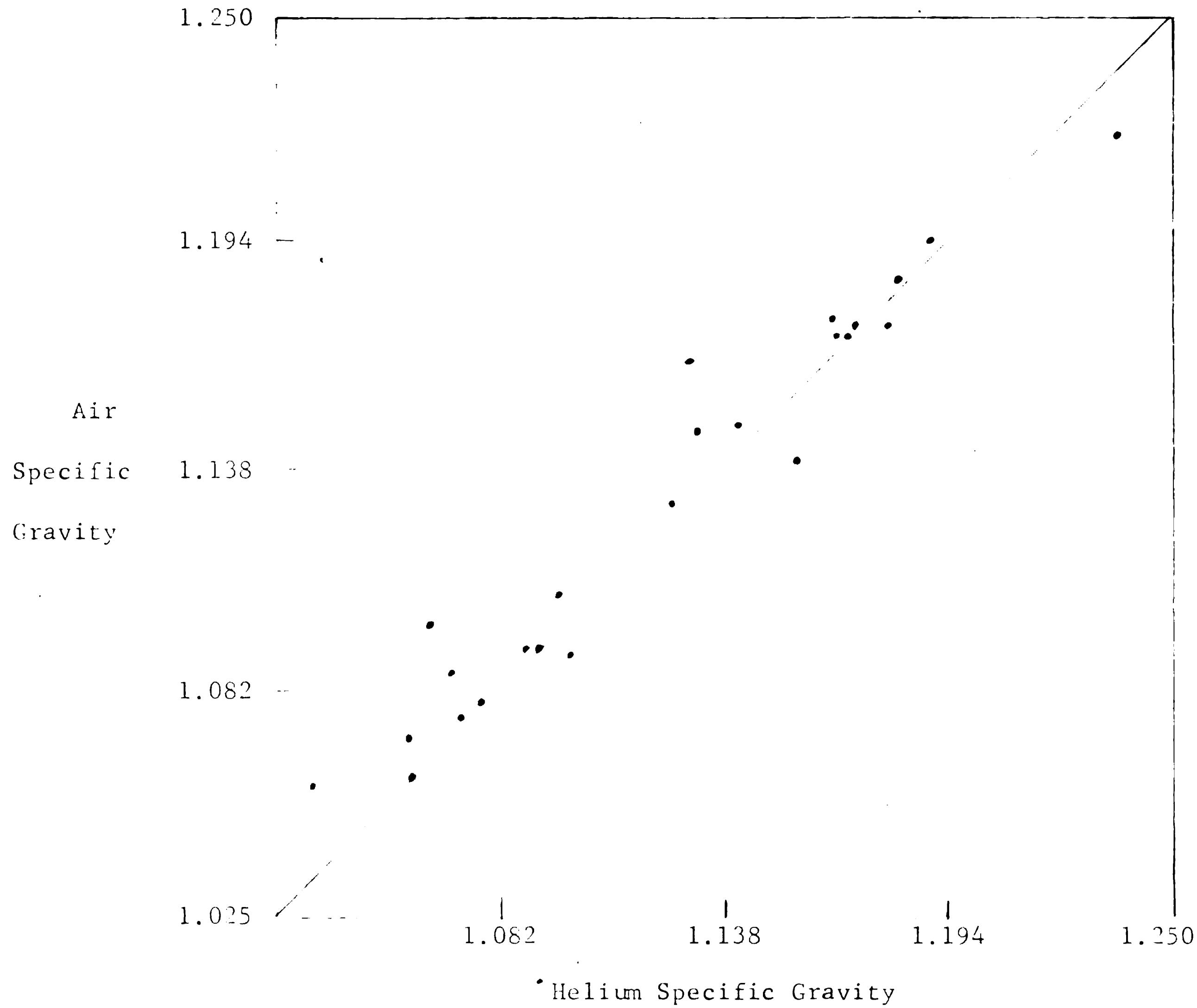


Figure 8. Air specific gravity versus helium specific gravity for men

Table 1. Summary of specific gravity data for men.

