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BODY HEAT LOSS IN MAN DUE TO HEATING AND HUMIDIFYING RESPIRED COLD AIR

By

Robert James Weir, Jr.

INDEX

Contents: caps & lower case

Most of the following report is made up of theses, somewhat modified, upon which graduate students obtained advanced degrees. It is understood that the research involved was made under the direction of Norman E. Phillips and Loyal Goff, principal investigator and research assistant on the contract.

Occasionally throughout this report there are tables which occupy more than one typed page. These have not been assembled but the pages pertaining to any one table are adjacent; it being left to the reader to properly arrange these tables as the report is being read.

INTRODUCTION

The respiratory tract is recognized as an important avenue of heat loss from the body. At ordinary room temperature approximately 15 percent of the total heat loss occurs through this pathway due to warming and humidifying inspired air. The remaining loss is accounted for by radiation, convection and conduction from the external body surface. Evaporation of moisture from the skin is another source of heat loss. This factor is dependent on the relative humidity of the surrounding air. A very small percentage of heat loss results from urination, defacation and the injestion of food. Du Bois (1) and Adolph (2) have reviewed the general problem of heat loss and temperature control.

By the proper choice of the type and amount of clothing, heat loss from the external surface of the body can be controlled to a considerable degree, irrespective of the ambient temperatures. The loss from urination, defacation and injestion of food is not important in low temperature exposures as this forms such a small fraction of total heat loss and because it is not dependent on external temperatures. Under conditions of low temperature, however, no adequate means is available for the control of heat loss from the respiratory tract.

At low ambient temperatures, if adequate clothing is provided to prevent an increase in heat loss due to radiation, conduction and convection from the external body surface, heat loss from the respiratory tract becomes relatively greater in importance.

Heat loss from the external body surface depends largely upon the temperature of the surrounding air, humidity and air movement. Loss through the respiratory tract depends, in addition to the above, on rate of breathing, depth of breathing and minute volume, and these in turn depend on the extent of exercise. The anatomical surface over which the air passes on inspiration and expiration constitutes an additional modifying factor. Little work has been conducted to determine to what extent various factors affect the magnitude of the loss in breathing cold air.

On a theoretical basis, Webster (3) has calculated the energy necessary to heat and humidify the breathed air and the fraction which this represents of the total energy metabolism. In addition, based on these calculations, he has hypothesised the ratio of survival time "with to without" conservation of the heat necessary to warm and humidify the inspired air.

Moritz and Weisiger (4) have caused dogs to breathe cold air by means of an oral cannula while the external body surface of the animal was exposed to ordinary room temperature. They observed the temperature of expired and inspired air and the region of the respiratory tract where the heating of the inspired air occurred. They also studied the pathological effect of cold air on the tissues of the respiratory system.

Smith (5) has described a syndrome of pain in the chest, cough, hemoptysis, patchy pulmonary consolidation and fever in flying personnel after high altitude missions in which intensely cold air was breathed. To this syndrome he gives the name "frostbite of the lungs". He has stated that the onset occurred more rapidly as a result of exercise, presumably because of increased ventilation.

The purpose of the present research was to study the extent of heat loss through the respiratory channel over a wide range of temperatures. The methods of exposure were so devised that the study included the effects of various factors such as the type of breathing and exercise. Conditions were controlled so that the external body surfaces of the subjects were exposed to normal room temperatures while the respiratory system was exposed to varying temperatures

METHODS AND PROCEDURES

In order to observe the heat loss resulting from the breathing of air of various temperatures independent of any heat exchange that might result from the simultaneous cooling of the body surface, the apparatus as diagramed in Fig. 1 was devised.

Air entered the system from the laboratory at ambient temperature through the inlet vent in a double, blower-type, electric fan. This blower was introduced to maintain a positive pressure in the system. As a result, on inspiration, an instantaneous flow of approximately 20 liters was available. This factor allowed a flow sufficient, during inspiration, to supply all of the respiratory needs with additional energy expenditure. The pressure of expiration and the spring load of the valve in the coil balanced the blower pressure so that air flowed through the system only during inspiration and hence volume of flow was registered at the meter only during inspiration.

The incoming air vent in the blower was placed some distance from the bath and was above it to prevent any possible contamination of incoming air with carbon dioxide from the dry ice in the bath.

The air was passed through a calcium chloride drying tube, 10 inches long and 2½ inches in diameter. This was interposed in the system to prevent blocking of the coil by the formation of ice. In addition, it prevented the subject from breathing humid air in exposures where the temperature of the bath was above freezing.

After being dried the air was passed through a dry test meter for measurement of volume. The meter had previously been compared for instantaneous volumes during respiration against a wet test meter and the instantaneous volume was found to be insignificantly in error for any interval of time.

The air passed to the cooling system which was composed of a coil of copper tubing immersed in an insulated varsol-dry ice bath. The coil was 27 feet long with an inside diameter of 7/8 inch. This made available a volume of 3 liters or 3 to 5 resting tidal volumes,

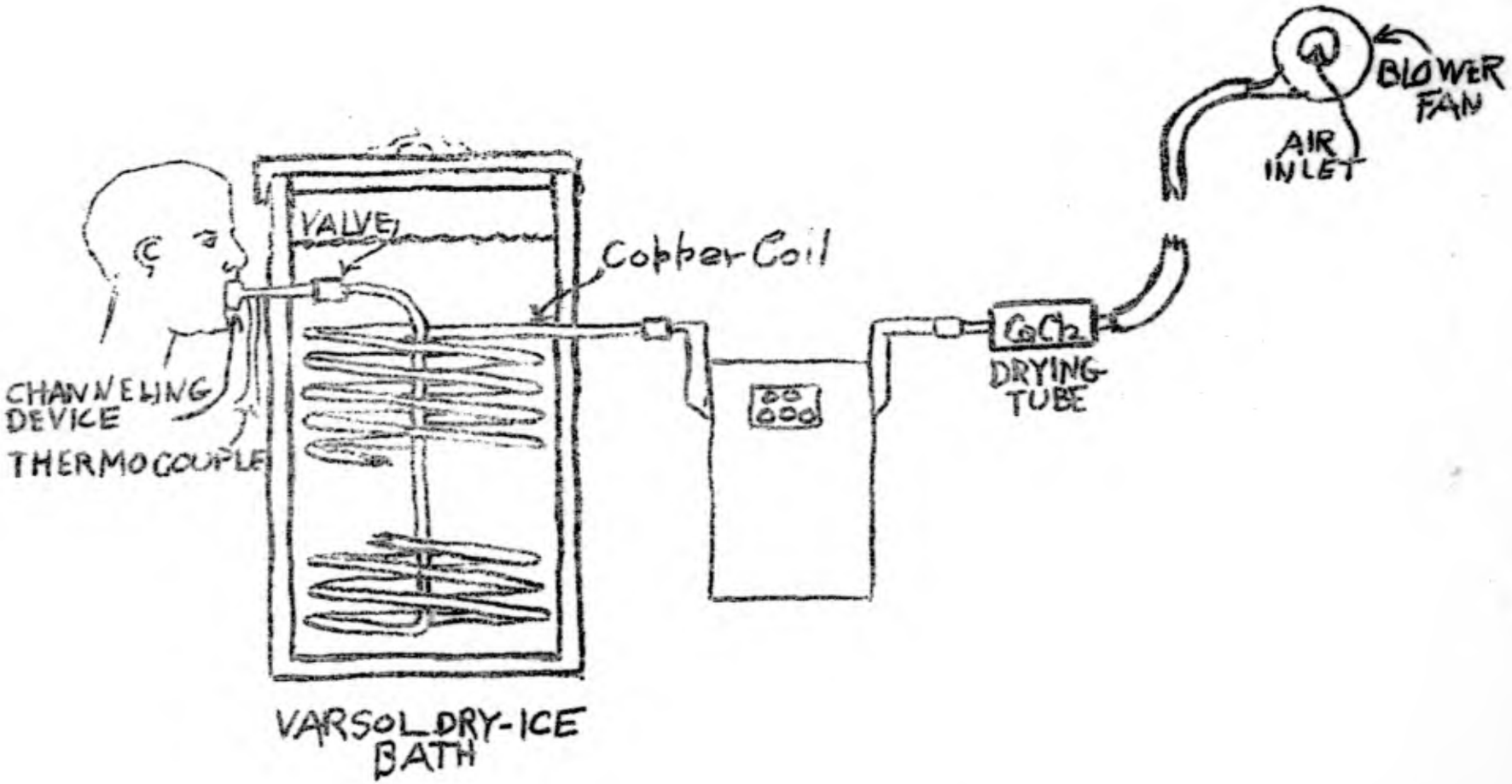


FIG.1 DIAGRAM OF EXPERIMENTAL APPARATUS.

so that the entire volume of cold air could not be depleted with one inspiration. In this way a constant low temperature supply of air was maintained.

The temperature of the bath was controlled by varying the amount of dry ice that was placed in the varsol. In this manner the air was delivered to the subject at a constant temperature throughout the entire test. The bath was insulated with saw dust and ground asbestos. This further helped maintain the equilibrium of the temperature at which air was delivered.

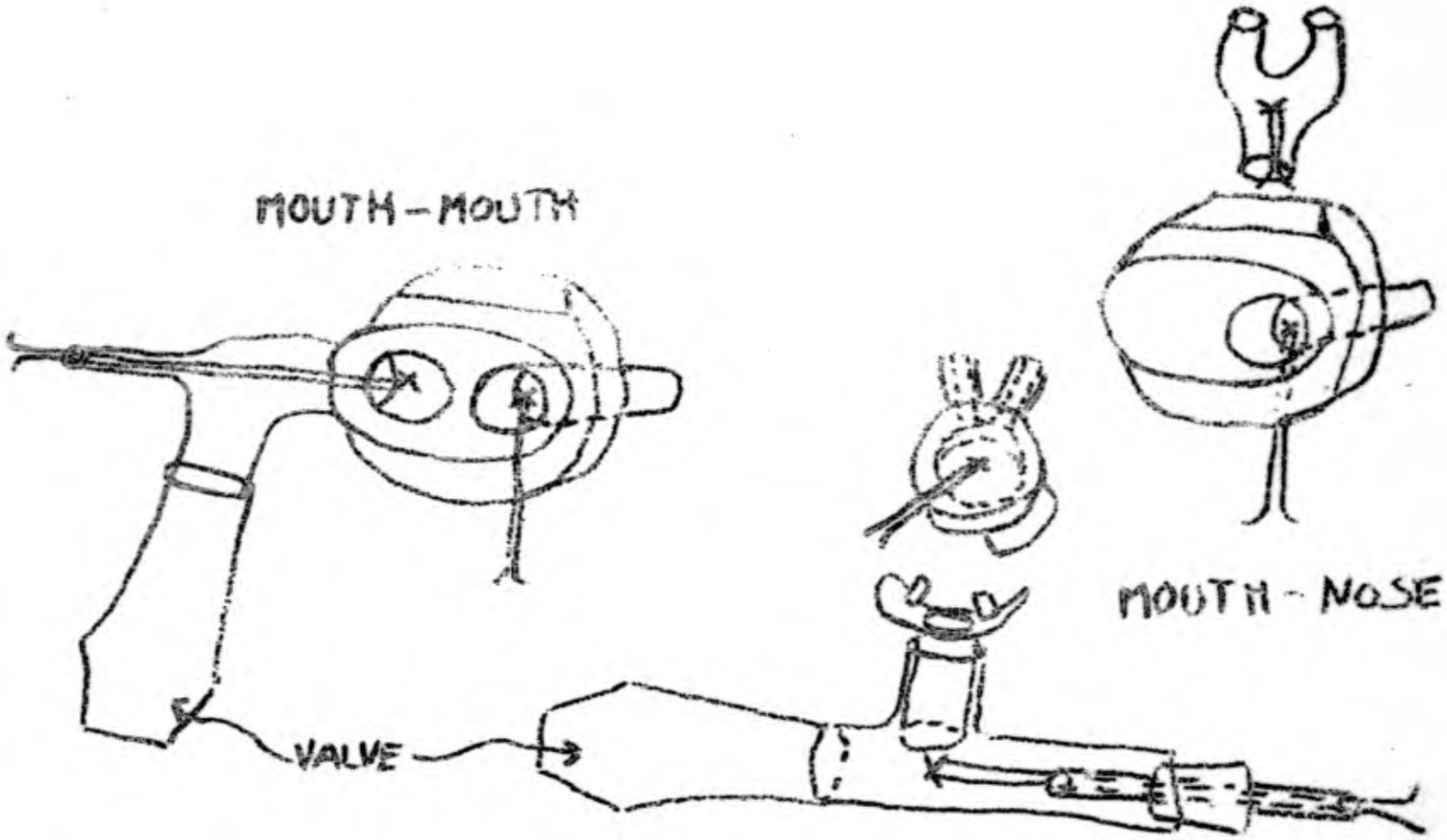
On inspiration, air passed through the check valve in the coil to be breathed by the subjects through an appropriate mouth or nose piece. These devices (details of which are shown in Fig. 2) were designed so that exposures could be made through different pathways to determine the most efficient heating surface of the upper respiratory tract. Pathways were as follows: (1) inspiration through the mouth and expiration through the mouth, (2) inspiration through the mouth and expiration through the nose, (3) inspiration through the nose and expiration through the mouth. These will be designated, hereafter, in the abbreviated form of mouth-mouth, mouth-nose and nose-mouth breathing respectively. The pathway of inspiration through the nose and expiration through the nose could not be accomplished due to difficulty in instrumentation. Since heat would be exchanged between the nose and the cold air, a proper nose piece could not be designed without involving errors in temperature measurement.

All subjects were trained to breathe in the patterns as stated above. In addition, valves were placed wherever necessary to facilitate the respiratory pattern and to prevent "blow back". In this way inspired cold air was not allowed to pass over the surface passed by the warmer expired air. Two different channels were available in all breathing devices, one for inspiration and one for expiration. The position of the valves and the double pathways of the devices are shown in Fig. 2.

No attempt was made to insulate the channeling devices (mouth or nose pieces) as the "spike effect", described by Henderson, Chillingworth and Whitney (6) as the phenomenon whereby air passing through a tube passes rapidly in the axial stream and more slowly in the peripheal stream, and the location of the thermocouples made this precaution unnecessary. All thermocouples were placed in the center of the stream. The thermocouples were placed as near to the subject's respiratory openings as possible to prevent any flow of heat between respired and ambient air.

The distance between mouth pieces and the point where the coil emerged from the insulation of the bath was kept as short as possible as it was found that heat lost from the system at this point made it impossible to acheive the desired low temperatures with a varsol-dry ice mixture if this distance was too great.

Inspiratory and expiratory temperatures were measured with copper-constantan thermocouples constructed of 30 gage wire. The thermocouples were connected with a Leeds and Northrup, type R, reflecting galvanometer having a period of 2.9 seconds (sensitivity 0.003 microamperes per millimeter, resistance 580 ohms). The gal-



THERMOCOUPLES INDICATED BY X

FIG. 2. Diagram of channeling devices and location of thermocouples.

vanometer deflection was measured by means of a mounted lamp and straight scale (Leeds and Northrup). A resistance of 1800 ohms was introduced in the system by means of a dial decade, resistance box (Leeds and Northrup). Two single pole, double throw knife switches were used in the system in order that any one of four thermocouples could be used at any time during the exposure. Fig. 3 shows the thermocouple wiring diagram.

A common thermocouple was used in the inspiratory pathway of the channeling devices. This thermocouple was inserted in the cooling coil a short distance from the mouth or nose as the situation warranted. The other three thermocouples were inserted in expiratory channels, one for each pathway of breathing. The thermocouples were so placed and supported that no contact was made with the sides of the containers. They were exposed to the straight flow of inspired or expired air. Care was taken to expose 4 to 6 inches of the insulated leads of the thermocouples themselves to prevent the conduction of heat through the leads.

The pattern of breathing and the rate per minute were recorded during the experiment by means of a pneumograph and recording kymograph. A signal magnet controlled by a simple key was used to designate the time interval.

In order to determine the affect of inspiratory temperature on expiratory temperature and calorie loss the subjects were exposed to six ranges of inspiratory temperatures. These temperatures were between -60° and 30°C. and were grouped in ranges of 15° increments as follows: -60° to -45°C., -45° to -30°C., -30° to -15°C., -15° to 0°C., 0° to 15°C., and 15° to 30°C.

Since Moritz and Weisiger (4) used anesthetized dogs as subjects in their experiment without any serious tissue damage or complications, it seemed safe to use human subjects in the research reported here. Six healthy male subjects were chosen. Two of these were deep, slow breathers, two were rapid, shallow breathers and the other two had an intermediate pattern of breathing.

The subjects were exposed at rest in a seated position to all of the temperature ranges through the breathing patterns of mouth-mouth, mouth-nose and nose-mouth. In addition, since Smith (5) stated that exercise precipitated the symptoms of "frost-bite of the lungs" more rapidly, all subjects were exposed to -30° to -45°C. inspiratory temperature when tested during exercise to determine the effect of exercise with the accompanying increased respired volume on the Calorie loss and expiratory temperature. And since under exercise conditions the mouth-mouth pathway is the most common, this was the only pathway used in the exercise tests.

Inspiratory temperatures were observed at each one minute interval and expiratory temperatures were taken on the intervening half minute in both exercise and rest exposures. Gas volumes were observed on the minute by a second operator who also operated the signal magnet. The respiratory tracings were counted for respirations per minute after the exposure. The ambient temperatures and barometric pressure were

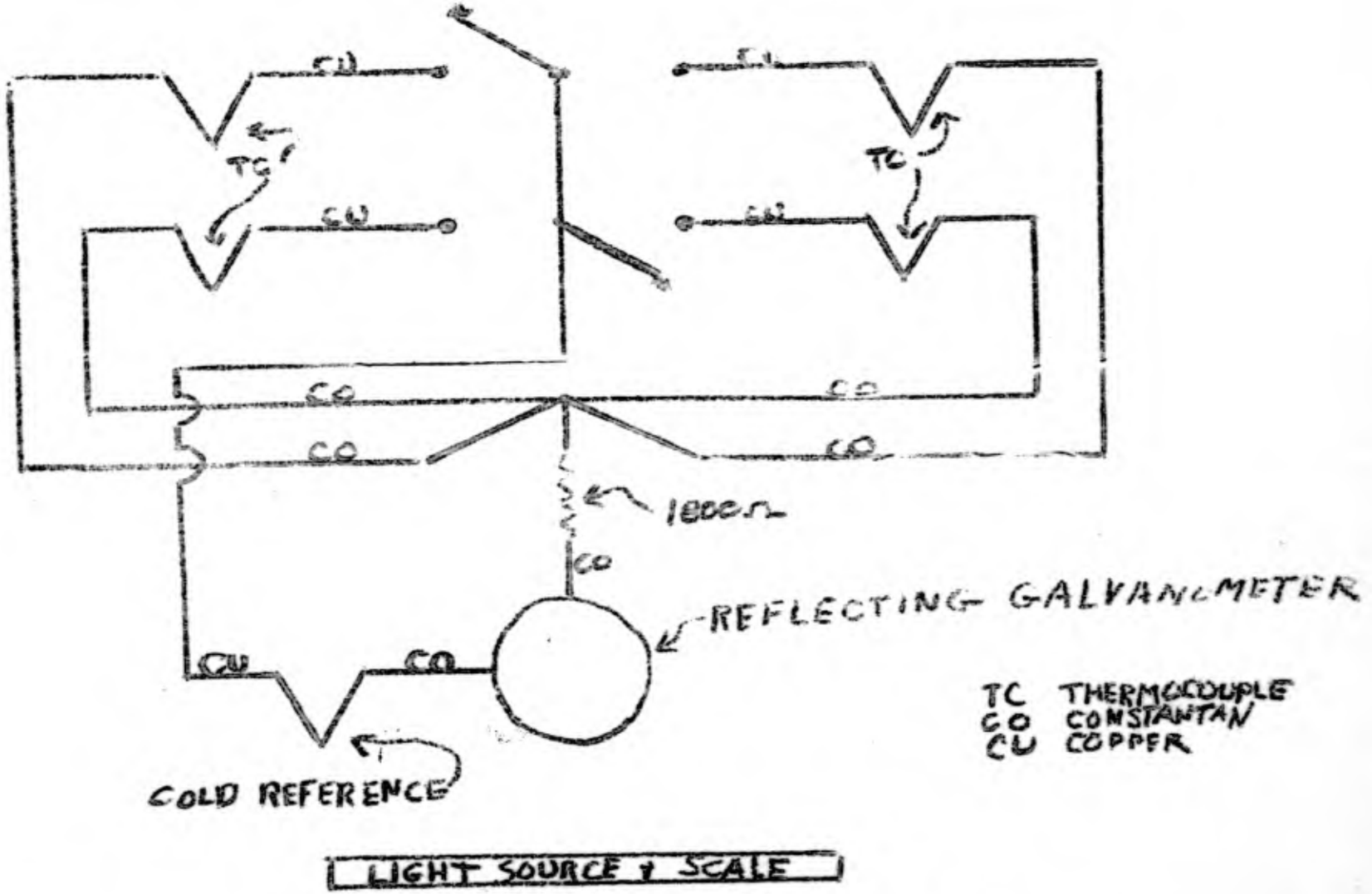


FIG 3 Wiring diagram of thermometry apparatus

also noted. The means of the last five minutes of exposure under rest conditions were calculated for the inspiratory and expiratory temperatures as well as respiratory rate. The volume respired for the last five minutes was calculated. In the exercise tests the above calculations were computed on the basis of the last four instead of five minutes.

Exercise was conducted on a stationary bicycle with 30 full revolutions of the pedals per minute. A metronome was used to keep the rhythm. All exposures at rest were conducted fora period of 10 minutes. Exercise exposures were conducted in two phases; (a) rest for two minutes (b) exercise for five minutes. The duration was chosen on the basis of the time necessary for the subject to arrive at a respiratory and thermal equilibrium.

All volumes were corrected to standard temperature and pressure and converted to moles. Heat capacity (Cp) was calculated from the equation for heat capacity of nitrogen gas as given by Johnston and Davis (7):

1) $Cp = 6.449 + (1.413 \times 10^{-3}T) - (.807 \times 10^{-7}T^2)$

The equation for Cp on the basis of nitrogen was chosen because the heat capacities of oxygen and carbon dioxide are very close to it and nitrogen gas is the largest component of atmospheric air. The error introduced by this choice was found to be less than that which had been imposed by instrumentation. The total heat capacity over the range T_1 to T_2 may be expressed in the form of the integral:

2) $Cp\ (total) = \int_{T_1}^{T_2} F(T)dt.$ where $Op = F(T)$.

Then:

3) $Cp = \int_{T_1}^{T_2} 6.449 + (1.413 \times 10^{-3}T) - (.807 \times 10^{-7}T^2)$

4) $Cp = \left[6.449T + \left(\frac{1.413 \times 10^{-3}T^2}{2}\right) - \left(\frac{.807 \times 10^{-7}T^3}{3}\right)\right]_{T_1}^{T_2}$

5) $Cp = \left[6.449\ (T_2 - T_1)\right] + \left[\frac{1.413 \times 10^{-3}}{2}(T_2^2 - T_1^2)\right] - \left[\frac{.807 \times 10^{-7}}{3}(T_2^3 - T_1^3)\right]$

Therefore:

6) $Cp = \left[6.449\ (T_2 - T_1)\right] + \left[.7065 \times 10^{-3}(T_2^2 - T_1^2)\right] - \left[.269 \times 10^{-7}(T_2^3 - T_1^3)\right] =$ Calories per molecular volume.

Equation 6 was found to be more satisfactory for the calculation of loss in Calories than the common method of using the specific heat and density of air. The product of the heat capacity and the molecular volume is the number of Calories lost in heating the respired cold air.

The rest of the heat lost from the respiration of cold air is due to that of evaporation of water. The number of grams of water per cubic meter of air at the expired temperature was found from a table in the Handbook of Chemistry and Physics (8) as was the heat of vaporization (Calories per gram). The product of these above two factors at the expired temperature and the volume in liters divided by 1000 is the amount of heat used in humidifying the respired cold air to 100 percent saturation. The saturation of expired air is not quite 100 percent but is close enough for this calculation. The sum of the heat lost in heating and that lost in humidifying the cold inspired air is the total respiratory heat loss in Calories.

RESULTS AND DISCUSSION

In Fig. 4 in which expiratory temperature is plotted against inspiratory temperature, it is shown that the lower inspiratory temperature resulted in a lower expiratory temperature in all three pathways of breathing. In no case, even in the inspired temperature range of 15° to 30°C., did the expired temperature reach normal body temperature (37°C.). Moritz and Weisiger found that with reported inspiratory temperatures as low as -100°C. the expiratory temperature as measured at the lower end of the trachea (22 centimeters below the larynx) averaged body temperature or above. However, by moving the expiratory thermocouple 12 centimeters closer to the larynx, they reported expiratory temperatures below body temperature, some of which were in the range of expired temperatures recorded in the present investigation.

Due to the methods and instrumentation employed by Moritz and Weisiger (4) there are grounds for criticism from the standpoint of exactness of temperature measurement. They reported that it was necessary to remove the cannula every 5 minutes during the exposure to clear an ice plug from the tip of the cannula. In addition a wire cage was used to protect the thermocouples in the trachea. They reported that mucus collected on this cage during the tests. These two conditions possibly allowed heat transfer to occur. In the former situation, heat could have been transferred between the inspired air and the ice plug. In the latter case heat could have been transferred from the warm trachea to the thermocouple by way of the mucus which collected on the protecting cage. From the diagrams and description in the report, it seems that Moritz and Weisiger failed to protect the leads to the thermocouples from the heat of the surfaces over which these leads passed. All of these conditions indicate that the expiratory temperatures reported by Moritz and Weisiger were higher than actually existed. It is agreed, however, that the general temperature changes that they report are correct and that these changes are in accord with those reported here.

It thus appears, from the results reported in this investigation and those of Moritz and Weisiger, that a gradient existed in the temperature of the air as it passed through the respiratory system when breathing cold air. The air passed into external respiratory openings and was progressively warmed by the upper respiratory passages cooling

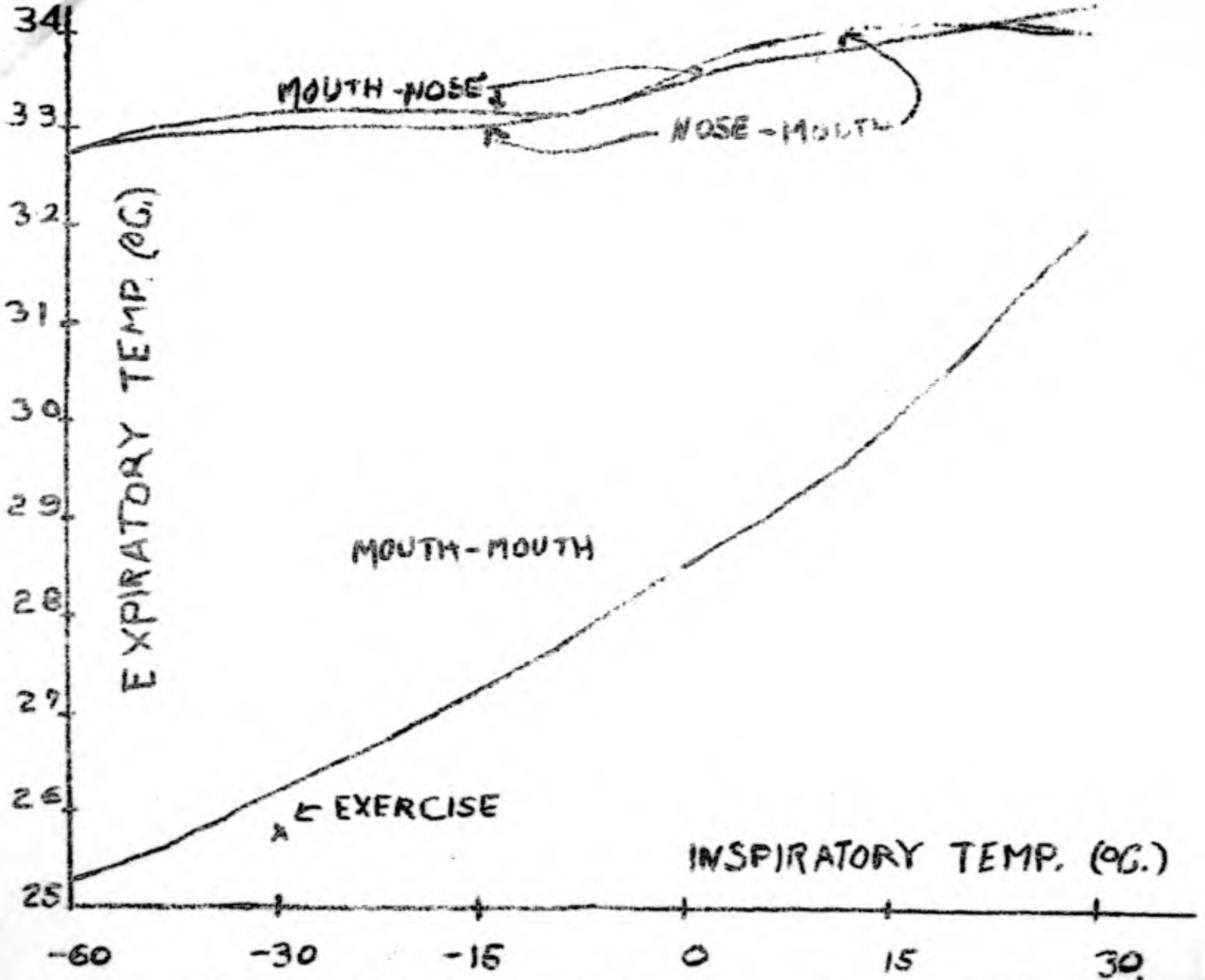


FIG 4. Expiratory temperature resulting from various inspiratory temperatures.

these organs below the normal temperature. When the air reached the lungs it had reached its maximum temperature. On expiration the air was progressively cooled by passing over those surfaces which had lost heat by the previous inspiration. This accounts for the low expiratory temperature reported here and the difference in results for two points in the trachea 10 centimeters apart as reported by Moritz and Weisiger.

The differences in the methods of the two experiments make them difficult to compare as in the present investigation human subjects were exposed by breathing cold air delivered to the normal respiratory openings so that the oral or nasal mucos was functional. In the experiment reported by Moritz and Weisiger the heating effects of the oral or nasal mucosa were absent as the air was delivered to the mid-portion of the mouth and to the larynx by an insulated cannula. In addition they used anesthetized dogs.

It is evident from Fig. 4 that the inspired air was heated equally as well by means of the pathway mouth-nose as by nose-mouth breathing. Since the respired gases passed across the same anatomical surface area in both cases, this relationship is not unexpected. The expiratory temperature was approximately 1.3°C. lower, for the inspiratory temperature -60°C., than the expiratory temperature resulting from the inspiratory temperature of air at 30°C.

In the case of mouth-mouth breathing in which the heating effect of the naso-pharyngeal surfaces was absent, it was found that the inspired air was not heated to as high a degree as in the cases of the other two pathways. The expiratory temperature was approximately 6.8°C. lower, for the inspiratory temperature -60°C., than the expiratory temperature of air inhaled at 30°C. The distance on the graph between the lines representing expiratory temperatures of mouth-mouth breathing and mouth-nose or nose-mouth breathing is nearly a measure of the heating ability of the naso-pharynx above that of the mouth surface (compared within the same inspiratory temperature range). The expiratory temperature for mouth-nose or nose-mouth breathing is 7.5°C. higher than mouth-mouth breathing for the inspiratory range of -45° to -60°C. and 2.0°C. higher in the inspiratory range of 15° to 30°C.

In the exercise test (inspiratory temperature of -30° to -45°C. and by means of the mouth-mouth pathway only) it was observed (as represented in Fig. 4) that the expiratory temperature was not influenced by a 2 to 3 fold increase in volume over that of rest. That is to say, despite the increased volume of air breathed due to exercise, the respiratory system was capable of heating this volume equally as well as it did the volume breathed at rest. The mean expiratory temperature was 25.75°C. while that of rest exposure was 26.2°C. for the inspiratory range of -30° to -45°C.

This ability of the respiratory system to heat a larger volume during exercise to practically the same expiratory temperature as the volume breathed at rest can be accounted for on the basis of increased circulation due to exercise. Hill (9) reported that body temperature increased from 1° to 4°F. during exercise. This, too, accounts for

the expiratory temperature during exercise.

In Fig. 5 where minute volume was plotted against Calories lost per hour (for the mouth-mouth pathway of breathing) it is seen that heat loss is directly proportional to the volume respired. This would be a natural conclusion since heat loss in Calories was calculated on the basis of the inspired volume. It is of interest to note the change of the slope of the lines in the temperature range between 0° and -30°C. as it was in this temperature range that subjects complained about the severe sensation of cold. This factor may have disturbed the respiratory rate and volume to such an extent as to have influenced the slope of the relationship of minute volume against Calorie loss as well as tidal volume against Calorie loss. In any event, many more exposures would be necessary to lend validity to this subjective observation.

There was also a tendency for the subjects to reduce the volume of respiration for the lower temperatures of inspired air.

The most important relationship depicted in Fig. 5 is the Calorie loss per unit of volume in each of the inspiratory temperature ranges. If the minute volume had been constant at 7 liters, then the Calorie loss for each inspiratory temperature range would have been as follows:

Inspiratory temperature range °C.	Calories lost per hour.	
15 to 30	8.8	
0 to 15	9.9	
0 to -15	10.9	Exercise
-15 to -30	12.6 x 3	
-30 to -45	13.8 x 3	41.4 = abt $\frac{1}{2}$
-45 to -60	14.7 x 3	44.1 = abt $\frac{1}{2}$

If the volume inspired were 12.2 liters per minute at temperatures of 15 to 30°C., then the Calorie loss would have been the same as it was for 8 liters at the temperature of -45 to -60. Therefore, Calorie loss is dependent on two factors: the volume and the inspiratory and expiratory temperatures and the difference between the two. If volume is constant, Calorie loss is entirely dependent on the temperatures. If the temperatures are constant, Calorie loss is entirely dependent on volume.

Fig. 6 shows the relationship of minute volume to Calories lost for all temperature ranges. In this graph only the mouth-nose pathway of breathing is depicted.

Fig. 7 shows the same relationship as Fig. 6 except the nose-mouth pathway is demonstrated.

Fig. 8 was drawn on the basis of a 7 liter minute volume. That is to say Calorie loss is plotted against inspiratory temperature with a constant minute volume of 7 liters. All three pathways of breathing are represented. It is seen that more heat is lost from the body in heating the same volume (7 liters per minute) by means of the mouth-nose and nose-mouth pathways than are lost by way of the mouth-mouth avenue of breathing. This relationship holds true for

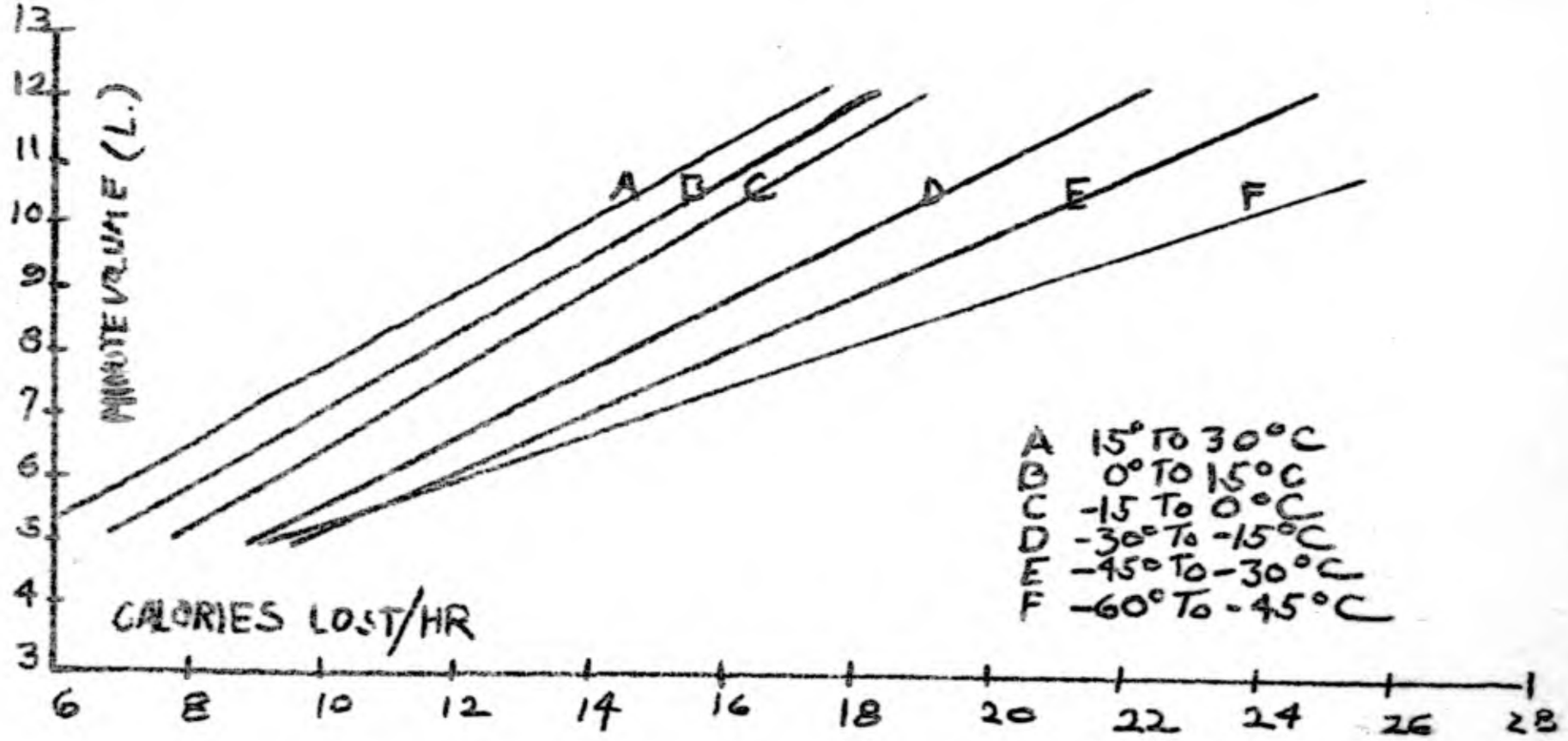


FIG. 5 Calorie loss based on minute volume for mouth-mouth breathing.

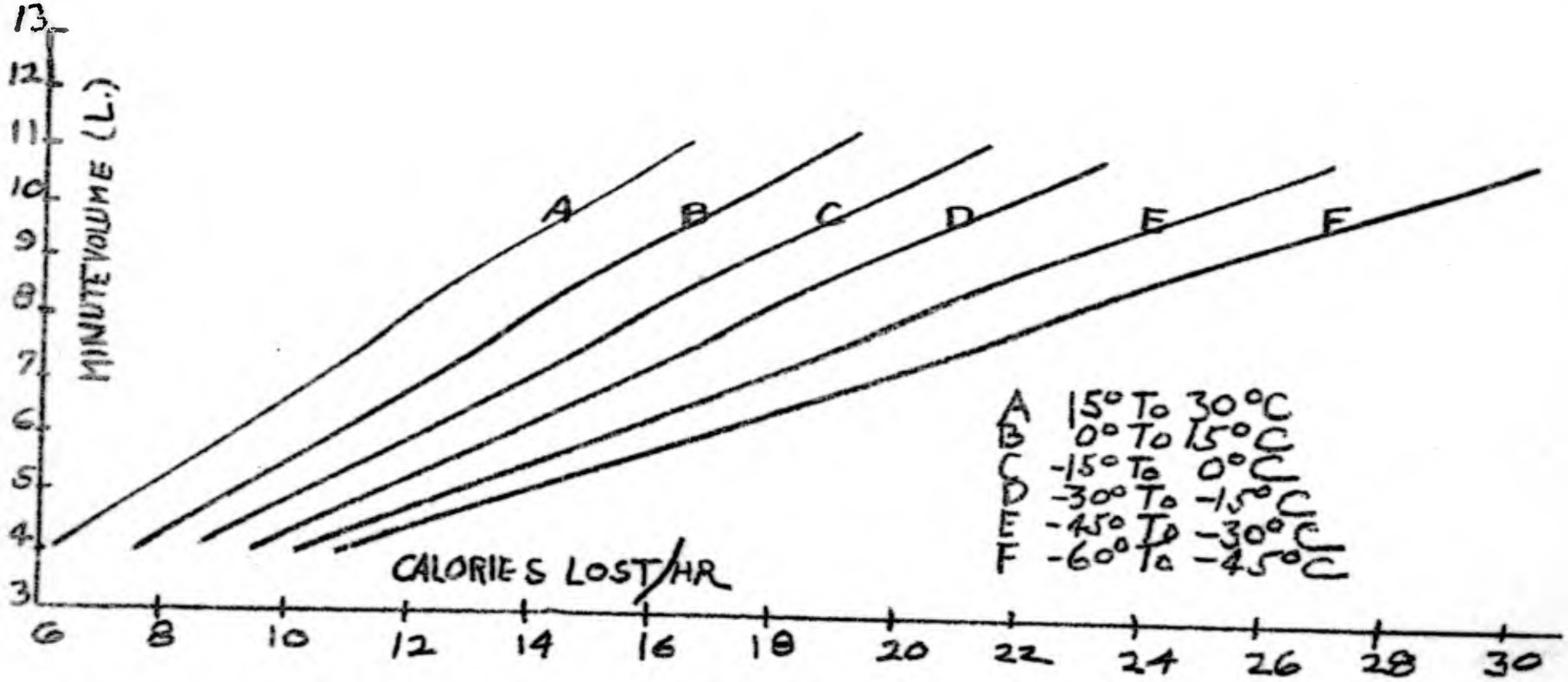


FIG. 6 Calorie loss based on minute volume for mouth-nose breathing

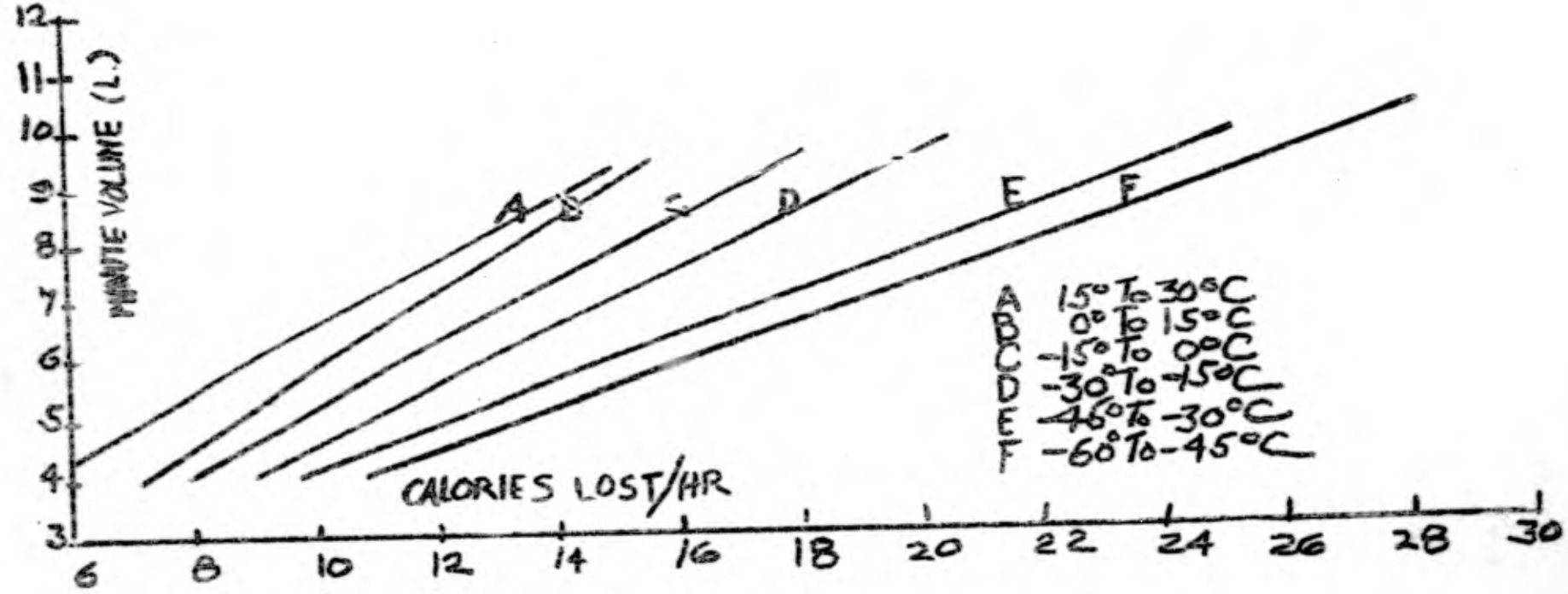


FIG. 7 Calorie loss based on minute volume for nose-mouth breathing.