Subject No.	Weight (lbs.)	Height (in.)	Volume Air	Volume Helium	Specific Gravity Air	Specific Gravity Helium
1	150.0	68.3	58.12	58.47	1.1717	1.1647
2	148.5	68.5	56.78	57.01	1.1874	1.1826
3	159.0	68.5	67.10	67.20	1.0759	1.0743
4	185.5	73.8	77.01	77.14	1.0936	1.0918
5	211.0	70.0	87.53	87.90	1.0944	1.0898
6	164.8	72.0	68.72	69.93	1.0885	1.0696
7	147.5	72.0	54.92	54.13	1.2192	1.2370
8	191.8	70.5	79.09	81.97	1.1006	1.0620
9	189.0	68.5	73.43	76.12	1.1684	1.1272
10	199.3	66.5	82.73	82.28	1.0934	1.0994
11	167.0	68.5	63.97	65.27	1.1852	1.1616
12	159.5	71.8	63.51	62.56	1.1401	1.1574
13	158.3	70.0	61.39	61.53	1.1704	1.1677
14	133.5	65.0	50.82	50.98	1.1926	1.1889
15	178.8	68.0	76.48	76.54	1.0611	1.0602
16	192.5	71.5	81.37	82.77	1.0739	1.0558
17	172.5	66.5	70.79	71.31	1.1064	1.0983
18	149.8	69.5	57.82	58.41	1.1759	1.1640
19	166.0	69.0	69.81	69.98	1.0795	1.0769
20	182.5	67.5	77.88	80.26	1.0638	1.0323
21	172.5	67.5	68.31	68.61	1.1464	1.1414
22	224.5	73.5	88.62	90.45	1.1500	1.1268
23	140.5	66.0	54.38	53.90	1.1730	1.1834
24	161.5	71.0	64.92	65.28	1.1294	1.1232
Mean	171.1	69.3	68.98	69.58	1.1309	1.1223

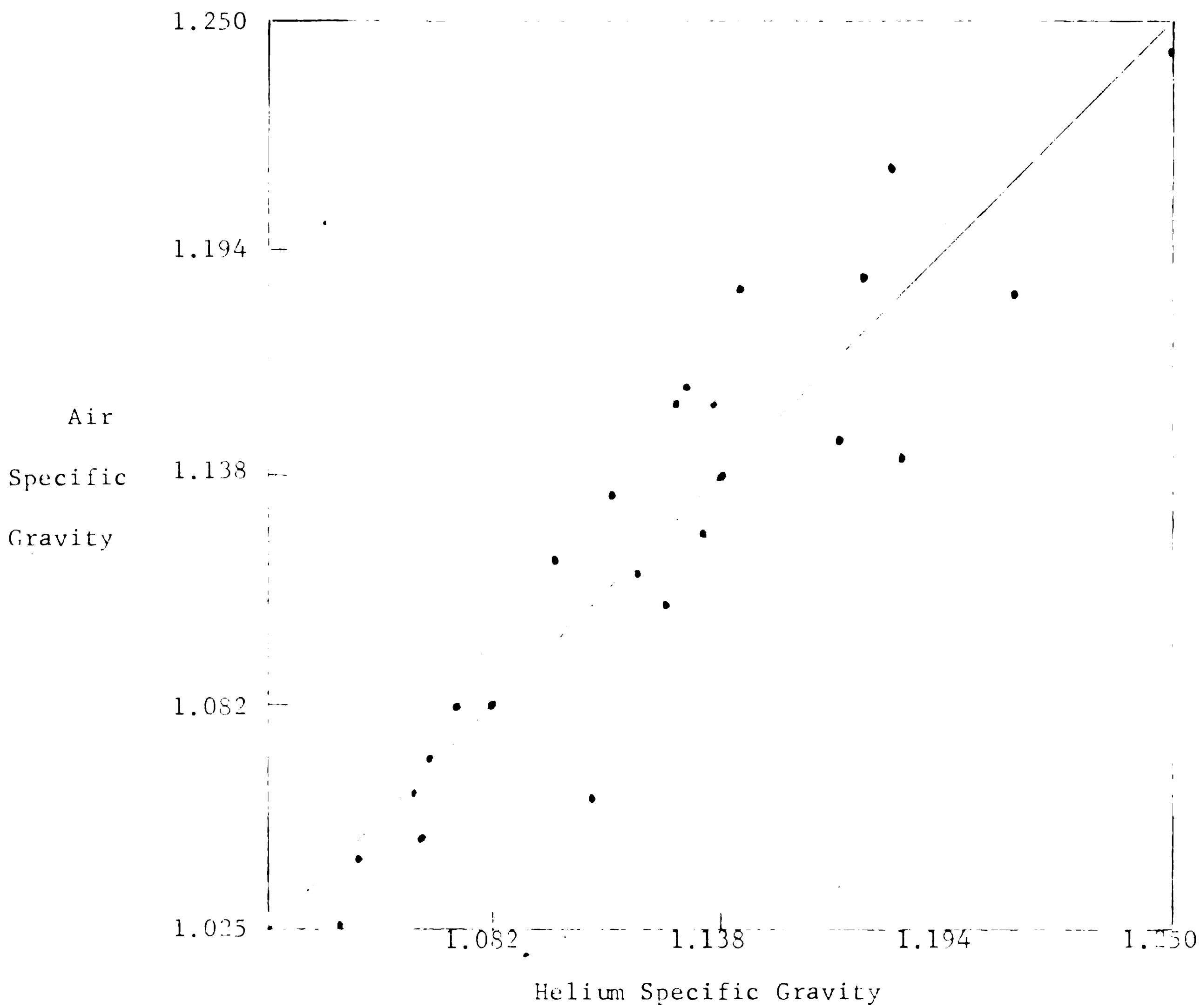


Figure 9. Air specific gravity versus helium specific gravity for women

Table 2. Summary of specific gravity data for women

Subject No.	Weight (lbs.)	Height (in.)	Volume Air	Volume Helium	Specific gravity Air	Specific gravity Helium
1	138.5	63.0	55.28	55.25	1.1375	1.1381
2	162.0	67.5	68.71	68.94	1.0704	1.0669
3	116.5	65.5	44.78	43.49	1.1811	1.2161
4	125.0	65.0	50.47	49.99	1.1244	1.1352
5	122.5	65.5	48.66	46.93	1.1430	1.1852
6	110.5	63.5	43.54	44.56	1.1523	1.1259
7	113.5	66.5	43.40	43.90	1.1873	1.1738
8	128.5	59.5	55.81	54.90	1.0453	1.0626
9	133.5	66.0	53.57	54.54	1.1314	1.1113
10	148.0	68.3	64.53	64.26	1.0412	1.0456
11	146.5	65.5	61.52	61.53	1.0811	1.0809
12	142.5	66.5	61.01	60.91	1.0605	1.0622
13	111.8	64.3	43.85	44.92	1.1569	1.1293
14	124.3	61.5	51.02	50.26	1.1056	1.1224
15	112.5	64.5	45.96	45.77	1.1114	1.1160
16	115.5	65.0	44.33	45.90	1.1829	1.1425
17	148.5	66.0	62.32	62.90	1.0818	1.0719
18	149.0	67.0	54.32	53.96	1.2452	1.2535
19	129.0	64.0	48.26	49.64	1.2136	1.1799
20	129.5	63.0	55.49	53.12	1.0595	1.1067
21	134.0	67.5	52.72	53.55	1.1540	1.1361
22	152.0	66.0	61.65	62.93	1.1194	1.0966
23	131.0	61.5	58.12	56.97	1.0232	1.0439
24	126.5	63.0	49.98	49.16	1.1491	1.1682
Mean	131.3	64.8	53.30	53.26	1.1233	1.1238

Correlations obtained with women between the two methods showed highly significant "r" values of 0.990 between volumes and 0.912 between specific gravity measurements. Differences in specific gravity values between the two methods showed more variation than was obtained with men. The differences between methods varied from 0.0002 to 0.0472, with a mean of 0.0185.

It seems probable that the decreased accuracy obtained with female subjects was due to the fact that, in most cases, their volume was below the range for which the helium dilution apparatus was calibrated (62.93 - 104.80). To test this theory, the women were arbitrarily divided into two groups using a volume of 55 liters as the dividing line. The 10 women whose volume exceeded 55 liters showed a mean difference in specific gravity values of 0.0128, which was the same value as was obtained for men. The 14 women whose volume was less than 55 liters showed a mean difference of 0.0226 in specific gravity. This indicates that a large proportion of the error in the computation of body volume of women by helium dilution was due to their small volume. It appears likely that calibration of the helium dilution apparatus in a range covering the actual volumes of the women would have improved the accuracy of the determination. The comparatively small volumes of the women also decreased the accuracy of the air displacement determination. This was due to the fact that the accuracy of the air displacement apparatus decreased as the chamber to subject volume ratio increased.

The average specific gravity values determined by combining the two methods were 1.1266 for men and 1.1236 for women. These values are somewhat higher than those reported in the literature.