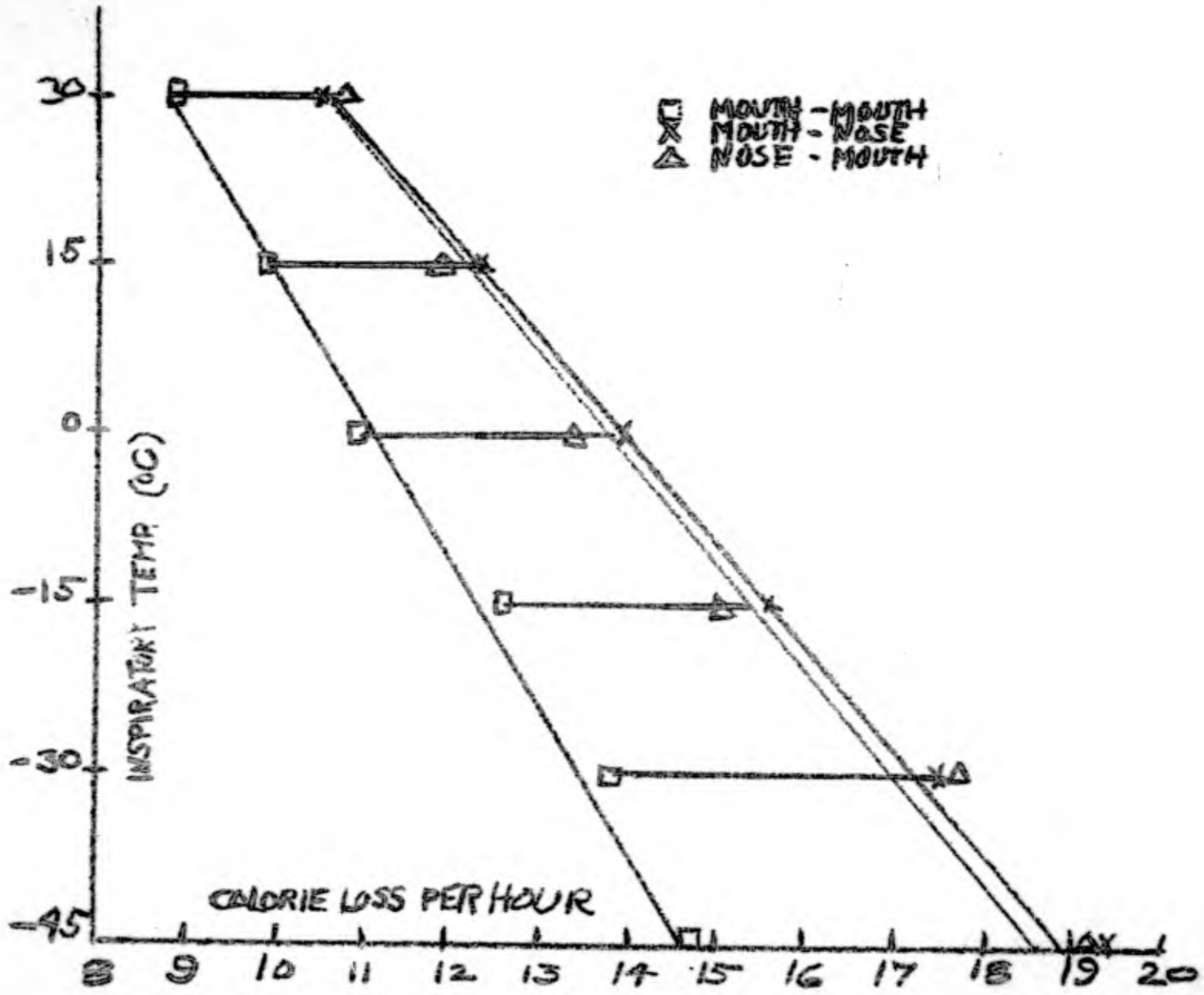


FIG. 8 Calorie loss per hour at various inspiratory temperatures with volume constant at 7 liters per minute.

all volumes, but the curve is shifted to the left or right as Calorie loss is dependent on volume. It was seen in Fig. 5 that the mouth-mouth pathway was not capable of heating the inspired air to as high temperatures on expiration as the nose-mouth or mouth-nose channels. It may, therefore, be stated that the mouth-mouth avenue of breathing is not as an efficient mechanism for heating inspired air as the nose-mouth or mouth-nose pathway. It may further be concluded that the nose-mouth and mouth-nose pathways are approximately equally capable of heating inspired cold air. This relationship holds true for any volume.

From these results it would appear that the cold air on passing the oral mucosa absorbs heat from this surface thus cooling the mucosa. On expiration, as the warm air passes the oral mucosa it looses heat to the oral surface thus being cooled. The expiratory temperature would be lower than body temperature and the Calorie loss, based on expired temperature, would also be low. However, in the nose-mouth and mouth-nose pathways of breathing, the breathed air does not pass the same surface on inspiration and expiration, so the heat transfer referred to above does not occur. Therefore, both the expiratory temperature and Calorie loss would be higher as a result of air passing by way of these channels than it would be for the mouth-mouth avenue.

On this basis nose-nose breathing, although there are no results from this experiment on this pathway, would appear to present approximately the same expiratory temperature-Calorie loss relationship as mouth-mouth, since in either type of breathing, the inspired and expired air pass the same surfaces.

In the foregoing the heating capacity of the trachea and bronchioles has been disregarded in discussing the advantages of the various avenues as these organs are common to all pathways. However, if the air is still cold by the time it reaches these organs, it will be warmed by them on inspiration. Expired air will give up its heat to the organ surfaces, which have previously been cooled on inspiration.

In situations where heat economy is paramount, it would be more advantageous to breathe by way of the mouth-mouth pathway as less heat is lost from the body by this avenue. (Theoretically, the nose-nose avenue is equally efficient.)

It is apparent that the degree of vascularization of the various surfaces over which the air passes is directly related to heat loss and temperature maintenance. If the blood supply to a cooled surface is minimal, it will remain cool as compared with a surface which has a maximal blood supply.

No subject reported subjective symptoms after exposure that would indicate severe pathological damage. However, there were several complaints of sinus headaches and irritation of the mucosa of the throat several hours after the exposure.

CONCLUSIONS

It is concluded from this experiment that with a decrease in temperature of inspired air there is a corresponding decrease in the temperature of expired air. The degree to which this results is dependent on the pathway over which the air is breathed. The mouth-mouth channel results in a lower expiratory temperature than nose-mouth or mouth-nose breathing. The expiratory temperatures of the latter two pathways is approximately the same for all inspiratory temperatures. At no inspiratory temperature between -60° and 30°C. will expiratory temperature reach normal body temperature. Expiratory temperature is essentially independent of the volume of air breathed.

Calorie loss due to heating and humidifying respired cold air is directly proportional to the volume of air respired if expired temperature is constant. Calorie loss is directly proportional to expired temperature if volume is constant.

The pathways of respiration have a significant effect on Calorie loss. There is little difference in Calorie loss between mouth-nose and nose-mouth breathing, but the mouth-mouth avenue results in a lower heat loss as demonstrated by Calories lost and expired temperature.

No severe pathological damage is inflicted on the respiratory system by respiration of cold, dry air at the temperatures and ventilation rates used.

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AN APPARATUS FOR THE DETERMINATION OF THE RESPIRATORY QUOTIENT AND METABOLIC RATE OF LABORATORY ANIMALS

By

Roscoe Gardner Bartlett

INTRODUCTION

New methods and apparatus for measuring the gaseous exchanges during metabolism of laboratory animals are of interest to the physiologist. Most of the apparatus and many of the methods used are too complicated and inaccurate for satisfactory results. It is, therefore, the purpose of this paper to describe a type of apparatus which is simple and dependable and which has been designed primarily for use with the common laboratory animals. A brief survey of the literature will serve to indicate the apparatus at present available for the determination of the respiratory quotient and the matabolic rate.

The use of the direct calorimeter of Atwater and Benedict (1) is precluded in the ordinary laboratory because of the elaborate nature of the apparatus. General laboratory facilities lend themselves more readily to indirect calorimetry, i.e., the determination of the heat production of an organism from its respiratory exchanges. The open-flow apparatus of Haldane (7) is readily constructed in the laboratory. However, serious error in the calculation of the respiratory quotient and metabolic rate is often encountered because of the many weighings that must be made. Since the gaseous exchanges of an animal during metabolism are a small percentage of the total gases handled by the animal, any apparatus of the open-circuit limits the accuracy of the results.

Apparatus used in the closed-circuit method are of several types. In the most common type CO_2 is determined by increase in weight of a sodalime tube and a known amount of CO_2 is introducted to replace that used by the animal. This type of apparatus is often combined with the direct calorimeter as used by Atwater-Rosa-Benedict. In some closed-circuit methods, Koehler (10), Davis and VanDyke (4), only O_2 consumption is measured. In these apparatuses, CO_2 is absorbed and O_2 metered into the chamber to replace that used by the animal. In another type the volume of CO_2 is measured by titration either of excess alkali or of carbonate, and a known volume of O_2 is introduced (18), (9), (15), (16). Werthussen (17) measures O_2 by current used in producing it electrolytically and CO_2 by rate of change of conduction. Lewis and Luck (13) measured CO_2 by conduction and O_2 by displacement. The apparatus designed by Chapman et al (3) which measures only O_2 consumption by displacement required that the animal be placed in a restraining jacket. Restraint of this nature has been shown by Phillips, Goff, Bartlett, and Grollman in unpublished data to effect a drop in body temperature which necessarily has a corresponding effect on metabolism. Various devices for recording body movement have been added to some apparatuses in order to ascertain the degree of activity (17), (13). In the use of most respiratory quotient apparatus, temperature critically affects the results. This may be a distinct disadvantage as temperature changes are hard to avoid and difficult to measure. In the apparatus to be described, temperature is not a critical factor. There is, moreover, only one piece of equipment to be weighed.

DESCRIPTION OF APPARATUS

The arrangement of the parts may be seen diagrammatically in Fig. 1. An electrolytic cell with drying tubes fitted with ground-glass joints comprises the electrolysis unit. The anode is shorter than the cathode and both are of platinum. The electrolysis unit is connected to the animal chamber by soft rubber tubing and ground-glass joint. The animal chamber is constructed of a large ground-glass joint approximately 2½ inches in diameter. The overall height of the animal chamber is approximately 6 inches. The CO_2 absorbing agent is placed in the well. A waxed screen wire cone with crumpled filter paper is placed on the supporting teats in the glass above the CO_2 absorber. The animal is placed on a hardware-cloth floor. The top of the large ground-glass joint is fitted with a thermometer. The entire animal chamber is immersed in a water bath.

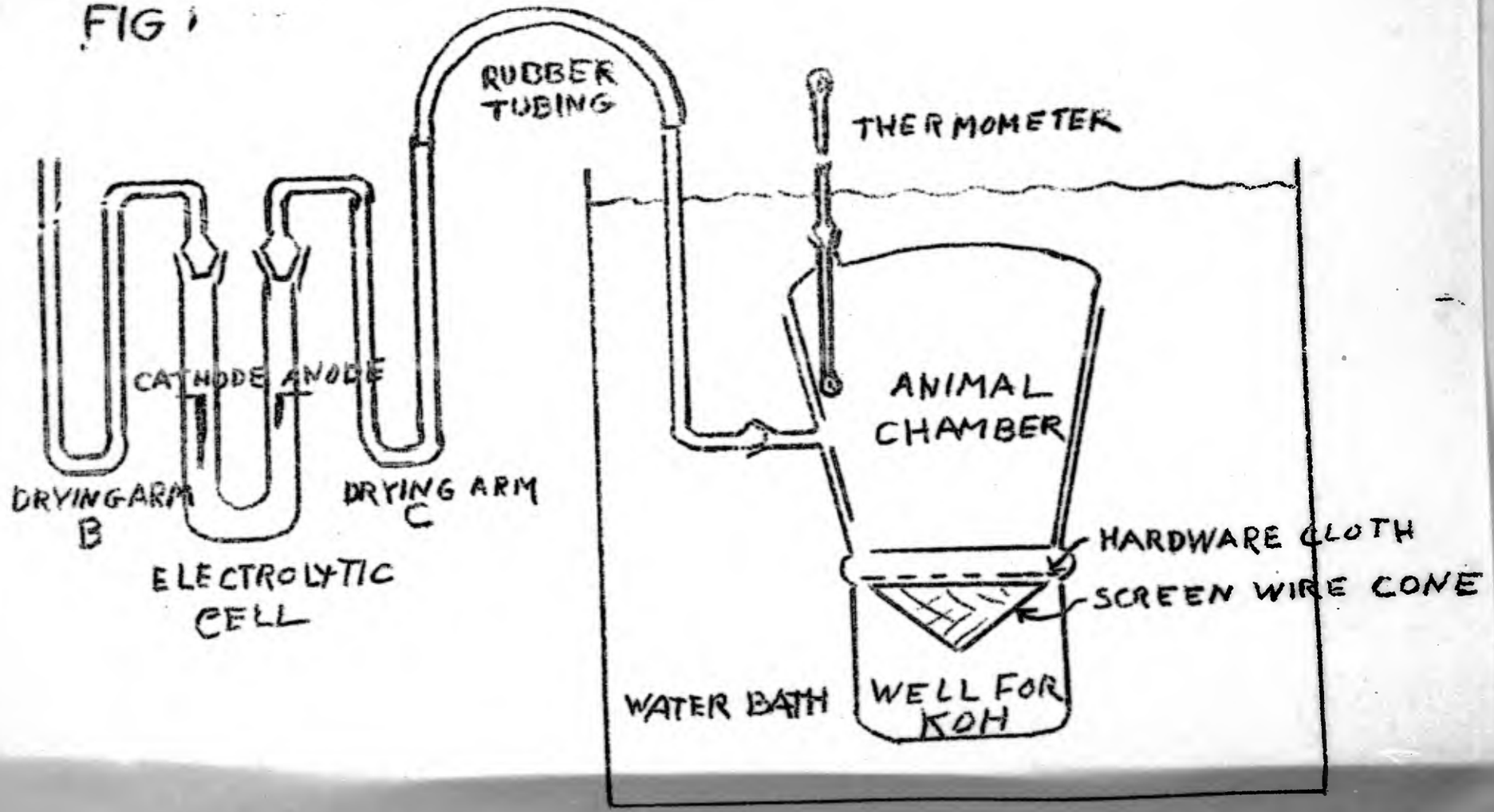
OPERATION OF APPARATUS

In operation, the apparatus worked as follows: As the animal, during metabolism, gave off CO_2, this gas was quantitatively absorbed by the KOH in the base of the apparatus. The used O_2 reduced the tension of the gases in the chamber below atmospheric pressure and thereby caused the level of the anolyte to rise on the anode, thus completing the electric circuit. O_2 was thereby released and by way of drying arm C and the soft rubber tubing reached the animal chamber and replaced that used by the animal. Subsequent titration of KOH in the chamber and weighing of the electrolysis unit made possible the determination of the gaseous metabolism of the animal.

It was necessary to carry out certain detailed procedures in the operation of the apparatus. For instance, at the beginning of the experiment the tip of the anode was just immersed in anolyte. After the electrolysis unit was weighed, placed in position and connected to the source of electric power, the rubber tube was connected to the drying arm C. Rubber tubing was used for this connection so that the animal chamber could be raised and lowered in the water bath. Then the alkali for absorbing CO_2 was placed in the well. This was done last to reduce absorption of CO_2 from the atmosphere. The screen wire cone and filter paper for collecting feces and absorbing urine, and the floor J were then put in place. The animal was introduced and the top of the chamber put into place <u>before</u> the ground-glass joint was closed. If the top of the animal chamber was placed on last, the increased air pressure forced the anolyte over into drying arm B. This formed a water plug and the apparatus would not function.

When the animal consumed O_2 and the produced CO_2 was absorbed in the alkali, the decreased air pressure raised the level of the anolyte on the anode and the O_2 thereby produced and released replaced the O_2 consumed by the animal. The anode for this reason was

FIG 1


RUBBER TUBING
THERMOMETER
CATHODE
ANODE
ANIMAL CHAMBER
DRYING ARM B
DRYING ARM C
ELECTROLYTIC CELL
HARDWARE CLOTH
SCREEN WIRE CONE
WATER BATH
WELL FOR KOH

made shorter than the cathode to keep the pressure in the animal chamber as near atmosphere as possible.

Temperature in the use of the apparatus was not a critical factor but had to be the same at the end as at the beginning of the run. If a temperature change occurred, the O_2 volume had to be corrected correspondingly. Any volume change caused by a temperature change was compensated for by the electrolysis unit. In order to correct the O_2 volume, the volume of the air in the system had to be known. For example, if there was 1 ml. expansion of the gases, the animal used 1 ml. more O_2 than was indicated by the change in weight of the electrolysis unit; and, likewise, if there was 1 ml. contraction due to a drop in temperature, the animal used 1 ml. less O_2 than was indicated by the weight changes. A correction factor for this effect could be established for use if such temperature changes occur. This need be no more accurate than the actual volume changes compared to the quotient of the chamber volume divided by 273 times each degree in temperature. There is an additional expansion of gases near the electrolysis unit caused by the generation of heat, but the volume changes were slight and were within the range of weighing accuracy.

The duration of the run depended on the amount of alkali and electrolyte. A run of one to two hours gave sufficient gaseous exchange for an accurate measurement.

DEVELOPMENT OF APPARATUS

In designing the apparatus, the size and shape of the animal chamber was of apparent importance. It had to be of such design that diffusion of gases would in no way be impeded and of such a size that the CO_2 unabsorbed at any time would not be of sufficient volume to affect the calculated R.Q. In the apparatus, the use of circulators or stirring meters in the animal chamber was avoided. This necessitated that the air in the chamber be close enough to the KOH that by diffusion alone the CO_2 would be quickly absorbed. Although there is no CO_2 tension above an alkalin solution of .2 normality yet there was of necessity some CO_2 on the way to the absorbing agent. To fulfill these requirements and to offer maximum ease of handling, a 71/60 ground-glass joint was pulled off to a well for the CO_2 absorber below and likewise above to hold a thermometer fitted with a ground-glass joint. This chamber contained no more than 300 cc of gases while it was in operation.

A similarly obvious problem was a means of catching urine and feces without interference with the adequate diffusion of gases. Urine and feces could not be allowed to drop directly into the alkali because excess alkali was titrated with standard acid for CO_2 volume and urine and feces would obviously change the hydrogen ion concentration. After several attempts with other types of wells, a cone made of wax coated screen wire proved adequate. Crumpled filter paper was placed in the tip. Feces, of course, could pass through the screen wire and the urine ran down the waxed wire to the tip where it would drop into the alkali below if it were not absorbed by

the filter paper. A small wad of filter paper adequately absorbed the urine from a mouse over a period of two hours. The screen wire cone offered practically no obstruction to the complete diffusion of gases.

The construction of the electrolytic cell and drying arms presented further problems. The entire electrolysis unit, electrolytic cell and drying arms, had to be light enough to be weighed accurately to the nearest milligram and yet contain sufficient electrolyte for the production of oxygen and calcium chloride for the absorption of water vapor. A unit which gave satisfactory results consisted of a U tube electrolytic cell and two U tube drying arms. The electrodes were of platinum sealed into pyrex. The anode was shorter than the cathode for reasons previously discussed. The vertical U tube drying arms prevented channeling. This reduced to a minimum the amount of calcium chloride that must be used for complete absorption of water vapor in the escaping gas. Since the animal used only .2 to .3 grams of water in a two hour run, it is easily seen that the unit had to be constructed on small dimensions.

Only certain electrolytes could be used in this electrolysis procedure. Electrolytes which under certain conditions of temperature, concentration, or electrolytic dissociations released gases harmful to the animal at the anode had to be avoided. Electrolytes which caused excessive foaming or which lost their conductive power could not be used. For example, when H_2SO_4 was used under certain conditions, it gave off SO_2 at the anode. When KOH was used, it lost its power of conduction in a relatively short time. One normal NaOH was found to be a suitable electrolyte. It did not lose its conductivity and no harmful substances were produced at the anode.

Alkaline solutions which formed an insoluble carbonate with CO_2 and tended to form a film on the surface of the alkali preventing further absorption of CO_2, could not be used to absorb CO_2. Further, the alkali had to be of a concentration that could be titrated accurately and yet could absorb CO_2 readily. .2 normal KOH was found to be suitable. Fifty milliliters absorbs the CO_2 produced by a mouse in a one and one-half hour run and could be titrated accurately.

The titration procedure itself posed some difficulties. When KOH and K_2CO_3 were titrated against an acid with phenophthalein as an indicator, the endpoint was difficult to determine accurately. As the solution approach a pH of seven the K_2CO_3 took on a hydrogen to become $KHCO_3$. This made an endpoint which faded and was difficult to ascertain. To check this endpoint, methyl orange was also added and the solution titrated to a second endpoint. This drives off the CO_2. This procedure, however, was abandoned because the phenopthalein endpoint was used in both calculations of CO_2 volume. The procedure finally adopted was the precipitation of the carbonate with $BaCl_2$ and titration of the remaining KOH to a phenophthalein endpoint. This was sharp and easily detected.

CALCULATION OF RESULTS

A. Oxygen consumption

Oxygen consumption was calculated from the loss in weight of the electrolysis unit. The entire loss of weight represented only a loss of H_2O as O_2 and H_2 since water vapor given off with these gases was absorbed in $CaCl_2$ in the drying tubes. Sixteen-eighteenths of this loss obviously was O_2. Since 32 grams of O_2 occupies 22.4 liters at standard conditions, 1 liter of O_2 weighs $\frac{32}{22.4}$ or 1.428 grams. Therefore, the volume of O_2 consumed by the animal calculated at standard conditions was computed from the proportion, 16/18 of the loss in weight of electrolysis unit: x : 1.428 : 1000 ml; when x equals the O_2 consumed. By simplification, the formula becomes volume of O_2 consumed equals $\frac{16000 \times \text{loss in weight of electrolysis unit}}{27.304}$.

B. Carbon-dioxide production

The carbon-dioxide produced was determined by titration of the remaining standard alkali with standard acid. Fifty ml. of N /5 KOH was originally pipetted into the chamber. At the completion of the run, the K_2CO_3 was reacted with an excess of $BaCl_2$ to give KCl and $BaCO_3$, a soluble neutral salt and an insoluble carbonate, neither of which took part in the subsequent titration of the remaining KOH with a standard acid. In the present experiment, N/10 H_2SO_4 was used as standard acid but a higher concentration of acid would have given equally acceptable results. The reaction in the absorption of CO_2 was as follows: KOH plus CO_2 ---> K_2CO_3. Therefore, 2 KOH reacted with 1 CO_2. Since one milliequivalent weight of CO_2 was equal to 2.24 ml. of the acid, therefore each milliequivalent of KOH reacted with 1.12 millliliters of CO_2. The volume of CO_2 then calculated at standard conditions was 1.12 times each milliequivalent of KOH bound in K_2CO_3. Since a known amount of standard KOH was used, the volume of CO_2 produced by the animal could be calculated after titration. Phenolphthalein was used as an indicator.

A typical example of the calculation will illustrate. An animal, #13, after a fast of unknown duration, was run in the apparatus for 1.5 hours. There was no temperature change. The weights of the electrolysis unit before and after the run were respectively 72.4394 and 72.2579. The loss in weight was .1815 grams. Sixteen-eighteenths of .1815 = .1613. $\frac{.1613}{x} = \frac{1.43}{1000}$ x = 112.82 cc of O_2 consumed by the animal. Fifty cc of N/5KOH were pipetted into the well just prior to the run. At the completion of the run, 3 grams of $BaCl_2$ (an excess) were placed in this KOH. A safe margin was added because the excess would not affect the titration. After thorough shaking, the excess KOH was titrated with N/10 H_2SO_4 of which 22.80 cc were used. Thus, 77.20 milliequivalents of KOH had reacted with CO_2. 77.20 x 1.12 = 86.46 cc CO_2 formed by animal. The R. Q. was,

therefore, $\frac{86.46}{112.82}$ or .76. Additional calculations would give the metabolic rate of the animal during the run.

STANDARDIZATION OF APPARATUS

Customary techniques were found to be unsuitable for the standardization of the present apparatus. The smallest alcohol or acetone lamp which would keep burning had an oxygen consumption far above the oxygen generating capacity of the electrolytic cell and further the heat generated in a 300 ml. chamber caused such expansion of gases as to completely inactivate the electrolysis unit which was in essence a water manometer, by preventing contact of the anolyte with the anode. The increased pressure not only forced all the water into the cathode arm of the electrolytic cell but also pushed water into the attached drying arm which formed a water plug with the $CaCl_2$, thus further inactivating the electrolysis unit. In calibrating apparatus where similar difficulties prevail, combustible vapors are commonly passed over heated platinized asbestos. Since the present apparatus had neither an air circulator nor a circuit where a tube of platinized asbestos could be inserted, this standardization technique also was not applicable. No oxidization process with a known R. Q. was found with either heat production or oxygen consumption small enough to be used in standardizing the present apparatus.

As an approximate standardization, both R. Q. and metabolic rates were calculated for runs on fasting and non-fasting animals. Fasting animals commonly have an R. Q. approximately .71 and the metabolic rate is generally about 16 cal/gr/hr. These figures are surprisingly constant. Non-fasting animals have R. Qs. and metabolic rates above those of fasting animals. These results appear in Table I. Both the R. Q. and metabolic rates were well within the range of possibility and approximated the theoretical values. As apparent in the table, they have small standard deviations. The results which appear in the table are not a selected group but are the results of all the completed runs.

The titration for CO_2 volume is standard procedure in other methods for R. Q. determination. The titration is accurate to 1 part in 4464 using N/10 H_2SO_4 and reading the burette to .02 ml. if the animal produces 100 cc of CO_2. All of the CO_2 produced by the animal was not immediately absorbed by the alkali. A fraction of the CO_2 produced by the animal had not yet reached the KOH at any given time. This CO_2 was, of course, not accounted for in the subsequent titration. It should be mentioned that this is an inherent error in this apparatus and method which cannot be altogether eliminated by design or technique. It is, however, possible to establish a correction factor for each set of apparatus. This correction factor is the volume of CO_2 which is not absorbed at any particular time. This volume is calculated from the % CO_2 present in the chamber times the chamber volume.

If this correction factor cannot be calculated because of lack of additional apparatus, it is noted that the error is a constant

TABLE I R. Qs. AND METABOLIC RATES OF FASTING AND NON-FASTING ANIMALS

Animal Number	Nutritive Condition	Wt.	R.Q.	cal/gr/hr	Metabolic Rate cal/sq.meter/hr	cal/hr
1	nonfasting	22.9	.873	17.86	23.559	409.20
10	"		.79			296.86
11	"		.76			300.20
12	"		.943			307.26
13	"		.78			296.86
16	"		.76			311.22
2	fasting	24.95	.719	14.92	20.234	372.301
3	"	26.6	.734	15.25	21.133	406.74
4	"	19.0	.733	19.62	24.107	372.8
5	"	20.3	.693	16.35	20.705	331.92
9	"	24.05	.729	16.28	21.864	391.594
17	"		.719			262.21
15	"		.72			297.23
14	"		.704			323.18
6	long fast	26.1	.66	15.36	22.879	400.62
7	"	22.2	.663	16.62	21.215	361.08
8	"	18.2	.64	20.06	24.488	365.13

$\bar{X}_{R.Q.}$ nonfasting .814

$\bar{X}_{R.Q.}$ fasting .719

$\bar{X}_{R.Q.}$ long fast .654

$\bar{X}_{cal/gr/hr}$ fasting 16.48

$\bar{X}_{cal/gr/hr}$ long fast 17.34

$\bar{X}_{cal/sq.\ m./hr}$ fasting 21.61

$\bar{X}_{cal/sq.\ m./hr}$ long fast 22.86

$\bar{X}_{cal/hr}$ 338.36

for each set of apparatus. Therefore, in comparative data this error will cancel out. It was found in the present apparatus that there was a 1% differential of CO_2 in the animal chamber, i.e., the CO_2 increased 1% during the run. This, of course, had an effect on the calculated R. Q. and metabolic rate. This effect is tabulated in Table II. It is seen that the metabolic rate is affected more than the R. Q. The effect on the R. Q. and metabolic rate decreased as the R. Q. approached unity. Since this error is a constant, in any experiment in which controls are used the error will be virtually eliminated.

TABLE II THE EFFECT ON R. Q. AND METABOLIC RATE WHEN CORRECTION FACTOR IS IGNORED

Correct R. Q.	Calculated R. Q.	Calculated Metabolic Rate cal/gr/hr	Correct Metabolic Rate cal/gr/hr
.700	.690	20.499	21.264
.750	.742	20.771	21.541
.800	.793	21.048	21.823
.850	.845	21.329	22.100
.900	.897	21.605	22.364
.950	.948	21.886	22.659
1.000	1.000	22.172	22.947

Assumed that the animals used 100 cc O_2
If the correction factor was ignored, it appeared that the animal used only 96.65 cc O_2
Run of 1 hr. duration
Animal assumed to weigh 22 grams

The 1 % increase in CO_2 concentration was found to be established after 5 to 10 minutes from the initiation of the run and then to remain constant. This permitted the computation and use of a correction factor. The unabsorbed CO_2 occupied the space that should have been filled by generated O_2 and the amount of CO_2 present in the air in the animal chamber was known, thus it was possible to correct the data in order to avoid the errors discussed above. In the present experiment, there was 3.35 ml. of CO_2 present after the first 5 to 10 minutes above that which was present at the initiation of the run. To correct the volume of O_2 and CO_2 as determined by means previously discussed, it was necessary only to add 3.35 ml. to each volume. In all previous discussions and calculations of results in this paper, this correction factor has been ignored.

The method for measuring O_2 consumption is, to the best of the author's knowledge, new and original for determining the R. Q. of laboratory animals. Therefore, past research could not be relied upon, as in the titration procedure, for establishing the validity of the results. The weighing procedure was accurate to 1 part in 185 if the chamber was weighed to the nearest milligram and if the animal used 137 ml. of O_2. It would have been impractical to measure the O_2 produced by the electrolysis unit by volume since common measuring techniques are no more accurate than the weighing procedure. Of course, great care had to be exercised in the weighing technique to avoid errors from moisture, heat, and finger prints. While not in use, the drying tubes were stoppered by corks so as to prevent water absorption by the $CaCl_2$. There was one possible error in the procedure for computation of O_2 consumption. As before mentioned, when the last ground-glass joint was closed in the initiation of the run, a positive pressure was created in the animal chamber, thus forcing the electrolyte down on the anode side of the electrolytic cell. If the anolyte was forced below the anode, the first O_2 consumed by the animal was not compensated for by the unit. This was avoided by using a small ground-glass joint in the side of the animal chamber and using care in

closing this joint so as to create as little positive pressure as possible. Also, after some practice, experience taught how far above the lower tip of the anode the anolyte must be in order to be forced to the tip of the anode by the above mentioned positive pressure to that the first O_2 consumed by the animal was immediately and totally compensated for by the action of the electrolytic cell. A stop-cock venting arm might have been used to avoid the above pressure change but this would have further complicated the apparatus.

Table III shows the change in calculated R. Q. and metabolic rate due to different errors in measuring O_2 consumption. It is obvious that neither the R. Q. nor metabolic rate was seriously altered by slight errors in measuring O_2 consumption.

TABLE III TABLE OF EFFECTS ON CALCULATED R. Q. AND METABOLIC RATE OF POSSIBLE ERRORS IN MEASURING O_2 CONSUMPTION

Error	O_2	CO_2	R.Q.	Metabolic Rate
no error	124.50	90.72	.729	19.559 cal/gr/hr
1° rise in temp.	125.97	90.72	.737	19.828 cal/gr/hr
1° drop in temp.	123.03	90.72	.720	19.283 cal/gr/hr
O_2 unit under-weighed 2 mg	123.28	90.72	.736	19.400 cal/gr/hr
O_2 unit over-weighed 2 mg	125.73	90.72	.722	19.717 cal/gr/hr

The animal assumed to weigh 30 grams
Run of 1 hr. duration

SUMMARY

An apparatus and method for the determination of the metabolic rate and the respiratory quotient of common laboratory animals are described.

The animal was placed in a closed chamber over a wall of KOH which absorbed the CO_2 produced. The O_2 consumed by the animal was replaced by O_2 from an electrolytic cell and was produced automatically at the rate used by the animal. The anode of the electrolytic cell was shorter than the cathode and as the pressure in the chamber fell below atmospheric because of the O_2 used and the CO_2 absorbed, the anolyte rose up on to the surface of the anode thus completing the electrical circuit to produce O_2 which raised the pressure to atmospheric.

O_2 was measured by the change in weight of the electrolysis unit. This unit consisted in a U-shaped electrolytic cell and two U-shaped drying arms filled with $CaCl_2$. Thus, the only loss in weight of the unit was the loss of water as H_2 and O_2. Sixteen-eighteenths of this loss in weight, therefore, represented the weight of O_2 used by the animal.

The CO_2 produced was absorbed in standard alkali, .2N KOH. The carbonate was precipitated as $BaCO_3$ by adding an excess of $BaCl_2$. The excess alkali was titrated against standard acid using phenolphthalein as an indicator. N/10 H_2SO_4 was used as the standard acid.

Several compounds were tried as electrolytes. Such electrolytes as H_2SO_4, KOH, Hcl, and $Cacl_2$ were found unsuitable. 1N NaOH was found to be entirely satisfactory and was used in all the final experiments.

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SOME PHYSIOLOGICAL EFFECTS OF ADDED RESPIRATORY DEAD SPACE

By

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INTRODUCTION

The composition of alveolar air is determined by several methods, The Haldane Priestly method, its modification by Henderson and Morris (1), the Plesch method, its modification by Higgins, and methods by Linhard, methods by Peters and Van Slyke (2). Such methods give the average composition of the alveolar gases. It is the purpose of this investigation, by increasing the dead space, to measure the composition of alveolar air in human subjects and to show the effects of increasing the dead space on breathing functions, such as rate of respiration, minute volume, effective tidal volume, alveolar ventilation, and physiological dead space.

Studies on the physiological changes in the venous blood of humans, breathing increased dead space, have been made by Grolman (3). The volumes of added dead space used were 1000, 2000, and 3000 ml. Prior to these studies the volumes of added dead space used experimentally were within the range of 75 to 800 mls. Tommaso (4) found that with added volumes up to 200 ml. there was little if any change in the CO_2 or O_2 tensions, and that with added volumes of from 200 to 600 ml. there was an increase in CO_2 tension of 30%. He also verified that the alveolar tension of O_2 diminished in proportion to the increase of the CO_2. Beyne and Bergeret (5) using added dead space volumes of 675 and 800 mls. and using pure O_2 found increased CO_2 tensions and increased O_2 tensions.

Stannard (7), working with oxygen breathing equipment design, found that all dead space above 100 mls. lead to significant increase in inspiratory and expiratory pressure.

The subjects in the present experiment breathed normal air and the increases in dead space were large enough to show variations in respiratory factors of a higher magnitude than were evident in any previous work. Earlier work was conducted with added dead space of 70-800 mls. In the present experiment, added volumes up to 3000 mls. were employed. Grolman (3), Phillips, et al., (6) all have shown that the use of added dead space, in volumes comparable to those in this study, is an effective method of producing alterations in the CO_2 and O_2 tensions of inspired and expired air and in many other physiological relationships.

METHODS AND PROCEDURES

A four hundred liter capacity Tissot type spirometer as shown in Figure 1 was used for collecting expired air. A mouthpiece with valves was connected to this spirometer. The added dead space was produced by using lengths of rubber tubing between the valves and the mouthpiece as indicated by the broken section in Figure 2. Each length of tubing nineteen and one-half inches in length and one inch inside diameter contained a volume of 250 mls. By using different

numbers of these lengths of tubing connected by glass couplings, the desired amount of dead space was obtained. Small metal baffles were inserted in each glass coupling. Between the mouthpiece and the interior of the bell of the spirometer, there was a volume which included the tube leading to the bell and a fan well in the spirometer as shown in Figure 1. This volume amounting to three liters had to be considered in all calculations involving expiratory volumes. The fan in the spirometer was used to insure sufficient mixing of the gases in the spirometer.

A pneumograph was fastened around the subject's chest, by means of which the respiratory movements were recorded on a kymograph. This showed the rate and amplitude of respiration. A nose clamp was used to insure that breathing occurred only through the mouth.

Three males, whose age, weight, and height appear in Table III served as subjects. They were previously trained to breathe on the apparatus used in this experiment and no anxiety was incurred and breathing was as regular and as normal as possible for each individual under the existing conditions. The subjects were seated as comfortably as possible without any hindrance or strain during respiratory movements, and without any cause for fatigue due to standing.

At zero dead space, the subject drew room air through the inlet valve and into his lungs. During expiration the air passed through the outlet valve and into the spirometer bell through the connecting tube. As dead space was added between the mouthpiece and the valves, the subject inspired fresh room air through the added tube. Upon expiration the fresh room air that remained in the added tube was forced through the outlet valve into the spirometer bell along with the alveolar air that the subject expelled from the lungs. The alveolar air that remained in the tube at the end of the expiration was drawn back into the lungs along with fresh room air at the next inspiration.

Due to the limited capacity of the spirometer, it was necessary to vary the duration of the exposures at different amounts of dead space. The durations of the exposures were 20 minutes at zero dead space, 15 minutes at 500 mls., 10 minutes at 1000 and 1500, and 5 minutes at 2000, 2500, and 3000 mls.

After the subject had breathed into the spirometer the designated length of time, the fan was run for two minutes in order to thoroughly mix the air in the bell. Five samples were then withdrawn through a rubber stopper. A hypodermic syringe of 10 ml. capacity with a twenty gauge needle was used for drawing samples. The syringe was lubricated with glycerin. The needle was cemented to the syringe for withdrawing from the rubber stoppers or rubber tubing. The five samples that had been withdrawn were put into a mixing apparatus as shown in Figure 3, from which one 10 ml. sample was drawn for analysis on the Haldane-Henderson gas analysis apparatus. Since the expired percentage of CO_2 with the increased dead space became very low, every precaution was taken to insure as accurate a reading as possible from the apparatus.

The volume and temperature of the gas in the spirometer, the barometric pressure, the temperature of the room, and the number of respirations were recorded. The volume of gas in the spirometer was corrected to standard temperature and pressure conditions. No correction for water vapor pressure was used, as all samples were analyed over water and the air in the spirometer bell was over water. The respiration per minute, the tidal volume of expired air, the expired minute volume, the tensions of CO_2 and O_2 in expired air, were calculated. The R. Q. was calculated from the volume of CO_2 expired and the volume of O_2 used. The calories produced per square meter of body surface per hour were calculated by the formula: Vol. O_2 used/hr x cal/liters O_2 ÷ surface area = calories/sq.meter/hr.

The withdrawal of alveolar samples was begun two minutes before the end of each exposure. After a normal inspiration, the subject expired fully but normally and a sample was drawn at the end of this expiration. These samples were thus drawn successively after every other expiration until five such samples were obtained. These were mixed and analyzed as described above for samples drawn from the bell. The tensions of the alveolar gases were calculated. Alveolar R. Qs. were calculated from the percentages of O_2 and CO_2 in the alveolar sample by the equation: $\frac{\%\text{ of } CO_2 \text{ in sample} - 0.03}{20.97 - \%\text{ of } O_2 \text{ in sample}} = \text{R. Q.}$

Each subject made either one or two tests a day. If two tests were conducted on the same day, one was made in the morning and one in the afternoon. With respect to dead space volumes, the tests were made in a given sequence in order to attain appropriate sampling and to avoid the possible effects of training or accomodation on the part of the subjects. The sequence used was as follows: consecutively, two exposures were made on each subject with zero dead space, then with 500, 1000, 1500, 2000, 2500, and 3000 mls. of dead space. Two consecutive exposures were made beginning at 3000 ml. down to zero dead space. Finally, one exposure was made at each increment of dead space up to 3000 mls., thus completing the five exposures at each designated volume.

RESULTS AND DISCUSSION

The Effects of Added Dead Space on the Breathing Rate, the Minute Volume and the Tidal Volume.

The breathing rate varied for each subject as the dead space was increased. Subject C showed a definite tendency to increase the number of respirations per minute with each increase in dead space from 8.8 respirations per minute at zero dead space to 13.4 respirations per minute at 3000 mls. of dead space. However, he had 13.4 respirations per minute at 2000 mls. of dead space. The other two subjects showed an increase from zero to 500 mls. then a decrease from 500 to 1000 mls. with intermittent increasing and decreasing of respirations per minute with further increases of dead space as shown in Table 1. These figures show no consistent varia-

tion in the rate in relation to added dead space. It is obvious that since with added dead space there is an increase in ventilation rate this increase must be due not so much to a change in breathing rate but to a change in tidal volume. Thus, with added dead space to control the ventilation rate, the breathing rate is the same as under ordinary conditions also in which rate plays a minor role.

The minute volume of expired air increased with increased dead space for all subjects. Subject A increased from 10.41 liters per minute at zero to 40.08 liters per minute at 3000 mls. of dead space. Subject B increased from 7.95 liters per minute at zero to 36.14 liters per minute at 3000 mls. of dead space. Subject C increased from 7.44 liters per minute at zero to 40.22 liters per minute at 3000 ml. of dead space. The average of all subjects showed an increase from 8.60 liters per minute at zero dead space to 38.81 liters per minute at 3000 mls. of dead space. The average minute volume increased by four and one-half fold from zero to 3000 mls. of added dead space as shown in Table II. Tommaso has shown that ventilation increases with the increase of the volume of dead space by 1.80% with a dead space of 600 mls. (4). The normal ventilation rate is about 7 liters of air per minute at rest which may be increased up to about 100 liters per minute during violent exercise. The subjects were not entirely at rest but they were performing no muscular exercise except that involved in breathing and yet their minute volumes increased from 8.60 liters to 38.81 liters per minute. Thus it would indicate that by breathing through added dead space, the minute volumes simulate those obtained by performing violent exercise.

Graph 1 shown that the ventilation increases with the increase of dead space. The curve is a straight line, therefore interpolating volumes from the mouth, as zero, back toward the lungs, it is found that about minus 800 ml. the expired volume of gases per minute would be zero. Since the volume of anatomical dead space has been determined to have an average volume of about 150 mls., the true zero point for expired minute volume should lie somewhere between the respiratory bronchioles and the blood capillaries in the alveoli. The capillary wall of the blood vessels where the exchange of gases occur would seem the logical zero point.

The volume of air moved into or out of the lungs during inspiration is called tidal air. With added dead space, the effective tidal volume is the tidal volume minus the amount of dead space. The average tidal volume of the three subjects increases two-fold at 1000 ml. of added dead space, three-fold at 2000 ml., and four-fold at 3000 mls. as shown in Table II.

From the discussion on breathing rate, it has been shown that the breathing rate did not increase in proportion to the increase in ventilation rate, therefore, the tidal volume must increase. The tidal volume did increase with dead space as shown in Tables I and II. However, this volume is used up overcoming the added dead space so that as the dead space increases the effective tidal volume decreases until at the higher volumes the effective tidal volume has a negative value.

Graph 2 shows that the effective tidal volume decreases as the dead space is increased. The decrease, from zero to 1000 mls. of dead space, is even but not rapid. From 1500 to 3000 mls. the decrease is constant and more rapid. At 2500 mls. the effective tidal volume is negative indicating that fresh air is not entering the lungs with each inspiration. If no fresh air is entering the lungs, then the p CO_2 (partial pressure of the CO_2) of the alveolar air should increase while the p O_2 decreases. The p CO_2 in the venous blood increases because it comes to equilibrium with the p CO_2 of the alveolar air. Therefore, in spite of an increase in the ventilation rate, a state of gaseous acidosis results because there is a decrease in the pH of the blood. This can be seen from the Henderson-Hasselbach formula: $pH = pK_1 + \log \frac{(HCO_2)}{CO_2}$. If there is an increase in the p CO_2 of the blood, there will be an increase in the HCO_3, thus the ratio $\frac{(HCO_3)}{(CO_2)}$ will decrease, and the acid-base balance of the blood will shift toward the acid. The acidosis described above will not increase the alkaline reserve since this occurs as HCO_3 increase of the blood when p CO_2 = 40 mm Hg as when there is an absorption of alimentary alkali or by elimination of fixed acids. Since the H_2CO_3 does not dissociate, the CO_2 in the tissues cannot enter the blood. Consequently, the metabolic activity of the tissues will decrease unless the CO_2 is removed from the blood, or allowance made to increase the CO_2 in the blood.

Since the duration of exposure with large amounts of dead space is short, the above may not occur as a period of compensation to CO_2 excess probably exists, because in this experiment little, if any, decrease in metabolic activity was found.

Alveolar Changes with Added Dead Space

From the data in Table I, all subjects showed an increase in the percentage of the CO_2 of alveolar air with increased dead space and a decrease in the percentage of O_2 in alveolar air. Two subjects, B and C, showed a decrease of CO_2 at 500 mls. while subject A showed a decrease at 1000 mls. of added dead space. The average composition of alveolar air varies somewhat in different subjects and in the same subject at different times. From the data obtained in this experiment, it can be seen that on a basis of fifteen exposures the average composition of the alveolar CO_2 at the end of a normal but full expiration as taken at the mouth showed little, if any, increase from zero to 1000 ml. of dead space. Therefore, if we interpolate from zero back to minus 500 to 1000 ml. of dead space which is somewhere within the alveoli. the composition of the CO_2 should be the same as shown in Graph 3. Since the subject is not getting any fresh air and composition of the alveoli remains constant in CO_2, there should be no physiological dead space.

Physiological dead space has been described as the total space within the lungs which just prior to expiration contains undiluted air, that is air which has not diluted the alveolar air or come into

contact with the respiratory epithelium. However, the fresh air that is drawn into the lungs passes down the respiratory tract in a turbulent fashion due to the branching of the respiratory passages. In the respiratory areas, it diffuses and mixes with the residual air in the alveoli. This mixture should come to equilibrium, due to the diffusion of O_2 into the blood. The percent of CO_2 in the mixture becomes increased and the percent of O_2 is reduced at the area lining the walls of the alveoli. Since the alveoli are microscopic in size and the diffusion of gases rapid, the composition of each gas within each alveoli should be uniform throughout the alveoli.

As expected from the preceeding discussion on tidal volumes, the alveolar CO_2 tensions increased and the alveolar O_2 tensions decreased for all subjects, from zero to 3000 mls. of added dead space. The average of all exposures showed the alveolar CO_2 tension increasing from 36.74 mm of Hg at zero to 53.16 mm of Hg at 3000 ml. of added dead space. The average O_2 tension of all exposures showed a decrease from 131.98 mm of Hg at zero to 78.46 at 3000 ml. of added dead space.

The average alveolar tensions in man, without dead space, range from 100 to 120 mm of Hg for O_2 and from 35 to 45 mm of Hg for CO_2. Conroe and Dripps (8) found variations in p O_2 between 86 and 104 mm of Hg at the end of quiet expirations and between 99 and 112 mm of Hg at the end of quiet inspirations. The tensions of O_2 and CO_2 in this experiment with dead space show values of CO_2 increasing above the normal range and values of O_2 decreasing below the normal range. Apparently, therefore, the tension of alveolar air can be modified by increasing the amount of dead space. How much each individual varies from these normal values depends upon his minute volume, tidal volume and vital capacity. This is to say his ability to overcome the effects of the added dead space.

The Effect of Added Dead Space on R. Q. Values and Metabolic Rate

Both alveolar R. Q.'s calculated from spirometer samples showed a drop as dead space was added. The alveolar R. Q.'s were lower than the spirometer values. The average alveolar R. Q. at 3000 mls. was .66. Many of the R. Q. values were over 1.00 especially those of spirometer samples at the smaller amounts of added space. These can be seen in Tables I and II. The decrease in alveolar R. Q. produces a curve as shown in Graph 4.