Bohnenkamp and Schmäh (1931) reported a mean value of 1.095 for men and 1.070 for women. Behnke _et al_. (1942) obtained a range in specific gravity of 1.021 - 1.097, with a mean of 1.068, on a large group of men. If the results of the present study are compared with the data of Behnke _et al_., (1942), only one man and three women would be considered obese (specific gravity < 1.060). It is quite possible that the subjects used in this study were not obese since they were not randomly selected from the population as a whole. Most of the subjects were graduate students, student wives, faculty members, secretaries, and technicians in the departments of Food Science and Animal Husbandry. None of these subjects were grossly overweight and many were of the athletic type. These factors undoubtedly contributed to the high specific gravity values for the population used in this study. However, the two methods used for measuring specific gravity in this experiment appeared to give considerably higher values than those obtained by Bohnenkamp and Schmäh (1931) and Behnke _et al_. (1942).

In general, specific gravity measurements appeared to be closely related to the height and weight of the subjects. For example, Table 1 shows that subject number 7 was one of the tallest men, but was among the lightest in weight, and therefore had a high specific gravity (1.228). Conversely, subject number 20 was among the shortest of the male subjects, but was near the top in weight, and consequently had a low specific gravity (1.0481). Table 2 shows that the same was true for women although the differences were not as pronounced, since there was less variation in height and weight.

Errors in Measuring Body Volume by Helium Dilution

Siri (1955) provided a detailed estimate of the errors involved in the instrumental design of the helium dilution method. He defined the limits within which each variable must be controlled in order to measure volume with a standard deviation of ± 0.1 liter. Gnaedinger (1962) reported that he could determine the volume of inert objects with a mean deviation of 0.81 liters. He concluded that the variation was mostly due to inaccuracy in adjustment of the current.

The problem of current adjustment was also encountered in the present study. The volume of inert objects in the present experiment was determined with a standard deviation of 0.75 liters for 8 determinations. The major part of the variation appeared to be a function of current setting. The power supply was quite stable at any particular setting; however, there was a lack of precision in adjusting the current to a specified value from day to day. This difficulty appeared to be the major source of error in determining the volume of inanimate objects. It could also be a source of error with live subjects, although probably not the major cause of variation.

When live subjects were used, other variables such as temperature and relative humidity became important. An examination of equations 12 and 13 shows that an increase in temperature and decrease in relative humidity causes a decrease in γ (the correction factor for temperature and relative humidity), and therefore an increase in helium concentration. These equations are based on the assumption that temperature and relative humidity remain constant or change at a constant rate throughout a run.

Any change in these variables would cause variation in the helium concentration curve and introduce uncertainty into its extrapolation to zero time.

In this study, the average temperature change during a run of 30 minutes duration was 1.42°C for men and 1.09°C for women. Relative humidity changes were not measured since they were assumed to be a function of temperature. Temperature changes did not appear to be high enough to be the major source of error. They could be further reduced, however, by maintaining the ambient temperature closer to the body temperature of the subject.

Another possible source of error could be the thermal expansion of the air-helium mixture in the chamber. If expansion of the gas mixture exceeded the rate at which it was being drawn through the cell, some of the helium would be forced out of the chamber. For example, a temperature increase of 0.5°C per minute would expand the gas at a rate of 1 liter per minute. Therefore, a flow rate of less than 1 liter per minute would cause some loss of helium. In this study, the average temperature increase was only 0.05°C per minute which would expand the gas at a rate of approximately 100 ml. per minute. Since the flow rate of the system (700-550 ml. per minute) was greater than 100 ml. at all times, this was not believed to be an important source of error in this study.

Gnaedinger (1962) reported that the major source of error in determining the body volume of hogs was the variable amount of activity when confined in the chamber. He also noted wide differences in respiration rate, which caused a great deal of variation in temperature and relative humidity during a run. In the present study, the subjects were instructed to lie as quietly as possible throughout the run. This resulted in minor changes in temperature as noted before. However, there did appear to be a marked difference in the respiration rates of the various subjects. Although these differences had a slight effect on temperature, they may have had a significant effect on the amount of carbon dioxide accumulated in the chamber. The accumulation of carbon dioxide and depletion of oxygen in the chamber would lower the thermal conductivity of the gas mixture and result in a change in the helium concentration curve. This change could cause an error in extrapolation of the helium curve to zero time.

The results of this experiment indicate that the major sources of error in the helium dilution procedure are the inaccuracy of current adjustment, temperature changes within the chamber and irregular accumulation of carbon dioxide.