The percent of CO_2 in the alveoli increased but the percent of CO_2 in the expired air decreased indicating that the CO_2 being produced is not being blown off. The data show that the R. Q. of both the expired and the alveolar air decreased with added dead space. Since the R. Q. decreased, it means that either the CO_2 and the O_2 used increased or the CO_2 decreased and the O_2 remained constant. However, if the CO_2 and O_2 both decreased, the CO_2 must have decreased relatively more than the O_2. This is the most likely explanation of the relationships found in the present experiment.

If the R. Q. decreases and the CO_2 in alveolar air content increases and O_2 used decreases, then the metabolic activity should

decrease. However, in this experiment, the metabolic rate did not decrease. Hence, there must have been a retention of CO_2 by the body. If so, did it occur in the buffer system of the blood or was it retained in the tissues? If it was retained in the blood, it caused an increase in the bicarbonate. This could have been possible as the duration of the exposures in this experiment was short. If the CO_2 was retained in the tissues and the metabolic rate did not decrease, then the metabolism taking place must have been anaerobic.

The metabolic rates did not show any great increase or decrease with added dead space.

Relationship of Vital Capacity with the Effect of Dead Space on Minute Volume and Tidal Volume

From Table III, the vital capacity of subject A was 5700. Subject B had a vital capacity of 4333. Subject C had a vital capacity of 5133 mls. Subject A who had the highest vital capacity had the lowest tidal volume and the lowest effective tidal at 3000 mls. of added dead space. Subject C who had the next highest vital capacity had the highest tidal volume and the highest effective tidal volume at 3000 mls. of dead space.

Subject A had the highest expired minute volume at zero dead space while Subject C had the lowest expired minute volume at zero dead space. At 3000, the minute volumes of both Subjects A and C were equal.

From the above results, it would seem that the vital capacity was not directly related to the respiratory functions. However, it did seem to have been involved in overcoming the dead space.

CONCLUSIONS

Due to breathing added dead space, the subjects showed the following physiological changes:

1. An increase in the amount of dead space produced little increase in the rate of breathing.

2. The minute volume increased as the dead space was increased. The volume increase, from zero to 3000 mls. was 4.5 times. Also, the place where there is zero minute volume is at about a value of minus 800 mls. This point would lie somewhere within the respiratory areas of the lungs, probably at the lining of the alveoli.

3. The tidal volume increased as the dead space increased. However, most of the increase in tidal volume was used up in overcoming the added dead space, so that the average effect in tidal volume decreased from 715 at zero to minus 180 mls. at 3000 mls. Thus indicating that the subjects were not getting fresh air into the lungs at 3000 mls. of dead space.

4. The percent of alveolar CO_2 increased from 4.8 at zero dead space to 7.0 at 3000 mls. of added dead space. The percent of alveolar O_2 decreased from 16.0 at zero to 10.3 at 3000 mls.

5. The tension of alveolar CO_2 increased from 36.74 mm of Hg at zero to 53.16 at 3000 mls. of added dead space. This is an increase of about 1.5 times. The tension of alveolar O_2 decreased from 122 mm of Hg at zero to 78.5 mm of Hg at 3000 mls. This is a decrease of about 1.5 times.

6. The R. Q. values decreased as dead space was added. The alvolar R. Q.'s were lower than the spirometer R. Q. Alveolar R. Q.'s at 3000 mls. of dead space were .7.0 or lower. Spirometer R. Q.'s were 1.0 or above for dead spaces of zero, 500, and 1000 mls. of added dead space. The decrease of R. Q. to .7.0 indicates that the subjects should have been metabolizing fats entirely. But since the metabolic rate did not increase or decrease, the subject may have been metabolizing proteins or carbohydrates anaerobically.

7. The metabolic rates did not increase or decrease with added dead space.

The data obtained in this experiment shows that the subjects did not receive fresh air into the lungs with higher volumes of added dead space, and that the percentages of alveolar CO_2 and O_2 were constant for dead spaces up to 1000 mls. Hence, there was no physiological dead space because the air in the alveoli was in equilibrium with air coming into the alveoli.

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Collected and Calculated

Subject	Added Dead Space mls.	Expired Volume at S.T.P. liters	Duration of exposure min.	Respirations per min.	Expired vol. per min. liters	Tidal Volume Liters	Effective Tidal Volume Liters	% CO_2 in expired air
	0	208.2	20	16.5	10.41	.631	.631	2.49
	500	261.0	15	17.0	17.40	1.024	.524	1.45
A	1000	202.0	10	13.5	20.20	1.496	.496	1.31
	1500	277.2	10	15.5	27.72	1.788	.288	.98
	2000	168.2	5	15.8	33.64	2.130	.130	.79
	2500	175.8	5	14.0	35.20	2.514	.014	.67
	3000	200.4	5	15.0	40.08	2.672	-.328	.42
	0	159.0	20	11.9	7.95	.668	.668	3.17
	500	197.2	15	12.3	13.15	1.067	.569	1.96
	1000	170.3	10	11.4	17.03	1.494	.494	1.51
B	1500	231.7	10	13.1	23.17	1.769	.269	1.01
	2000	116.1	5	10.8	23.22	2.150	.150	.96
	2500	145.3	5	12.4	29.06	2.344	-.156	.59
	3000	180.7	5	13.0	36.14	2.780	-.220	.53
	0	148.9	20	8.8	7.44	.845	.845	3.33
	500	169.8	15	8.9	11.32	1.272	.772	2.47
	1000	159.7	10	9.9	15.97	1.613	.613	1.71
C	1500	230.5	10	12.2	23.05	1.889	.389	1.12
	2000	149.7	5	13.4	29.94	2.234	.234	.84
	2500	163.0	5	12.8	32.60	2.547	.047	.77
	3000	201.1	5	13.4	40.22	3.001	.001	.66

TABLE I

Data Added Respiratory Dead Space Experiments

% O_2 in expired air	Tension CO_2 in expired air mm. Hg.	Tension O_2 in expired air mm. Hg.	R.Q.	Calories per square meter per hour	Alveolar CO_2 %	Alveolar O_2 %	Tension Alveolar CO_2 mm. Hg.	Tension Alveolar O_2 mm. Hg.	R. Q. Alveolar
18.45	19.058	142.04	1.03		4.24	16.78	32.478	128.535	1.01
19.65	11.042	149.62	1.12		4.42	16.85	33.658	128.313	1.07
19.67	9.986	149.93	1.00	42.859	4.27	16.69	32.550	127.228	1.00
20.12	7.510	154.47	1.19		5.20	15.83	39.931	121.599	1.01
20.18	6.033	153.76	.99	43.668	5.72	14.43	43.575	109.928	.87
20.22	5.123	154.16	.86	43.086	5.89	13.80	44.905	105.211	.82
20.50	3.224	155.88	.85	30.777	6.90	10.91	52.468	82.960	.69
17.71	24.273	135.59	.96	41.780	4.93	15.72	37.749	120.368	.94
19.12	15.041	146.73	1.07		4.88	15.91	37.449	122.093	.96
19.40	11.526	148.11	.95	42.918	5.04	15.43	38.470	117.777	.91
19.94	7.669	151.31	.97	38.295	5.81	14.19	44.115	107.745	.86
19.90	7.317	151.37	.87	40.256	5.90	13.13	44.875	99.867	.75
20.12	4.503	153.01	.64		6.79	10.30	51.652	78.352	.64
20.36	4.022	155.07	.83	35.557	6.95	9.86	52.945	75.113	.63
17.53	24.473	134.54	.96	34.997	5.12	15.25	39.987	117.044	.91
18.24	18.825	139.26	.88	42.079	5.07	15.26	38.704	116.495	.89
19.16	13.037	145.08	.93	39.354	5.40	14.43	41.170	110.014	.83
19.76	8.567	151.11	.90	37.897	5.76	14.35	44.058	109.763	.87
19.98	6.391	152.02	.81	40.429	6.30	12.63	47.930	96.089	.76
20.01	5.836	152.29	.76	42.658	6.58	11.46	59.065	87.199	.69
20.16	5.019	153.09	.77	44.267	7.12	10.18	54.062	77.297	.66

Collected and Calculated Data Added

Added Dead Space mls.	Expired Volume at S.T.P. liters	Duration of exposure min.	Respirations per min.	Expired vol. per min. liters	Tidal Volume Liters	Effective Tidal Volume Liters	% CO_2 in expired air	% O_2 in expired air
0	172.0	20	12.5	8.60	.715	.715	3.00	17.93
500	209.3	15	12.7	13.96	1.121	.622	1.96	19.00
1000	177.3	10	11.6	17.73	1.534	.535	1.51	19.41
1500	246.3	10	13.6	24.65	1.815	.315	1.04	19.94
2000	144.7	5	13.3	28.93	2.171	.171	.86	20.02
2500	161.4	5	13.1	32.29	2.468	-.032	.68	20.12
3000	194.1	5	13.8	38.81	2.818	-.182	.54	20.34

TABLE II

espiratory Dead Space Experiments Average of Three Subjects

ension CO_2 in xpired air m. Hg.	Tension O_2 in expired air mm. Hg.	R. Q.	Calories per square meter per hour	Alveolar CO_2 %	Alveolar O_2 %	Tension Alveolar CO_2 mm. Hg.	Tension Alveolar O_2 mm. Hg.	R. Q. Alveolar
22.601	137.39	.98		4.79	15.92	36.738	131.982	.95
14.969	145.20	1.02		4.79	16.01	36.604	122.300	.97
11.516	147.71	.96	41.710	4.90	15.52	37.397	118.340	.91
7.915	152.30	1.02		5.59	14.79	42.701	113.022	.91
6.580	152.38	.89	41.451	5.95	13.40	45.460	101.961	.79
5.154	153.15	.75		6.42	11.85	48.874	90.254	.72
4.088	154.68	.82	36.868	6.99	10.32	53.158	78.457	.66

TABLE III

Description of Subjects

Subject	Age years	Height inches	Weight lbs.	Vital Capacity mls.	Compli-mental mls.	Supple-mental mls.
A	31	74	150	5700	4374	1349
B	34	68	180	4333	3208	1117
C	25	73	230	5133	3783	1266

DIAGRAM OF SPIROMETER

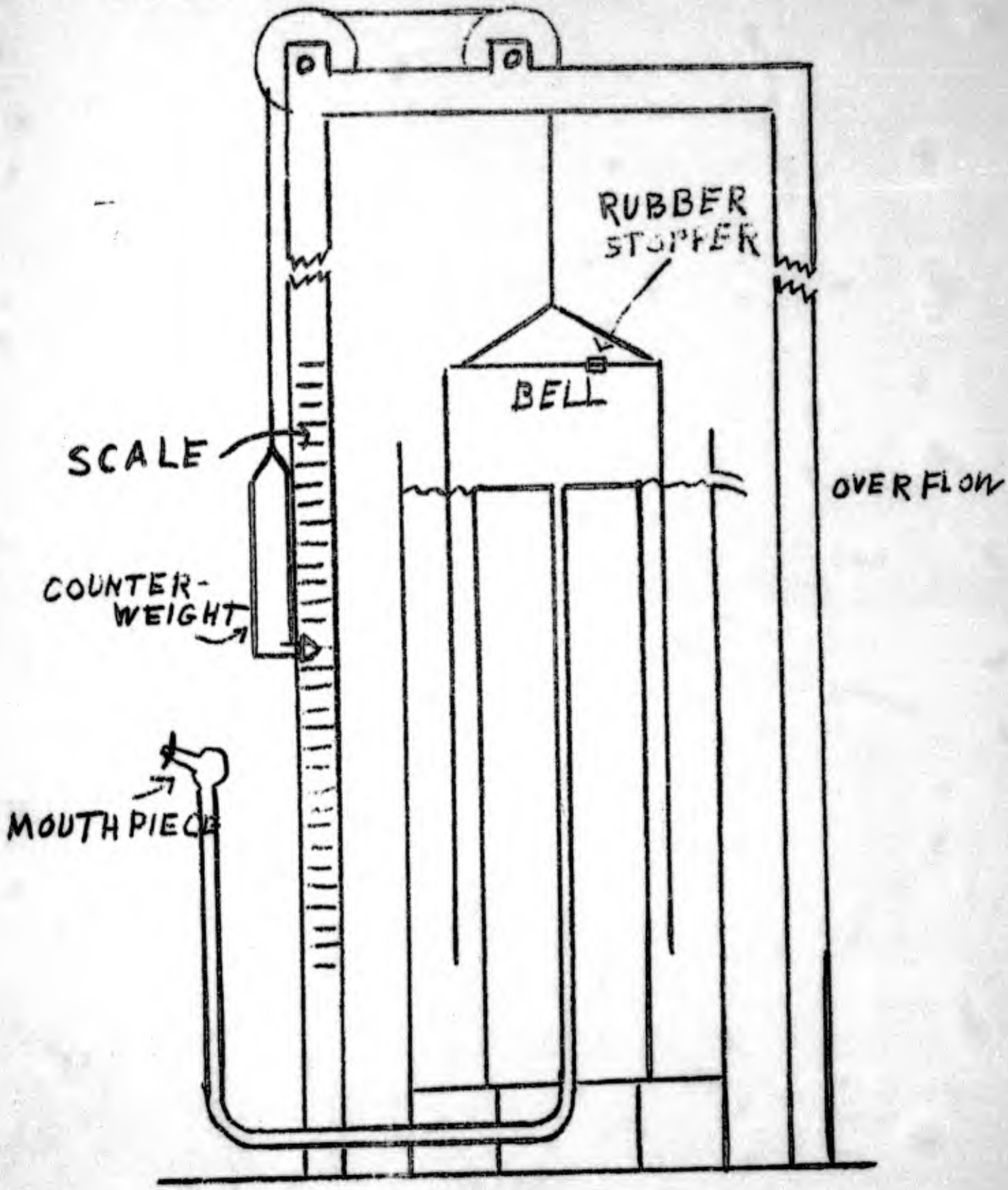


FIG. 1

DIAGRAM OF BREATHING VALVE

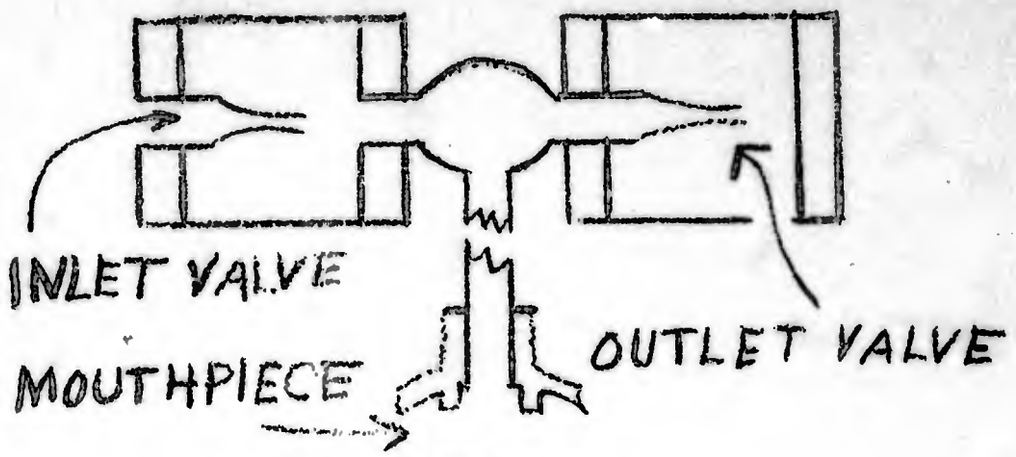


FIG 2

MIXING CHAMBER

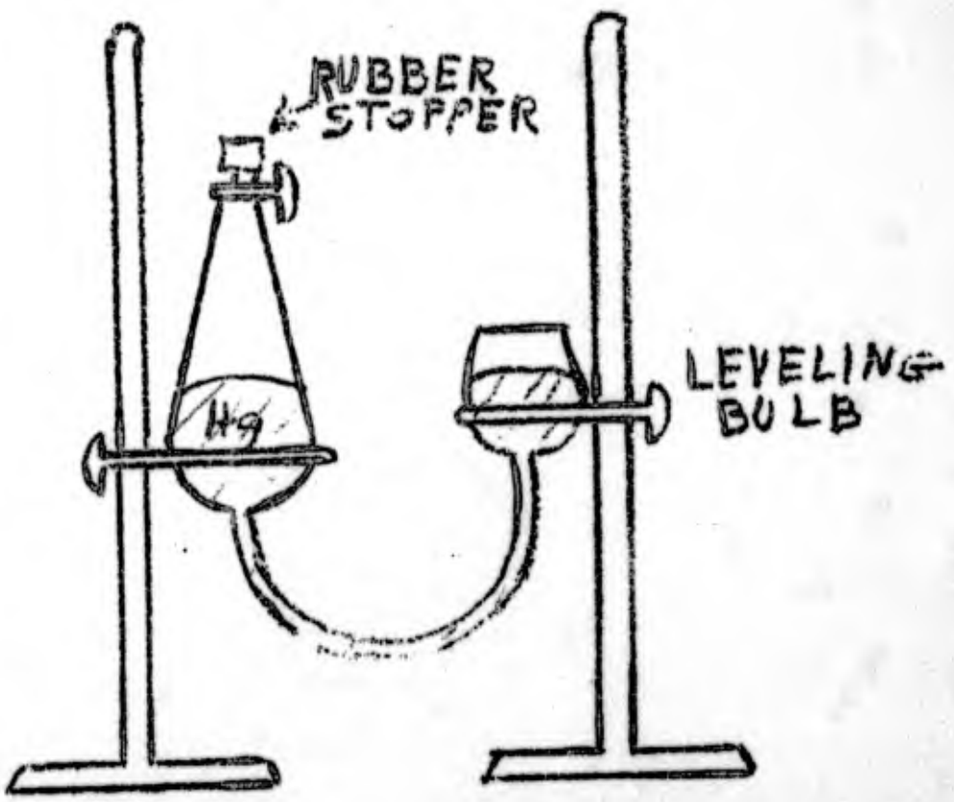
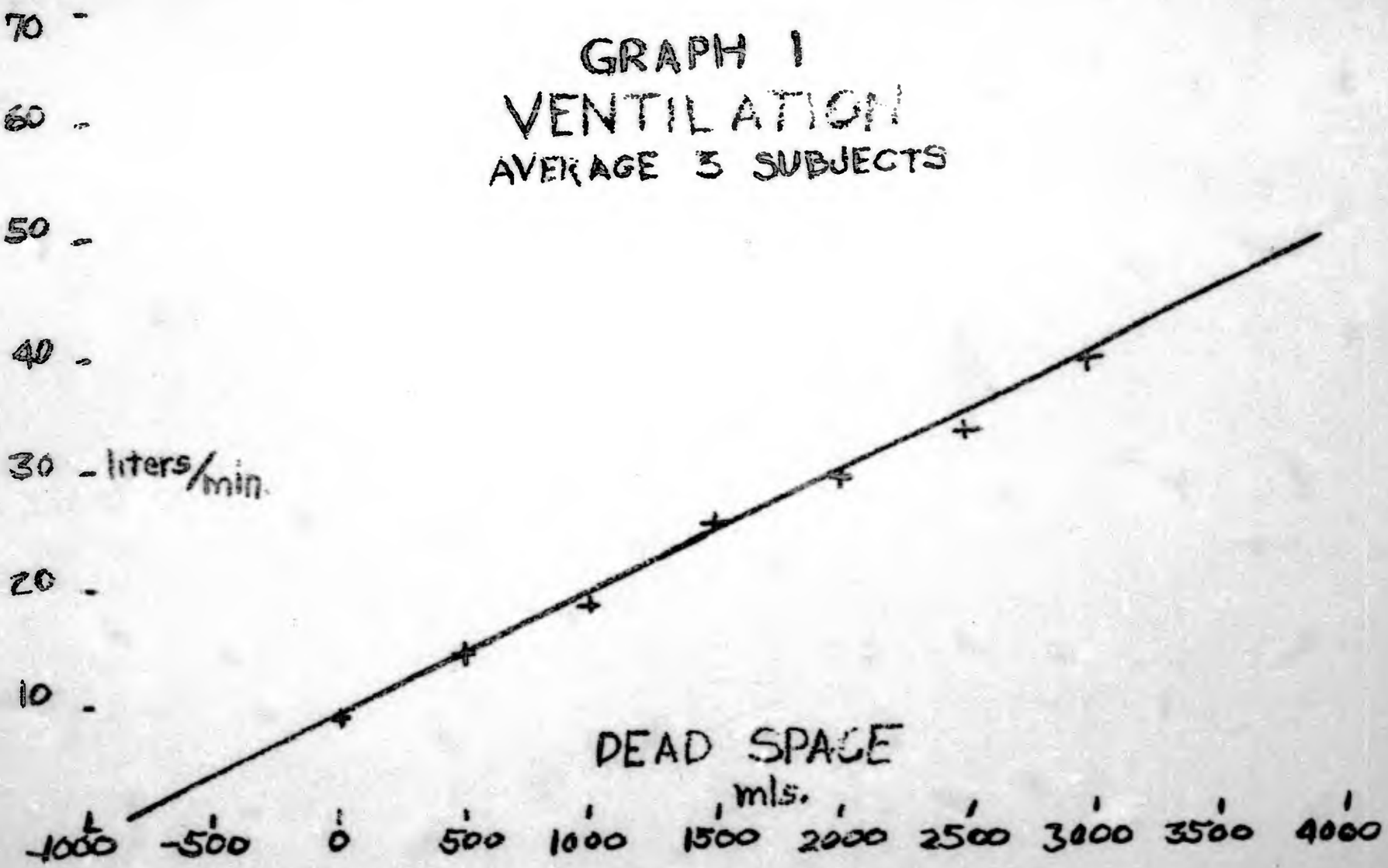
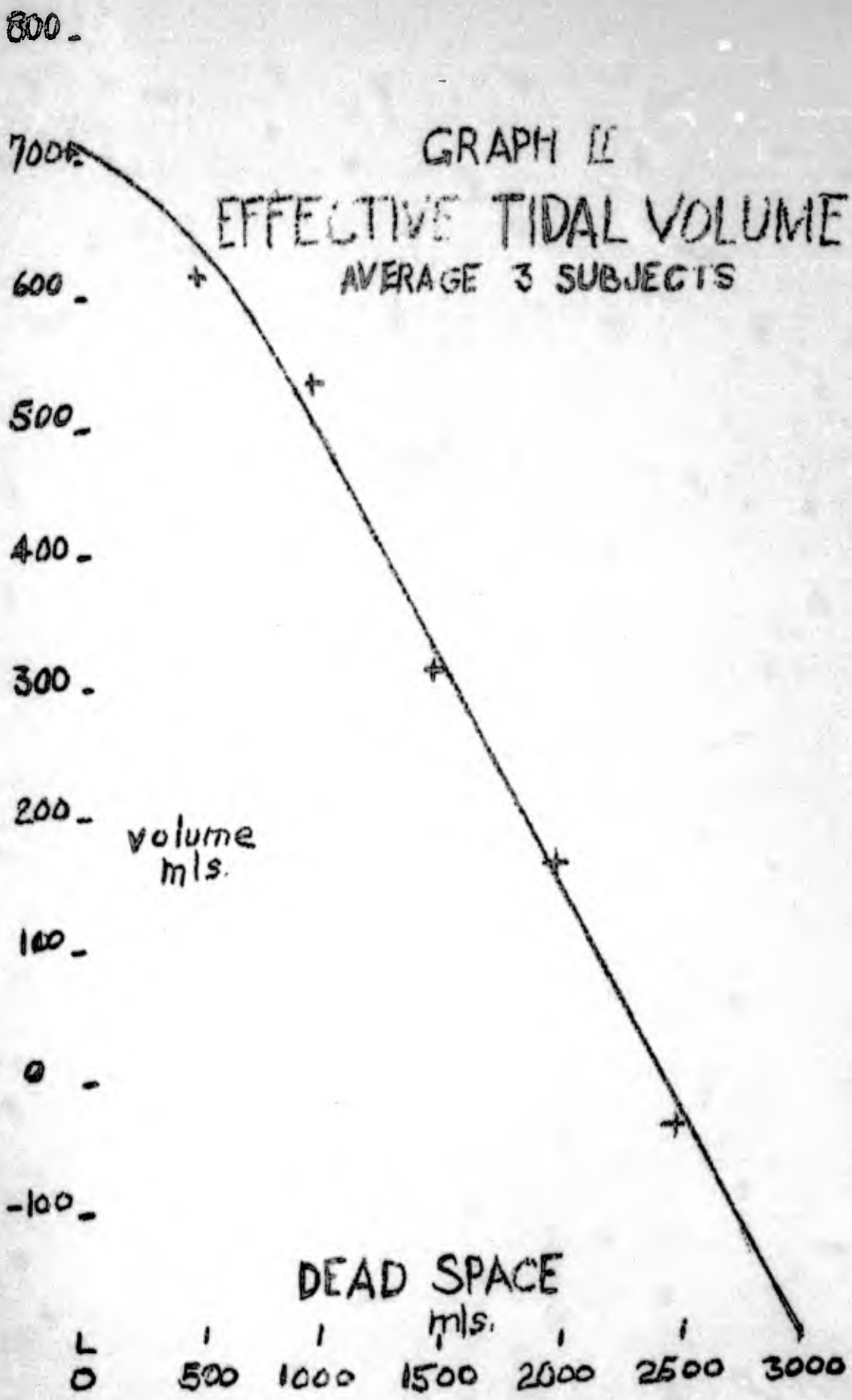