Errors in Measuring Body Volume by Air Displacement

Gnaedinger (1962) reported that the major source of error in the air displacement method was the lack of precision in measuring volumes from day to day. In an attempt to correct for this variation, he measured the volume of the empty animal chamber each day prior to making a run. In the present study this procedure was also used. In addition, the ambient temperature was maintained above 27°C throughout the course of the experiment. This temperature level served to minimize day to day temperature differences. This is undoubtedly the main reason for the improved results in this study over previous work on air displacement by Gnaedinger (1962). These improved results are demonstrated by a statistical analysis of the empty chamber volumes calculated each day. The values obtained ranged from 461.59 to 466.60 liters with a mean of 464.01 liters and standard deviation of ± 1.06 liters. Gnaedinger (1962) reported empty chamber volumes ranging from 454.71 to 470.83 liters with a mean of 464.61 liters and a standard deviation of ± 4.7 liters.

Analysis of variance of the empty chamber volumes obtained in the present study is shown in Table 3. Results showed that there was a significant difference ($P < .05$) between the mean values obtained on different days. However, there was a non-significant difference between the two values which made up the mean for each day. This would seem to indicate that replicate volume measurements are unnecessary in the air displacement procedure.

Table 3. Analysis of variance of values obtained for the volume of the empty subject chamber.

Source of variation	Degrees of freedom	Mean square	F value
Total	43		
Between means	21	1.63	2.59*
Within means	1	.18	$.29^{ns}$
Error	21	.63	

In a preliminary study, the volume of inert objects was determined with a standard deviation of 0.45 liters for 15 determinations.

Gnaedinger (1962) performed an estimation of errors on the equation for computing body volume (equation 15) to determine the sources of variation which consistently occurred. Since the same apparatus was used in the present study, the same estimation of errors was calculated. The following conditions were set up in order to estimate the maximum error inherent in the apparatus: (1) The maximum error in reading pressure was ± 0.1 mm. on the cistern-type manometer; (2) the maximum error in reading temperature was ± 0.1°C.; (3) the relative humidity was assumed to be constant throughout a run so that no corrections were required. Under these conditions the maximum percentage error was computed to be 0.72%.

If, however, relative humidity corrections were included in the equation for computing body volume (equation 15), the maximum error increased to 2.54%. This was due to the poor accuracy of the relative humidity sensing device, which was only accurate to ± 2% of full scale. Since the sensing device was not accurate, less error would be introduced into the computation of body volume if the relative humidity corrections were deleted from the equation. This would be true only if the changes in relative humidity were minor.

It was observed in this experiment that the relative humidity changed only as a function of temperature. For example, a temperature increase of 1.5°C would cause a 0.25% decrease in relative humidity. For this reason, no corrections were made for relative humidity in this study. However, since the average temperature increase observed in the present experiment was 1.42°C for men and 1.09°C for women, it is possible that changes in relative humidity introduced some error into the computation of body volume.

Some error may also have been due to the fact that temperatures were measured with a single thermistor. With live subjects in the chamber, it seems likely that temperature errors as great as ± 0.5°C could have occurred. However, these errors would tend to be decreased due to the fact that ambient temperature was maintained near body temperature.

In the present study the major sources of error in the air displacement method appear to be due to changes in temperature and relative humidity, and to inaccuracies in reading the manometer.

SUMMARY

The specific gravity of 24 men and 24 women was determined by air displacement and helium dilution procedures. Correlation coefficients of 0.964 for men and 0.912 for women were obtained between specific gravity values computed by the two methods. The volumes from which the specific gravity values were calculated also showed high correlations of 0.997 for men and 0.990 for women. Although the specific gravity values appeared to be somewhat high, they were closely related to the height and weight of the subjects.

Differences in specific gravity values between the two methods were greater for women than for men. This was probably due to the fact that the body volume of the female subjects was lower, and in most cases, fell below the range for which the helium dilution apparatus was calibrated. The accuracy of the air displacement apparatus also decreased as the ratio of empty chamber to subject volume increased.

The major sources of error with the helium dilution procedure were the inaccuracy of current adjustment and changes in temperature and relative humidity within the subject chamber. Results indicated that temperature changes due to the spread between ambient and body temperature were also responsible for much of the error in the air displacement technique. Changes in relative humidity probably also caused some error with this method.

In general, specific gravity values obtained by the two methods were closely related and showed excellent agreement.

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