FIG. 3

GRAPH 1
VENTILATION
AVERAGE 3 SUBJECTS

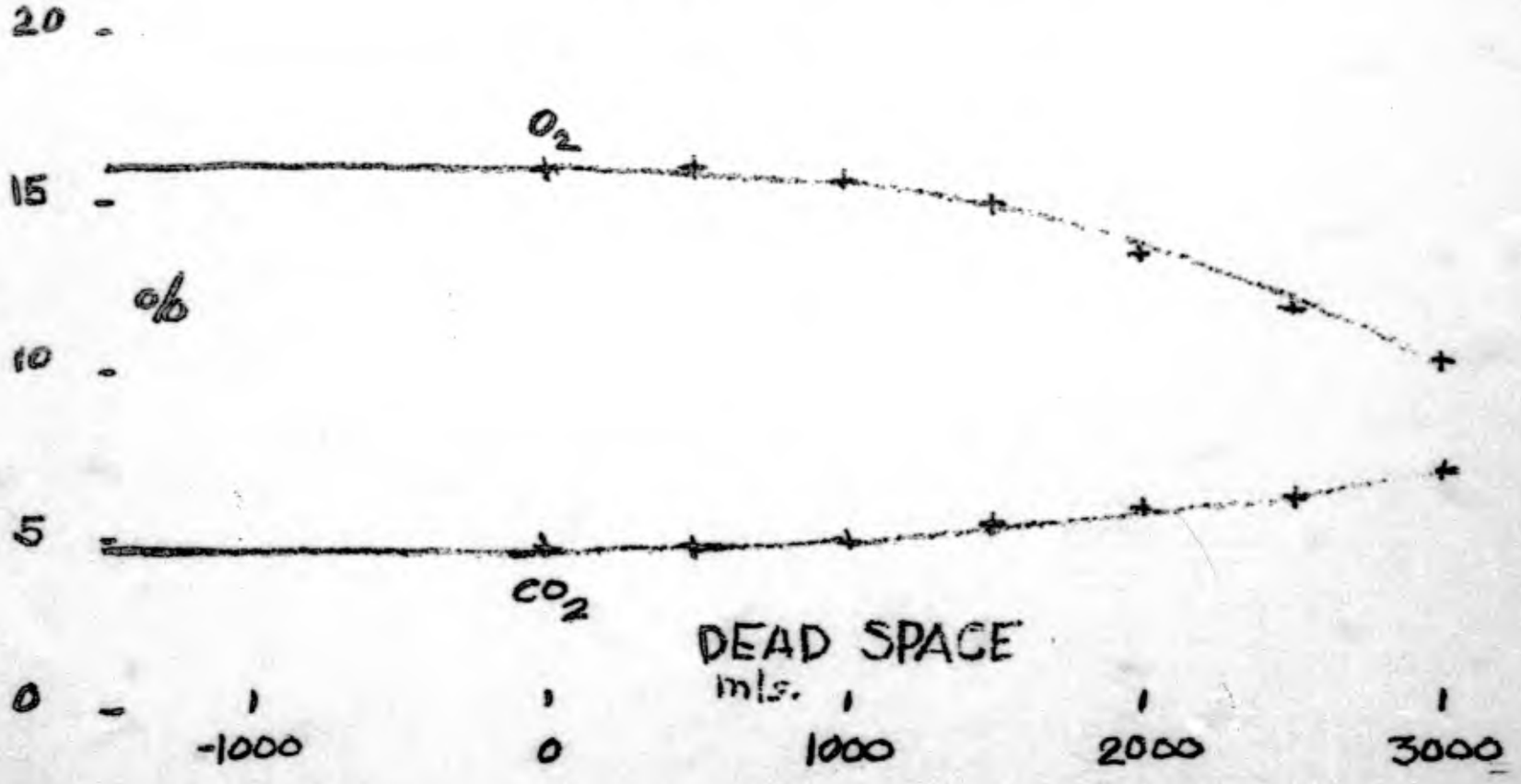



GRAPH II
EFFECTIVE TIDAL VOLUME
AVERAGE 3 SUBJECTS
800
700
600
500
400
300
200
100
0
-100
volume mls.
DEAD SPACE mls.
0
500
1000
1500
2000
2500
3000

GRAPH III

ALVEOLAR COMPOSITION

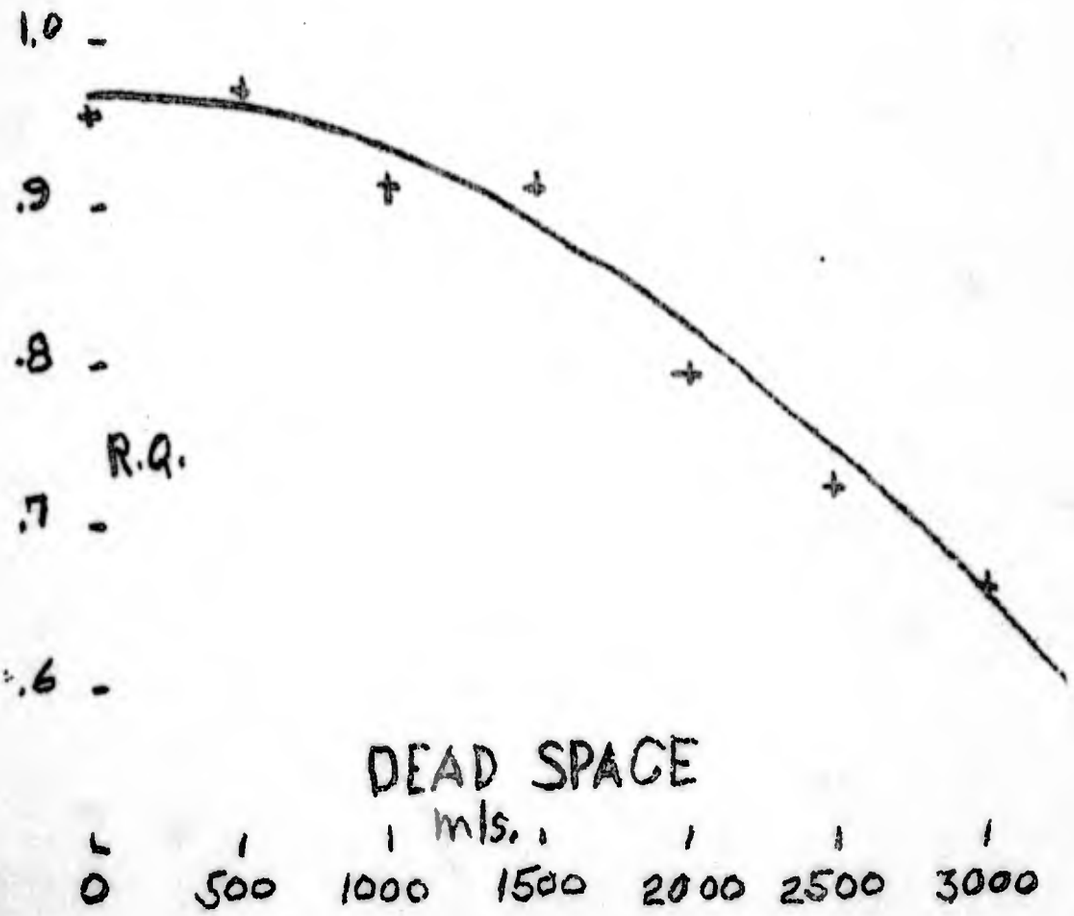
AVERAGE 5 SUBJECTS



GRAPH IV

ALVEOLAR R.Q.

AVERAGE 3 SUBJECTS



THE EFFECTS OF ADDED DEAD SPACE ON RESPIRATORY RATES AND VOLUMES

By

Frank A. Dolle

INTRODUCTION

Breathing added respiratory dead space has been shown to result in an increase in ventilation rate because of an increase in both tidal volume and breathing rate. Up to 1500 cc. of added dead space does not appreciably change the alveolar pCO_2 or pO_2 and at 2000 cc. of added dead space the tensions of these gases are not critically changed, as found by Swann (32). Deep breathing accomplishes the same physical expansion of the thorax and depression of the diaphragm but results in a lowering of the pCO_2 of alveolar air to the detriment of other phsiological processes. Exercise attains the same muscular activity and expansion of the lungs but introduces the factor of fatigue. It then became of practical as well as theoretical interest to determine the effects of prolonged and repeated use of the breathing mechanism without alterations in alveolar composition and without fatigue. The breathing of added dead space afforded a means of accomplishing this determinition.

During normal inspiration in the human an average of 500 cc. of fresh are inhaled. This volume is known as tidal air and of this amount a certain portion, about 150 cc., occupies the respiratory tract from the nostrils to and including the terminal bronchioles. This portion is the anatomic dead space. The air in this region does not enter into gaseous exchange with the blood since the lining of the air passages which contain it are relatively thick and not richly supplied with blood vessels. Loewy (20) in 1894 measured the dead space in the air passages in a cadaver and reported this anatomic volume to be approximately 144 cc. for the adult. Siebeck (30) was the first to approach the problem in the living individual. He observed that expired air issuing from the nose or mouth was a mixture of "lung air" from the alveoli and atmospheric air from the air passages which had never been subjected to the true respiratory process.

Physiologic dead space is defined as the volume of fresh air in the lungs, that before expiration, has not come into contact with the respiratory epithelium or diluted the alveolar air. It is also termed the effective dead space and Enghoff (10) affixed to it the name "volumen inefficax."

An increase in the anatomic dead space produces an effect similar to the condition found during polypnea. The anatomic and physiologic dead spaces are about equal at rest, but during polypnea the physiologic dead space is increased because the inspired air does not remain in the lungs long enough to come into equilibrium with the blood as shown by Main (22). Blickenstorfer (4) found that the addition of more dead space results in dead space hyperventilation without any change of the arterial gases. This was substantiated by Tomasso (34) who stated that hyperventilation compensates for the additional dead space up to a limit of 200 cc.

Disagreement arose among investigators concerning the actuality

of the anatomic dead space as compared with the physiologic or effective dead space. Zuntz (36) in 1882 assumed that dead space was an unvarying volume at rest and during exercise. Campbell, Douglas and Hobson (5) claimed dead space was a physiologic rather than an anatomic concept. Henderson, Chillingworth and Whitney (15) and Haldane (13) working independently reached the same conclusions that dead space increases with an increase in the depth of breathing, excluding hyperpnea and any other causes which might also affect the dead space. Krogh and Linhard (20) definitely stated that the dead space was constant in volume following an adoption of the method of Zuntz in determining dead space. Pearce and Hoover (26) concluded that dead space was a fixed anatomic and physiologic entity when respirations are controlled by the respiratory center and not of extreme depth. They also claimed that the variations which Haldane (13) and Henderson and co-workers (15) found in the volume of dead space on deep breathing were due to faulty methods and artificial modes of breathing. Aitken and Clarke-Kennedy (1) found that the dead space varied during muscular exercise and that it bore a definite relationship to the volume of the tidal air. Moncrieff (23) by his work on 20 subjects upheld this latter finding.

Thus it seems apparent that the great variation in the estimates of dead space was due to the relative degree of accuracy of the methods used by the investigators for obtaining the samples of alveolar air, especially during exercise. Enough evidence has been presented to show that the physiologic and anatomic dead spaces do exist, but the volumes of these dead spaces are variable and not well defined. It is quite evident, however, that dead space volume expressed as a percentage of the tidal air is a more meaningful term since the anatomic dead space varies but little in capacity. No attempt was made in this experiment to ascertain the dead space as a percentage of the tidal air, the primary concern being an observation on the effect of repeated breathing of added anatomic dead space on such physical entities as vital capacity, pulse rate, chest expansion, and respiration rate.

The volume of deepest inspiration after normal inspiration is known as the complemental air. The maximum expiration after quiet expiration is termed the supplemental or reserve (33) air. The complimental air, including the tidal air, and the supplemental air comprise the vital capacity. Another means of delineating vital capacity is to say that it is the maximum expiration after maximum inspiration.

Hutchinson (17), who invented the spirometer, measured the vital capacity of 2000 individuals and came to the conclusion that vital capacity varied more closely with differences in height than with any other known measurable variable. His method of using height as a factor in determining the vital capacity normal for each individual has received much criticism on the basis that individual variations in vital capacity for each height are too great. In 1919, Dreyer (8), found a relationship between sitting height, which he called stem height, and vital capacity. He also noted a relationship between vital capacity and both weight and body surface. Jackson (18), in 1927, showed that the correlation between vital capacity and chest expansion is relatively low. In 1928, Jackson and Lees (19) found the mean vital

capacity in 100 male university students to be 4406 ±575 cc. They then correlated vital capacity to age, stature, sitting height, body weight, pulse rate, respiration rate, surface area, chest girth and chest expansion. Surface area yielded the highest correlation, but it was not significantly higher than body weight, stature and chest girth. Sitting height was somewhat lower with chest expansion having a very much lower correlation. Cordero, Narciso and Ocampe (6) in a study of 2600 male university students in the Phillippine Islands used two prediction standards. The first was based on standing height, and the second was based on height in centimeters times the weight in grams or, in other words, surface area. The second proved to be the more accurate standard. West (35) found that vital capacity varied more uniformly with body surface area than with any other factor. Muller (24), in 1931, in an analysis of 500 school boys whose ages ranged from 10.6 to 16.4 years and whose heights ranged from 132 to 177 cm., discovered that with heights under 166 cm. the increase in vital capacity after a 100 meter run averaged about 50 cc. per centimeter and in heights over 166 cm. the increase was about 120 cc. per centimeter. He also concluded that chest expansion was not a good criterion of pulmonary condition. Reddy and Sastry (27) in a study of 310 Indian women and 1038 Indian men found a high correlation between sitting height and vital capacity. The mean vital capacity for the men was 2982 cc.

Ocaranza (25) measured the vital capacity of 5000 Mexican Indians, mestees and whites whose ages ranged from 6 to 80 years, and reported that vital capacity increased slowly unti 15 years and rapidly thereafter. From 30 to 65 years, there was a slow decrease which became rapid after the age of 65.

Amar (2) and Engelhardt (9) working independently postulated that exercise does not alter the vital capacity of the lungs and was therefore thought to be a constant for the individual. Engelhardt also stated that exercise increased the supplemental air. Gukelberger(12) made a study of 30 subjects who were entered in a ski race and discovered that the better skiers had a greater vital capacity before entering competition than those rated as less skilled skiers. His analysis of the athletes' pulse rate and blood pressure showed these measurements were too irregular to be significant. Hamilton and Morgan (14) and Stephen (31) presented evidence to show that vital capacity decreased in the recumbent posture. Amar (2) demonstrated that vital capacity remained constant through a wide range of respiration frequencies and that it was independent of posture, being lowered only by positions interfering with the movements of the ribs or diaphragm.

The usual respiration rate in the adult, in general, is stated to be from 12 to 20 per minute. This number, however, is subject to variations such as those caused by muscular exercise, altitude, higher body temperatures, posture, age and sleep. In the reclining position, the rate is usually about 13 per minute. Sitting raises this rate to about 18 per minute and 22 per minute is not unusual in the standing posture.

The work of Schneider and Ring (28) bore out the fact that the trained individual breathes less air for the same accomplishment than does the untrained subject. This is partly because the chest in the expiratory position becomes larger and the resting breathing becomes slower and deeper. As a result, alveolar ventilation is more effective even at rest and a smaller increase is sufficient to produce the same oxygen intake during moderate exertion.

The pulse is the result of the pressure changes which occur in an artery as a consequence of the ejection of blood from the heart during systole into an already filled aorta. The walls of the arterial system expand with these pressure changes and the pulse may be felt at the accessible arteries thus being an adequate measure of the heart rate.

The heart of a trained individual pumps more blood with fewer beats. One of the reasons for this may be a better return of the venous blood. Henderson, Haggard and Dolley (16) and Schneider, Clarke and Ring (29) report lowered pulse rates for athletes or trained individuals. Cotton (7) found, in a comparison of athletes in various sports, that swimmers had the lowest basal pulse rate with a mean of 47 per minute. Griffith and co-workers (11) discovered a seasonal change in the basal pulse rate over a two year period with the summer minimum being 7 beats per minute lower than the winter minimum.

METHODS

Sixteen normal, healthy male students at the University of Maryland were subjects for this experiment. Their ages ranged from 19 to 26 years and their weights from 149 to 235 pounds. Nine were actively in training and seven were moderate to sedentary in activity. At the beginning of the experiment, the subjects were examined for their usual blood pressure, pulse rate, chest expansion and vital capacity. Respiration rate was determined for six subjects. The subjects were cautioned to maintain the same general routine and degree of activity during the experimental period. This served to make the effects of exercise a constant for each individual.

Measurements

All the measurements, except chest expansion, weight and height, were taken with the subject in a sitting position and always after the subject had rested for not less than ten minutes to allow him to reach resting equilibrium. Height was measured with the subject in stocking feet and his back against a wall; head, scapula, buttocks and heels touching the wall. Weight was established by means of a spring scale and sitting height was taken with the subject sitting erect in a straight backed chair, the distance from the top of his head to the seat being accepted. Blood pressure was determined at the brachial artery using a Tycos sphygomanometer and the pulse taken at the radial artery. Chest expansion was measured by means of a tape held at the level of the nipples while the subject stood erect and at ease. The reading for the initial girth was taken just after

the subject had completed a normal expiration and the expansion noted after maximum inspiration. Vital capacity was recorded with the use of a Tissot spirometer without valves and calibrated in cubic centimeters. When three readings agreed they were accepted as the measurement. Respiration rate was established by means of a pneumograph, the subject being allowed to breathe normally for five minutes and an average rate per minute was taken.

Equipment and Procedure

The work was done in three four-week periods; March to May, July to August and October to November in 1949. In the March to May period an attempt was made to determine the general physical fitness of the subjects by means of the Step Test devised by the Harvard Fatigue Laboratory. This test, however, was found to be unsuited to the experiment and was, for this reason, eliminated.

For a period of four weeks the subjects breathed through increasing amounts of dead space produced by the use of rubber tubing. This tubing is constructed with a 1 inch inside diameter and is open at both ends. Sections of this tubing were used so that each contained 500 cc. volume and were connected by a glass tube coupling of the same inside diameter and equipped with a baffle to insure adequate dissipation of the inspiratory and expiratory air and to prevent the formation of axial spikes of flowing air. A mouthpiece was fitted to the tubing in a manner that would not alter the dead space volume and a nose clamp was worn by the subject to assure his breathing only through the tube. As the amount of dead space was to be increased, more sections were added to the tubing. The subjects breathed 500, 1000, and 2000 cc. of dead space successively for a period of five days at each volume, fifteen minutes per day. On the same day each week the subjects were examined using a standardized procedure. The subject would rest for not less than ten minutes; his pulse rate, blood pressure and respiration rate were then taken. This was then followed by the measurement of chest expansion and finally vital capacity was recorded. In this way, the subject began with no exertion and ended with the measurement of vital capacity which required quite a bit of respiratory output.

RESULTS AND DISCUSSION

Effects of Added Dead Space on Vital Capacity

From Table I it may be seen that there was an increase in the vital capacity of all the subjects ranging from a minimum increase of 50 cc. to a maximum increase of 1200 cc. The mean total change was 450 ±352 cc. Breathing the large amounts of added dead space produced, in the subjects, a deeper and more regular respiratory pattern, which in effect, trained the muscles of respiration so that there was greater inspiration. Since the lungs play a purely passive role in respiration, this may have resulted in the utilization of al-

veoli that had been inactive and therefore the subjects were able to make a greater respiratory thrust culminating in an increased vital capacity.

During expansion, the thoracic cage is not enlarged equally in all directions. The lower portion increases more than the upper and more anteriorly than posteriorly (3). Thus it is evident that the increase in lung volume is mainly caused by the descent of the diaphragm. Exercise of the type afforded by breathing added dead space would bring about an increased efficiency of the anatomic regions involved in diaphragmatic breathing. This would allow a greater excursion and a concomitant increase in lung volume.

The increase in vital capacity may have been caused by an increase in either the supplemental or complemental airs or both, but since the exact determination of these components of total lung volume was complicated by the emotional subjectivity of the individuals examined, differentiation would not be accurate.

Effects of Added Dead Space on Chest Expansion

In all but 3 of the 16 subjects there was an increase in chest expansion of from .25 inches to 1.0 inch with a mean total change of .469 ± .227 inches. (Table II) This increase follows the results shown in Table I, but cannot be interpreted as meaning that a greater chest expansion produces a greater vital capacity since it has been shown by Jackson (17) and Jackson and Lees (18) that the correlation between chest expansion and vital capacity is relatively low. It may also be seen that the subjects who evidenced the greater increase in vital capacity did not have the greater increase in chest expansion.

In all probability, it means that the respiratory muscles allowed a greater expansion of the thoracic cage resulting in a great excursion of the diaphragm that would be evidenced by an increase in vital capacity. The area of the diaphragm is approximately 270 square centimeters. During eupnea, its excursion is about 1 cm. and during exercise this increases to about 3 cm. Thus forcible inspiration would add 810 cc to the vital capacity of the individual, but the amount would be variable because of the individual differences in the area of the diaphragm and its excursion.

Effects of added Dead Space on Pulse Rate

Although the changes in pulse rate are statistically insignificant, there is a definite trend toward a decrease in rate with only 4 of the 16 subjects showing either no change or an increase. These results, in conjunction with the two previously discussed, lead to the belief that within limits breathing large amounts of added dead space comprises a form of training which is reflected in the tendency toward lowered pulse rates.

The releasing ability rather than the oxygen carrying capacity is the true measure of the efficiency of the blood as a transporting medium. This efficiency can be measured as the ratio of the oxygen consumed by the tissues to the oxygen supplied and is called the coefficient of oxygen utilization. During moderate exercise, oxygen demand is satisfied by an increase in circulation rate rather than in an increase in the coefficient of oxygen utilization. It is thought that, as a result of training, the coefficient of oxygen utilization rises during exercise and thus spared the work load of the heart. It has been shown by work of Henderson, Haggard and Dolley (15) that the pulse rate of an athlete will rise proportionately as much as the pulse rate of an indivudual not in training during exercise, but that the trained person has the advantage of starting off at a lower pulse rate. There is, however, reason to believe that the heart output of the trained individual is increased by an increase in stroke volume rather than an augmentation of heart rate. Possible justification of this may be that excessive pulse rate tends to decrease stroke volume during exercise, resulting in an increased expenditure of energy.

Effects of Added Dead Space on Respiration Rate

The measurement of the changes in the respiration rates of 6 subjects varied from an increase of 3 per minute to a decrease of 2 per minute making these results statistically insignificant. It is interesting to note that the initial respiration rates of the individuals not in training were lower than the respiration rates of those actively in training, but with such a small number of samples no valid conclusions may be drawn.

Comparison of changes in vital capacity of individuals in training and individuals not in training

In Table VI, it is evident that the initial vital capacity of those subjects in training was somewhat lower than the initial vital capacity of those not in training. This supports the general observation that there is no important relationship between vital capacity and athletic status.

It may also be seen that the vital capacity of those individuals not in training increased less than the vital capacity of those in training. It is possible that breathing added dead space together with training produces a greater increase in vital capacity than would either one alone. In contrast to the postulation of Amar (2) and Engelhardt (8) that vital capacity is unaffected by exercise, the increase in the non-training group not unreasonably implies vital capacity may be influenced by some forms of training.

Comparison of Chest expansion changes in individuals in training and individuals not in training

The chest expansion of all the 9 subjects in training evidenced an increase of from .25 inches to .75 inches with a mean increase of .5 ± .17 inches. Of the 7 subjects not in training, 3 remained constant and 4 showed an increase of from .5 inches to 1.0 inch with an overall mean of .43 ± .41 inches. Since those individuals not in training, whose chest expansions remained constant, had increases in vital capacity, it is probable that an increase in the excursion of the diaphragm was responsible for this gain.

The increases of those in training may have been due either to their athletic training or to the training incident to breathing added dead space, but it is more plausible that a combination of the two would bring about those increases.

Comparison of changes in pulse rate of individuals in training and individuals not in training

As shown in Table VIII, the pulse rates of those subjects in training decreased in 7 out of 9 instances with 1 showing an increase and 1 remaining constant. Of the 7 individuals not in training, 2 had an increased pulse rate, 1 remained constant and 4 showed a decrease. Since there was no significant change in the non-training group, and since the group also served as a control for those in training, the decreases in the training group were probably due to either training or a combination of athletic training and breathing added dead space but not breathing added dead space alone.

CONCLUSIONS

In normal human male subjects breathing amounts of added dead space increasing successively from 500 cc. to 2000 cc. in a period of four weeks, it was observed that:

1. There was an increase in the vital capacity of all the subjects that may have been produced by a training of the muscles of respiration and the utilization of alveoli that had been inactive. It is questionable whether the increase was due to an increase in the supplemental or complemental airs or both.

2. In 13 of the 16 subjects there was an increase in chest expansion which, in all probability, was caused by a greater excursion of the diaphragm.

3. There was a definite tendency toward a decrease in pulse rate, which, in addition to the two observations previously mentioned, lead to the belief that within limits breathing large amounts of added dead space comprises a form of training.

4. The measurements of the changes in the respiration rates of 6 subjects were found to be insignificant.

5. The vital capacity of those individuals not in training increased less than the vital capacity of those in training. In support of the fact that there is no important relationship between vital capacity and athletic status, it was found that the initial vital capacity of those subjects in training was somewhat lower than the initial vital capacity of those not in training.

6. There was a significantly greater increase in chest expansion in the training group. Since those subjects not in training experienced increases in vital capacity with no increase in chest expansion, it is probable that an increased excursion of the diaphragm was responsible for this augmentation.

7. The pulse rates of those subjects in training decreased more than the pulse rates of those not in training. The decreases in the training group may have been due either to training or a combination of training and breathing added dead space, but not breathing added dead space alone.

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TABLE I

THE EFFECTS OF BREATHING ADDED DEAD SPACE ON VITAL CAPACITY

Subject	Age	Wgt.	Hght.	Sitting Height	Vital Capacity Initial cc.	Final cc.	Increase cc	% Change
1	19	165	6'0"	3'0"	4000	4350	350	8.70
2	24	170	6'0"	3'0"	5150	5500	350	6.70
3	23	235	6'1"	2'11"	5500	5600	100	1.81
4	22	168	5'11½"	2'10½"	4800	4850	50	1.04
5	25	155	5'8"	2'10½"	4300	4900	600	13.95
6	23	185	6'0"	3'0"	4300	4700	400	9.30
7	24	155	5'11"	2'10"	3800	4200	400	10.52
8	23	170	5'8½"	2'9"	3800	4450	650	17.10
9	26	170	6'0"	2'11"	5250	5600	350	6.66
10	23	149	5'5"	2'8½"	3950	4300	350	8.86
11	22	194	6'2½"	3'1"	4600	5800	1200	26.08
12	24	214	5'8"	2'10"	3900	4050	150	3.84
13	24	170	5'8½"	2'11"	5200	5300	100	1.92
14	21	155	5'9"	2'10½"	3350	4300	950	28.35
15	22	190	6'0"	3'0"	4800	5500	700	14.58
16	21	155	5'9"	2'11"	4700	5200	500	10.63
Mean					4463	4913	450	10.63
Standard Deviation					± 193	± 181	± 352	± 7.16

TABLE II

THE EFFECTS OF BREATHING ADDED DEAD SPACE ON CHEST EXPANSION

Subject	Age	Wgt.	Hght.	Sitting Height	Chest Expansion Initial In.	Final In.	Change In.	% Change
1	19	165	6'0"	3'0"	2.0	2.5	± .5	25.00
2	24	170	6'0"	3'0"	1.5	2.5	±1.0	66.60
3	23	235	6'1"	2'11"	1.5	1.5	0.0	0.0
4	22	168	5'11½"	2'10½"	1.5	1.75	± .25	16.66
5	25	155	5'8"	2'10½"	1.25	2.0	± .75	60.00
6	23	185	6'0"	3'0"	2.0	2.5	± .5	25.00
7	24	155	5'11"	2'10"	1.0	1.5	± .5	50.00
8	23	170	5'8½"	2'9"	2.25	2.75	± .5	22.22
9	26	170	6'0"	2'11"	2.0	2.5	± .5	25.00
10	23	149	5'5"	2'8½"	1.75	2.0	± .25	14.28
11	22	194	6'2½"	3'1"	2.0	2.75	.75	37.50
12	24	214	5'8"	2'10"	2.75	3.75	±1.0	36.36
13	24	170	5'8½"	2'11"	3.0	3.0	0.0	0.0
14	21	155	5'9"	2'10½"	2.25	2.25	0.0	0.0
15	22	190	6'0"	3'0"	2.0	2.5	± .5	25.00
16	21	155	5'9"	2'11"	2.25	2.75	± .5	22.22
Mean					1.94	2.41	.469	26.62
Standard Deviation					±.48	±.56	± .227	±14.73

TABLE III

THE EFFECTS OF BREATHING ADDED DEAD SPACE ON PULSE RATE

Subject	Age	Wgt.	Hght.	Sitting Height	Pulse Rate Initial	Final	Change	% Change
1	19	165	6'0"	3'0"	80	74	-6	7.50
2	24	170	6'0"	3'0"	72	68	-4	5.55
3	23	235	6'1"	2'11"	90	90	0	0
4	22	168	5'11½"	2'10½"	86	80	-6	6.97
5	25	155	5'8"	2'10½"	88	82	-6	6.81
6	23	185	6'0"	3'0"	60	60	0	0
7	24	155	5'11"	2'10"	78	86	+8	10.25
8	23	170	5'8½"	2'9"	80	72	-8	10.00
9	26	170	6'0"	2'11"	96	92	-4	4.16
10	23	149	5'5"	2'8½"	92	86	-6	6.52
11	22	194	6'2½"	3'1"	72	76	+4	5.55
12	24	214	5'8"	2'10"	76	74	-2	2.63
13	24	170	5'8½"	2'11"	76	78	+2	2.63
14	21	155	5'9"	2'10½"	84	74	-10	11.90
15	22	190	6'0"	3'0"	86	74	-12	13.95
16	21	155	5'9"	2'11"	76	74	-2	2.63
Mean					80.8	77.5	-3.25	6.07
Standard Deviation					±8.7	±8.0	±4.81	±3.27

TABLE IV

THE EFFECT OF BREATHING ADDED DEAD SPACE ON RESPIRATION RATE

Subject	Age	Wgt.	Hght.	Sitting Height	Respiration Rate Initial	Final	Change	% Change
11	22	194	6'2⅜"	3'1"	18	16	-2	11.00
12	24	214	5'8"	2'10"	13	16	+3	23.07
13	24	170	5'8⅜"	2'11"	12	14	+2	16.66
14	21	155	5'9"	2'10½"	16	14	-2	12.50
15	22	190	6'0"	3'0"	16	16	0	0
16	21	155	5'9"	2'11"	13	13	0	0

TABLE V

THE EFFECT OF BREATHING ADDED DEAD SPACE ON THE RESPIRATION RATE OF INDIVIDUALS IN TRAINING AND INDIVIDUALS NOT IN TRAINING

Subject	Age	Wgt.	Hght.	Sitting Height	Respiration Rate Initial	Final	Change	% Change
T								
11	22	194	6'2⅜"	3'1"	18	16	-2	11.00
15	22	190	6'0"	3'0"	16	16	0	0
NT								
14	21	155	5'9"	2'10½"	16	14	-2	12.50
12	24	214	5'8"	2'10"	13	16	+3	23.07
13	24	170	5'8⅜"	2'11"	12	14	+2	16.66
16	21	155	5'9"	2'11"	13	13	0	0

T . . . . Training
NT. . . . Non-training

TABLE VI

THE EFFECTS OF BREATHING ADDED DEAD SPACE ON THE VITAL CAPACITY OF INDIVIDUALS IN TRAINING AND INDIVIDUALS NOT IN TRAINING

Subject	Age	Wgt.	Hght.	Sitting Height	Vital Capacity Initial cc.	Final cc.	Increase cc.	% Change
T								
1	19	165	6'0"	3'0"	4000	4350	350	8.70
4	22	168	5'11½"	2'10½"	4800	4850	50	1.04
5	25	155	5'8"	2'10½"	4300	4900	600	13.95
6	23	185	6'0"	3'0"	4300	4700	400	9 30
8	23	170	5'8½"	2'9"	3800	4450	650	17.10
9	26	170	6'0"	2'11"	5250	5600	350	6.66
10	23	149	5'5"	2'8½"	3950	4300	350	8.86
11	22	194	6'2½"	3'1"	4600	5800	1200	26.08
15	22	190	6'0"	3'0"	4800	5500	700	14.58
Mean					4422	4938	516	11.80
Standard Deviation					±450	±484	±157	±6.76
NT								
2	24	170	6'0"	3'0"	5150	5500	350	6.70
3	23	235	6'1"	2'11"	5500	5600	100	1.81
7	24	155	5'11"	2'10"	3800	4200	400	10.52
14	21	155	5'9"	2'10½"	3350	4300	950	28.35
16	22	155	5'9"	2'11"	4700	5200	500	10.63
12	24	214	5'8"	2'10"	3900	4050	150	3.84
1	24	170	5'8½"	2'11"	5200	5300	100	1.92
Mean					514	4878	364	9.11
Standard Deviation					± 61	±617	±280	±8.56

T. . . . . .Individuals in training
NT. . . . . .Individuals not in training

TABLE VII

THE EFFECT OF BREATHING ADDED DEAD SPACE ON THE CHEST EXPANSION OF INDIVIDUALS IN TRAINING AND INDIVIDUALS NOT IN TRAINING

Subject	Age	Wgt.	Hght.	Sitting Height	Chest Expansion Initial In.	Final In.	Change In.	% Change
T								
1	19	165	6'0"	3'0"	2.0	2.5	±.5	25.00
4	22	168	5'11½"	2'10½"	1.5	1.75	±.25	16.66
5	25	155	5'8"	2'10½"	1.25	2.0	±.75	60.00
6	23	185	6'0"	3'0"	2.0	2.5	±.5	25.00
8	23	170	5'8½"	2'9"	2.25	2.75	±.5	22.22
9	26	170	6'0"	2'11"	2.0	2.5	±.5	25.00
10	23	149	5'5"	2'8½"	1.75	2.0	±.25	14.28
11	22	194	6'2½"	3'1"	2.0	2.75	±.75	37.50
15	22	190	6'0"	3'0"	2.0	2.5	±.5	25.00
Mean					1.86	2.36	.5	27.85
Standard Deviation					±.42	±.44	±.17	±12.90
NT								
2	24	170	6'0"	3'0"	1.5	2.5	±1.0	66.66
3	23	235	6'1"	2'11"	1.5	1.5	0	0.0
7	24	155	5'11"	2'10"	1.0	1.5	± .5	50.00
12	24	214	5'8"	2'10"	2.75	3.75	±1.0	36.36
13	24	170	5'8½"	2'11"	3.0	3.0	0	0.0
14	21	155	5'9"	2'11"	2.25	2.25	0	0.0
16	21	155	5'9"	2'11"	2.25	2.75	± .5	22.22
Mean					2.04	2.46	.43	25.03
Standard Deviation					±.78	±.85	± .41	±24.90

TABLE VIII

THE EFFECT OF BREATHING ADDED DEAD SPACE ON THE PULSE RATE OF INDIVIDUALS IN TRAINING AND INDIVIDUALS NOT IN TRAINING

SUBject	Age	Wgt.	Hght.	Sitting Height	Pulse Rate Initial	Final	Change	% Change
T								
1	19	165	6'0"	3'0"	80	74	- 6	7.50
4	22	168	5'11½"	2'10½"	86	80	- 6	6.97
5	25	155	5'8"	2'10½"	88	82	- 6	6.81
6	23	185	6'0"	3'0"	60	60	0	0
8	23	170	5'8½"	2'9"	80	72	- 8	10.00
9	26	170	6'0"	2'11"	96	92	- 4	4.16
10	23	149	5'5"	2'8½"	92	86	- 6	6.52
11	22	194	6'2½"	3'1"	72	76	+ 4	5.55
15	22	190	6'0"	3'0"	86	74	-12	13.95
Mean					82.2	77.3	-6.3	5.59
Standard Deviation					±8.85	±8.65	±2.91	±2.74
NT								
2	24	170	6'0"	3'0"	72	68	- 4	5.55
3	23	235	6'1"	2'11"	90	90	0	0
7	24	155	5'11"	2'10"	78	86	+ 8	10.25
12	24	214	5'8"	2'10"	76	74	- 2	2.63
13	24	170	5'8½"	2'11"	76	78	+ 2	2.63
14	21	155	5'9"	2'10½"	84	74	-10	11.90
16	21	155	5'9"	2'11"	76	74	- 2	2.63
Mean					78.8	77.7	-1.14	1.96
Standard Deviation					±5.64	±7.13	±2.43	±2.29

T . . . . . Individuals in Training
NT. . . . . Individuals not in Training

STUDIES ON THE DENSITIES OF ANIMAL TISSUES

by

Barbara Jane Smith

INTRODUCTION

The specific gravity of animal tissues has received little attention, possibly because of the absence of an accurate and simple method of determination. The method presented here is an adaptation and modification of that developed by Phillips, et al (13) for the determination of the specific gravity of blood.

The objectives of the investigation reported here were: (1) to determine whether the method as outlined by Phillips, et al (13) was adaptable for use with tissues, (2) to establish the normal ranges of the specific gravities of certain tissues of the albino mouse and the albino rat, (3) to measure the effect of water deprivation on the specific gravities of tissues, and (4) to ascertain whether any measurable relationship existed between age and the specific gravities of tissues.

METHODS AND PROCEDURES

The specific gravity measurements were carried out by dropping pieces of a tissue individually into a graded series of aqueous copper sulphate solutions of known specific gravities until the solution was found which matched the tissue in density, as indicated by the suspension of the tissue within the body of the liquid.

The graded series of copper sulphate solutions was made by dilution of a standard solution of specific gravity 1.100. This standard solution may be prepared or, as in the present investigation, purchased already standardized from a chemical supply house.[1] The dilutions were made according to the recommendation of Phillips, et al (13) for whole blood. The degree of dilution of those solutions used for tissues, the specific gravity of which lay outside of the range given by Phillips, et al (13) for blood, was determined by extrapelation. A 100 ml. burette graduated in 0.2 ml. was used for measuring both the water and the copper sulphate standard. The solutions during use were kept tightly covered in 4 oz., wide-mouth jars equipped with plastic caps.

In the experiment, 174 animals were used including 60 albino mice and 114 albino rats. The mice were housed in groups of six in wooden cages with sawdust litter. The rats were housed in groups of four in wire cages. The animals had free access to standard laboratory Purina chow, and to water at all times except during an experiment in which the animals were deprived of water. They were sacrificed either by rupture of the cervical cord or by concussion. All of the animals were of the strain developed at the Albino Farms, Red Bank, New Jersey, and all came from there except the very young rats (those up to thirty days of age) which were bred from animals obtained from the Albino

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[1] The Hartman-Leddon Company, Philadelphia, Pennsylvania

Farms. A period of adjustment of one week or more was allowed after receipt of the animals before experimental work was begun.

Immediately after an animal was sacrificed, the dissection was made and tissue samples were removed from thirteen different organs in the following order: spleen, pancreas, adrenal gland, gonad, liver, kidney, heart, skeletal muscle, stomach, small intestine, large intestine, cerebellum, and cerebrum. When possible, those specimens were removed from the same region of the organ or, in the case of the ovary and adrenal gland, the entire organ was used. The heart sample was taken from the apex below the floor of the ventricles. The skeletal muscle samples were taken from ventral thigh muscles. The gastro-intestinal tract was freed of its contents by gentle pressure before samples were taken. Stomach pieces were cut from the pyloric end of the fundus, small intestine pieces from just above the ileo-caecal valve, and large intestine pieces from just above the rectum. In no case was the tissue washed. All apparent fat and fascia were removed, and any excess blood was lightly blotted off with filter paper slightly moistened with physiological saline. The size of the tissue pieces varied, but most were cubes of about 5 mm on a side.

During a preliminary test, it was found that allowing a piece of the spleen to remain uncovered and therefore exposed to evaporation caused a considerable increase in the density of the tissue. During the dissection, therefore, the animal was kept covered with a cloth dampened slightly with physiological saline to prevent evaporation. Also, a fluorescent lamp was used to reduce heat which might hasten evaporation, and care was taken to eliminate drafts from open windows. Air bubbles which occasionally formed on the surface of the tissue segments were gently forced off with a glass rod against the side of the copper sulphate container.

As each tissue sample was dissected from the animal, it was dropped into one of the solutions of known specific gravity. If the tissue rose or fell in the solution, another piece was taken and put in a less dense or more dense solution as the previous trial indicated. This was repeated until the solution was found in which the tissue remained suspended for several seconds. The movement of the tissue was noted immediately after the momentum of the fall was lost, because a copper proteinate precipitate soon forms causing the tissue to increase in density. Often, however, since the solutions were in gradations of .002 specific gravity units, the specific gravity of the tissue fell between that of two successive solutions in the graded series. In this case, the value was taken as being midway between the two.

The copper sulphate solutions were renewed five times during the experiment. Phillips, et al (13) found that the solutions remained without significant change in specific gravity for fifty drops of blood per 100 cc. of copper sulphate solution. In the present study, any error introduced by the repeated use of the same solutions by comparison with the use of fresh solutions was found to be of no significance.

The experimental study was divided into three parts. First, the specific gravities of the tissues of twenty adult female mice were determined to establish the normal range for these tissues. Secondly, twenty female mice were used to determine the effects of water deprivation on tissue densities. This group was deprived of drinking water for an average of twenty-seven hours (ranging from 23 to 31 hours) following which the tissue densities were determined immediately. Another group of twenty female mice was deprived of water for an average of 48 hours (ranging from 42 to 51 hours). Finally, the relationship between age and tissue densities was studied with a group of 114 albino rats, male and female, at various known ages. The ages of these animals ranged from birth to 110 days, and were known accurately to within two days.

RESULTS

The data collected on the specific gravities of the tissues of normal albino mice is presented in Table I. Table II represents the data collected during the experiment on water deprivation. Both of these series of data are summarized in Table III. In cases where an insufficient amount of tissue was available, because of the small size of the organs, the specific gravity was estimated on the basis of the data available, a question mark was placed by the figure, and it was not incorporated in the calculations of the averages for those tissues as used in the graphs or summaries. In Table III the values designated for the adrenal gland, pancreas, and liver are not accurate because the extremes for these tissues fell outside of the range of the copper sulphate series being used at the time, i.e., 1.046 to 1.080. The specific gravities of those tissues lying outside this range were indicated as being greater than (>) 1.080 or less than (<) 1.046. The pancreas and liver tissues were actually more dense and the adrenal gland less dense than the figures indicate. Additional standards were added to cover the increased range before subsequent studies were undertaken. The apparent lightness of the adrenal gland was undoubtedly due to improper removal of connective tissue during the early phase of the experiment, which condition also was later corrected.

The data collected during the age study are tabulated in Table IV. Figures I and II are graphic presentations of these data, the former showing the relationship between tissue density and age and the latter showing the relationship between tissue density and weight. Since over the time period studied, weight is in general a function of age, there is a similarity between these curves. The specific gravity-weight curves have been incorporated only because quite often the ages of animals are judged by firms supplying animals solely on a weight basis.

The specific gravity-age data from Table IV were grouped into classes of five days each up to the age of thirty days, above which the class range was established on a ten-day basis. The mean value of each class was plotted on the graph, and the best smooth curve was

Table I

The Specific Gravities of the Tissues

Animal Number	Body Weight	Spleen	Liver	Pancreas	Adrenal	Ovary	Kidney	Heart
9	32.5	1.069	1.079	1.074	1.049	1.055	1.063	1.065
10	35.7	1.068	>1.080	1.080	<1.046	1.055	1.063	1.065
11	33.8	1.069	1.071	1.079	<1.046	1.055	1.066	1.064
12	35.6	1.071	1.073	1.076	<1.046	1.055	1.062	1.065
29	35.3	1.063	1.068	1.069	1.046	1.051	1.057	1.064
30	25.6	1.069	>1.080	>1.080	<1.046	-	1.066	1.064
31	24.5	1.069	>1.080	1.077	-	-	1.060	1.064
32	29.7	1.069	1.077	1.080	<1.046	1.057	1.065	1.065
33	32.6	1.067	1.079	1.079	1.050	1.053	1.056	1.063
34	26.3	1.067	>1.080	>1.080	1.047	-	1.060	1.064
35	28.1	1.067	1.079	>1.080	1.053	1.054	1.063	1.065
36	29.7	1.068	>1.080	1.078	1.053	-	1.069	1.061
37	28.1	1.069	1.070	>1.080	1.054	1.055	1.061	1.061
38	29.1	1.069	>1.080	>1.080	1.050	1.055	1.061	1.065
39	30.9	1.068	>1.080	1.070	1.047	1.053	1.057	1.063
40	28.7	1.069	1.073	1.079	-	1.055	1.063	1.063
41	26.7	1.072	>1.080	>1.080	1.052	1.055	1.061	1.066
42	34.3	1.066	1.079	1.077	<1.047	1.053	1.053	1.063
43	32.8	1.069	1.077	>1.080	1.047	1.051	1.061	1.063
44	31.8	1.067	1.079	1.077	<1.046	1.051	1.058	1.059
45	30.5	1.065	1.080	1.071	1.056	1.051	1.057	1.061

of Normal Albino Mice

tomach	Muscles	Small Intestine	Large Intestine	Cerebellum	Cerebrum
1.063	1.068	1.064	1.062	1.049	1.047
1.062	1.068	1.066	1.062	1.047	1.047
1.064	1.071	1.071	1.061	1.049	1.047
1.064	1.072	1.071	1.063	1.049	1.049
1.067	1.071	1.062	1.059	1.049	1.047
1.071	1.071	1.063	1.065	1.047	1.047
1.075	1.069	1.063	1.068	1.048	1.047
1.072	1.079	1.069	1.067	?1.049	1.047
1.065	1.069	1.063	1.067	<1.046	1.047
1.068	1.079	1.066	1.067	1.047	1.047
1.070	1.067	1.065	1.064	<1.046	1.047
1.068	1.070	1.069	1.069	1.049	1.047
1.062	1.071	1.069	1.069	1.047	1.046
1.062	1.067	1.071	1.071	1.047	1.047
1.066	1.069	1.069	1.063	1.048	1.047
1.060	1.067	1.065	1.071	1.047	<1.046
1.079	1.065	1.067	1.071	1.051	<1.047
1.069	1.068	1.068	1.073	1.049	1.047
1.067	1.071	1.067	1.067	1.049	1.047
1.071	1.067	1.065	1.067	1.049	?1.049
1.063	1.066	1.065	1.063	1.049	1.047

TABLE II

The Specific Gravities of the Tissues

Animal Number	Body Weight	Dehydration Period	Spleen	Liver	Pancreas	Adrenal	Ovary	Kidney
22	30.5	23.00	1.069	>1.080	>1.080	-	1.054	1.063
23	29.1	24.25	1.071	1.077	>1.080	1.047	1.055	1.065
18	20.6	25.00	1.073	>1.080	>1.080	1.048	1.070	1.067
24	28.6	25.25	1.071	>1.080	>1.080	<1.046	1.055	1.070
13	29.0	25.50	1.071	1.077	>1.080	1.053	1.057	1.059
5	27.7	25.75	1.070	>1.080	>1.079	?1.054	1.054	1.067
25	30.0	26.25	1.071	1.078	>1.080	1.051	1.055	1.069
19	28.1	26.50	1.069	>1.080	1.079	1.049	1.057	1.062
14	31.8	27.00	1.069	1.077	>1.080	1.051	1.057	1.061
6	27.4	27.25	1.069	>1.080	>1.080	-	1.053	1.064
20	26.9	27.50	1.071	>1.080	>1.080	<1.046	1.053	1.067
26	29.7	28.00	1.069	-	1.079	1.047	1.057	1.077
21	27.6	28.50	1.071	>1.080	>1.080	<1.046	1.055	1.069
27	31.7	29.00	1.070	>1.080	>1.080	-	1.057	1.060
7	32.5	29.25	1.069	1.079	>1.080	1.049	1.056	1.065
15	29.9	29.25	1.071	1.077	>1.080	1.051	?1.054	1.065
28	30.3	30.00	1.069	1.079	1.077	<1.046	1.057	1.061
16	29.6	30.50	1.071	>1.080	>1.080	1.048	1.056	1.067
8	20.75	30.25	1.069	>1.080	>1.080	1.049	1.057	1.067
17	26.8	31.25	1.070	>1.080	>1.080	?1.052	1.057	1.069
46	18.3	42.75	1.070	>1.086	>1.086	1.064	1.056	1.079
63	28.6	44.75	1.070	1.083	1.083	1.062	1.054	1.067
47	20.1	45.00	1.069	1.082	1.081	?1.056	1.061	1.062
64	27.3	45.75	1.070	1.077	1.083	1.053	1.058	1.065
48	24.0	46.50	1.071	1.085	1.083	?1.055	1.059	1.065
57	18.5	46.50	1.071	1.082	1.081	1.057	1.059	1.067
59	19.2	47.00	1.071	1.089	1.088	1.057	1.057	1.071
65	25.5	47.00	1.070	1.081	1.078	1.049	1.057	1.063
49	21.5	47.50	1.069	>1.086	1.082	1.056	1.056	1.067
58	24.1	48.00	1.071	1.083	1.083	1.055	1.057	1.065
60	22.9	48.00	1.071	1.087	1.087	1.055	1.054	1.071
50	25.0	48.50	1.071	1.083	1.077	1.055	1.055	1.071
54	22.0	48.50	1.069	1.077	1.069	1.055	1.055	1.065
51	21.90	49.50	1.071	1.083	1.081	1.056	?1.055	1.070
52	22.70	49.50	1.070	1.084	1.083	1.063	-	1.065
61	23.3	49.25	1.071	1.089	1.087	1.057	1.056	1.071
62	24.5	50.00	1.067	1.080	1.079	?1.058	?1.058	1.064
55	23.3	50.00	1.069	1.085	1.085	1.057	1.056	1.065
53	25.9	50.75	1.067	1.081	1.086	?1.053	1.057	1.070
56	-	51.00	1.071	1.085	1.084	1.055	1.053	1.072

of Albino Mice Deprived of Water

Heart	Stomach	Muscles	Small Intestine	Large Intestine	Cerebellum	Cerebrum
1.065	1.071	1.071	>1.080	1.075	1.049	1.047
1.067	1.070	1.075	1.075	1.073	1.051	1.049
1.067	1.074	1.074	>1.080	1.075	1.049	1.047
1.066	1.066	1.075	1.077	1.073	1.047	1.047
1.067	1.071	1.069	1.073	1.069	1.046	1.047
1.067	1.067	1.072	1.066	1.067	1.051	1.047
1.068	1.063	>1.080	1.062	1.077	1.051	1.049
1.066	1.071	1.069	1.068	1.071	1.047	1.047
1.066	1.063	1.071	1.064	1.067	1.047	1.066
1.065	1.067	1.072	1.066	1.072	1.051	1.049
1.073	1.073	1.073	1.071	1.069	1.049	1.047
1.067	1.064	1.072	1.071	1.073	1.049	1.047
1.071	1.069	1.069	1.064	1.069	?1.050	1.047
1.068	1.072	1.076	1.077	1.069	1.047	1.049
1.068	1.069	1.070	1.067	1.066	1.047	1.049
1.067	1.067	1.071	1.065	1.071	1.049	1.046
1.067	1.069	1.073	1.071	1.069	1.049	1.049
1.067	1.071	1.074	1.064	1.073	1.049	1.049
1.067	1.063	1.069	1.067	1.065	1.050	1.048
1.069	1.073	1.071	1.071	1.073	1.047	1.047
1.067	1.075	?1.074	1.080	1.076	1.052	1.050
1.069	1.077	1.079	1.083	1.073	1.053	1.049
1.065	1.081	1.073	1.075	1.068	1.053	1.049
1.069	1.079	1.075	1.087	1.069	1.053	1.049
1.065	1.081	1.077	1.067	1.069	1.051	1.049
1.067	1.073	1.078	1.079	1.067	1.055	1.049
1.068	1.078	1.081	1.073	1.071	1.053	1.052
1.065	1.075	1.079	1.071	1.068	1.051	1.049
1.065	1.075	1.079	1.073	1.069	1.052	1.049
1.069	1.071	1.073	1.077	1.071	1.050	1.049
1.067	1.071	1.079	1.075	1.074	1.051	1.049
1.065	1.075	1.077	1.077	1.075	1.051	1.049
1.067	1.081	1.079	1.074	1.072	1.051	1.049
1.067	1.076	1.079	1.069	1.075	1.051	1.049
1.066	1.077	1.079	1.075	1.071	1.051	1.049
1.067	1.075	1.085	1.071	1.079	1.053	1.051
1.065	1.082	1.077	1.077	1.075	?1.052	1.049
1.065	1.081	1.075	1.075	1.071	1.053	1.051
1.066	1.081	1.079	1.071	1.071	1.051	1.049
1.069	1.085	1.079	1.075	1.073	1.053	1.049

TABLE III

A Comparison of the Specific Gravities of the Tissues of Normal Albino Mice With Those of Mice Deprived of Water

		Period of Water Deprivation	
Organ	Normal	27 hrs.	48 hrs.
Spleen	1.068 ± .002	1.070 ± .001	1.070 ± .003
*Liver	1.077 ± .004	1.079 ± .001	1.083 ± .003
*Pancreas	1.077 ± .004	1.080 ± .001	1.082 ± .004
*Adrenal	1.049 ± .003	1.049 ± .002	1.056 ± .003
Ovary	1.054 ± .002	1.056 ± .002	1.056 ± .002
Kidney	1.061 ± .004	1.066 ± .004	1.068 ± .004
Heart	1.063 ± .002	1.067 ± .004	1.067 ± .002
Stomach	1.067 ± .005	1.069 ± .003	1.077 ± .004
Muscle	1.070 ± .004	1.072 ± .003	1.078 ± .003
Small Intestine	1.067 ± .003	1.070 ± .005	1.075 ± .004
Large Intestine	1.066 ± .004	1.071 ± .003	1.072 ± .003
Cerebellum	1.048 ± .002	1.049 ± .002	1.052 ± .001
Cerebrum	1.047 ± .001	1.048 ± .001	1.049 ± .001

* This data is not valid. See the text.

TABLE IV

The Specific Gravities of the Tissues of Albi

Animal Number	Weight in Grams	Age in Days	Sex	Spleen	Pancreas	Adrenal	Testes	Ovary	Liver
203	6.5	1		1.064	-	-	-		1.053
204	6.6	1		1.062	-	-	-		1.058
205	8.9	3		1.065	-	1.053		-	1.057
206	8.4	3		1.065	-	1.052			1.059
209	8.9	4		1.063	-	-	?1.045		1.070
210	8.3	4		1.064	-	-	-		1.071
207	17.5	7		1.065	-	1.053	1.047	-	1.072
211	12.4	7		1.064	-	-		-	1.075
212	11.2	7		1.065	-	-	?1.046		1.077
208	19.6	8		1.065	-	1.053	1.047		1.071
214	18.2	10		1.064	1.064	-	-		1.074
213	18.4	Q		1.065	1.068	?1.056		-	1.071
170	-	11		1.065	-	1.057	-		1.073
215	29.0	12		1.065	-	?1.058	1.045		1.068
216	21.3	12		1.064	?1.066	1.060		-	1.071
172	18.9	13		1.065	?1.060	1.057		-	1.069
173	20.0	13		1.065	?1.053	1.057		-	1.069
177	26.4	16		1.067	-	1.055	1.047		1.073
178	25.2	16		1.065	?1.065	1.057		-	1.071
179	26.2	16		1.065	1.065	1.057		-	1.073
186	34.5	18		1.066	1.060	1.057	1.045		1.077
187	31.3	18		1.065	1.060	1.056	1.046		1.073
185	32.6	18		1.065	1.065	1.057		-	1.075
188	35.4	20		1.065	1.065	1.059		?1.055	1.077
189	34.5	20		1.065	1.065	1.059		?1.057	1.084
190	27.1	20		1.065	1.065	1.059		1.055	1.077
191	25.5	22		1.067	1.065	1.058	1.043		1.079
183	29.5	22		1.066	1.063	1.057		-	1.085
184	24.8	22		1.065	1.064	1.056		-	1.078
192	35.9	23		1.065	1.071	1.059		1.052	1.079
193	39.1	23		1.067	1.075	1.058		1.051	1.081
194	38.4	23		1.066	1.070	1.058	1.045		1.079
201	55.9	26		1.067	1.072	1.059	1.045		1.081
200	59.5	26		1.067	1.071	1.059	1.042		1.079
171	65.3	26		1.067	1.072	1.057		-	1.082
168	83.0	27		1.067	1.071	?1.060	1.045		1.078
169	81.7	27		1.068	1.075	1.057		-	1.075
100	42.7	30		1.064	-	1.055		-	1.085
102	54.3	31		1.065	1.067	1.055	-		1.075
103	54.3	31		1.07	1.072	1.056	-		1.071
101	70.6	31		1.069	1.067	1.055		1.054	1.079
104	61.6	31		1.065	1.072	1.055		1.051	1.077
105	33.0	31		1.065	1.074	1.055		1.055	1.075
106	65.0	34		1.063	1.075	1.054		1.055	1.077
107	63.1	34		1.065	1.075	1.055	1.043		1.080
108	54.3	34		1.065	1.071	1.054	1.045		1.071
109	60.5	35		1.065	1.070	1.055	?1.040		1.087
110	75.4	37		1.065	1.065	1.049	1.041		1.081
111	70.9	37		1.065	1.067	?1.049		1.053	1.077
112	79.0	38		1.067	1.073	1.053		1.055	1.083
113	91.6	38		1.065	1.070	1.055	1.041		1.082
115	103.7	40		1.065	1.073	1.055	1.041		?1.090
114	93.2	40		1.065	1.074	1.049		1.051	1.089

nc Rats at Various Ages

Kidney	Heart	Muscles	Stomach	Small Intestine	Large Intestine	Cerebellum	Cerebrum
1.050	1.053	?1.046	1.059	?	1.057	1.033	1.033
1.049	1.051	1.044	?1.065	-	1.066	-	?1.032
1.050	1.051	1.047	1.069	-	1.055	?1.032	1.032
1.054	1.055	?1.046	1.071	-	1.054	1.033	1.032
1.047	1.051	?1.048	1.066	-	1.059	1.031	1.032
1.050	1.052	1.048	1.065	-	1.059	1.034	1.032
1.051	1.049	1.053	1.072	-	1.056	?	?1.032
1.049	1.052	1.051	1.067	-	1.058	?	1.030
1.051	1.053	1.051	1.065	-	1.059	1.033	1.032
1.049	1.050	1.055	1.071	-	1.059	1.033	1.033
1.051	1.051	1.060	1.070	-	1.060	1.033	1.033
1.051	1.050	1.053	1.067	-	1.063	1.032	1.034
1.053	1.049	1.057	1.064	1.059	1.059	1.034	?1.034
1.052	1.050	1.065	1.065	-	1.059	1.037	1.035
1.052	1.055	1.062	1.062	-	1.059	1.037	1.033
1.049	1.051	1.054	1.067	1.059	1.059	1.037	1.035
1.053	1.051	1.056	1.069	1.068	1.058	1.041	1.035
1.055	1.054	1.062	1.062	1.069	1.060	1.044	1.039
1.056	1.055	1.063	1.067	1.071	1.063	1.044	1.036
1.058	1.055	1.063	1.059	1.069	1.062	1.044	1.039
1.059	1.052	1.065	1.065	1.066	1.060	1.045	1.041
1.056	1.053	1.065	1.063	1.064	1.063	1.044	1.041
1.055	1.053	1.063	1.066	1.066	1.061	1.045	1.041
1.054	1.057	1.065	1.065	1.065	1.055	1.046	1.043
1.059	1.054	1.065	1.068	1.064	1.062	1.045	1.043
1.057	1.055	1.067	1.065	?	1.062	1.046	1.041
1.059	1.057	1.065	1.067	1.066	1.058	1.047	1.041
1.057	1.053	1.064	1.074	1.065	1.058	1.046	1.043
1.057	1.055	1.058	1.065	1.064	1.064	1.045	1.043
1.059	1.057	1.067	1.069	1.069	1.065	1.048	1.043
1.059	1.057	1.067	1.065	1.065	1.059	1.047	1.043
1.058	1.054	1.064	1.065	1.067	1.060	1.046	1.041
1.059	1.059	1.067	1.065	1.067	1.061	1.047	1.043
1.061	1.058	1.067	1.064	1.066	1.056	1.047	1.043
1.059	1.059	1.065	1.067	1.063	1.062	1.048	1.045
1.062	1.058	1.071	1.067	1.063	1.061	1.048	1.045
1.060	1.057	1.071	1.069	1.063	1.063	1.049	1.045
1.063	1.059	1.065	1.073	1.065	1.059	1.049	1.045
1.063	1.055	1.063	1.066	1.065	1.063	1.048	1.045
1.061	1.055	1.065	1.061	1.061	1.063	1.048	1.045
1.061	1.053	1.068	1.071	1.067	1.061	1.047	1.045
1.063	1.055	1.063	1.065	1.059	1.061	1.047	1.045
1.068	1.057	1.061	1.063	1.061	1.065	1.047	1.043
1.060	1.057	1.066	1.070	1.073	1.063	1.047	1.045
1.064	1.057	1.069	1.073	1.071	1.064	1.049	1.047
1.059	1.057	1.065	1.071	1.064	1.063	1.048	1.045
1.059	1.056	1.069	1.061	1.066	1.059	1.049	1.045
1.060	1.055	1.065	1.061	1.065	1.059	1.047	1.045
1.056	1.057	1.065	1.066	1.059	1.059	1.047	1.045
1.061	1.055	1.065	1.069	1.062	1.059	1.049	1.045
1.063	1.057	1.065	1.066	1.071	1.057	1.049	1.045
1.063	1.057	1.069	1.065	1.067	1.063	1.049	1.045
1.061	1.059	1.069	1.071	1.065	1.059	1.048	1.045

116	112.6	40	1.066	1.071	1.049			1.085
117	91.7	41	1.065	1.073	1.056		1.051	1.063
118	102.9	41	1.066	1.073	1.061	1.041		?1.092
119	87.3	42	1.063	1.071	1.059	1.042		1.081
120	100.8	42	1.067	1.079	1.055		?1.052	1.081
121	82.7	42	1.067	1.077	1.051		1.051	1.081
123	100.9	44	1.065	1.069	1.053		1.056	1.091
124	124.6	45	1.065	1.075	1.055	1.038		1.081
125	145.2	48	1.067	1.077	-	1.041		1.087
126	115.5	48	1.071	1.076	1.053		1.051	1.087
127	92.3	48	1.067	1.075	1.053		1.049	1.087
128	156.4	49	1.067	1.078	-	1.040		1.075
129	147.6	49	1.069	1.075	1.053	1.041		1.079
130	89.6	49	1.072	?1.086	1.051		1.053	1.075
131	140.0	51	1.073	1.075	1.053		1.051	1.083
132	161.0	52	1.067	1.075	1.057	?1.040		1.087
133	139.6	52	1.067	1.077	1.053		1.051	1.083
134	165.7	54	1.066	1.073	1.057	?1.040		1.079
135	137.7	54	1.071	1.078	1.053		1.055	1.083
136	136.3	55	1.068	1.075	1.056		1.050	1.085
137	141.4	55	1.067	1.075	1.053		1.051	1.087
138	169.0	57	1.067	1.075	1.056		1.053	1.091
139	198.9	57	1.067	1.070	1.055	1.039		?1.094
140	238.7	58	1.067	1.079	1.060	1.039		1.083
141	183.5	59	1.067	1.083	1.057	1.039		1.083
142	213.2	59	1.067	1.075	1.059	1.039		1.087
143	141.2	59	1.069	1.077	?1.052		1.053	1.087
144	156.5	61	1.069	1.075	1.058		1.051	1.087
145	231.4	61	1.067	1.077	1.059	1.038		1.087
146	134.2	64	1.069	1.075	1.053		1.053	1.087
147	248.1	65	1.067	1.083	1.059	1.039		1.089
148	182.1	66	1.069	1.076	1.055		1.051	1.083
149	239.8	66	1.067	1.080	1.056	1.039		?1.053
150	262.4	69	1.069	1.076	1.059	1.038		1.087
151	259.4	69	1.069	1.082	1.059	?1.031		1.085
152	165.9	69	1.068	1.075	1.057		1.055	1.077
153	141.2	69	1.069	1.083	1.051		1.057	1.077
154	167.0	72	1.071	1.083	1.057		1.053	1.086
155	297.1	73	1.067	1.076	?1.054	1.039		1.087
156	262.7	76	1.067	1.078	1.057	1.039		1.087
157	203.5	77	1.071	1.080	1.054		1.053	1.081
158	319.8	77	1.069	1.081	1.053	1.039		1.079
159	202.6	77	1.071	1.081	1.051		1.053	1.083
160	174.3	77	1.070	1.083	1.052		1.053	1.081
161	280.2	79	1.069	1.075	1.057	1.038		1.091
162	186.0	83	1.069	1.075	1.051		1.055	1.077
163	339.6	83	1.068	1.072	?1.054	1.039		1.089
164	324.7	84	1.065	1.081	1.056	1.039		1.087
165	200.0	84	1.069	1.083	1.059		1.052	1.079
166	192.4	86	1.069	1.079	1.055		1.054	1.079
167	310.7	90	1.068	1.081	1.054	1.037		1.083
174	303.7	97	1.069	1.077	1.057	1.041		1.081
175	234	97	1.069	1.079	1.052		1.055	1.085
176	219.7	97	?1.079	1.081	1.053		1.051	1.087
180	369.4	98	1.069	1.079	1.057	1.041		1.081
181	362.5	98	1.068	1.077	1.057	1.041		1.085
182	241.1	99	1.071	1.083	1.051		1.050	1.079
195	201.6	104	1.071	1.079	1.055		1.051	1.085
196	234.4	104	1.069	1.077	1.057		1.053	1.075
197	288.4	105	1.070	1.081	1.056	1.037		1.089
198	382.5	105	1.069	1.083	1.058	1.039		1.077
199	267.7	105	1.067	1.077	?1.053	1.039		1.077

1.059	1.057	1.071	1.067	1.065	1.063	1.049	1.045
1.063	1.057	1.069	1.071	1.067	1.060	1.049	1.045
1.063	1.057	1.071	1.069	1.071	1.063	1.049	1.047
1.061	1.053	1.071	1.069	1.069	1.063	1.049	1.044
1.061	1.059	1.071	1.071	1.069	1.059	1.049	1.047
1.059	1.059	1.069	1.070	1.069	1.062	1.048	1.045
1.057	1.059	1.067	1.071	1.063	1.062	-	-
1.059	1.057	1.071	1.069	1.063	1.059	1.048	1.045
1.055	1.061	1.071	1.073	1.071	1.061	1.047	1.045
1.055	1.061	1.071	-	1.063	1.065	1.048	1.045
1.065	1.057	1.071	1.065	1.065	1.065	1.048	1.045
1.065	1.059	1.073	1.069	1.067	1.065	1.047	1.045
1.059	1.059	1.071	1.069	1.067	1.062	1.047	1.045
1.061	1.059	1.073	1.073	1.065	1.063	1.047	1.045
1.061	1.059	1.071	1.069	1.071	1.061	1.046	1.045
1.059	1.059	1.073	1.064	1.068	1.061	1.046	1.045
1.059	1.059	1.073	1.065	1.071	1.058	1.047	1.045
1.065	1.060	1.071	1.073	1.066	1.063	1.047	1.045
1.059	1.057	1.073	1.069	1.066	1.063	-	-
1.061	1.057	1.073	1.069	1.065	1.059	1.048	1.045
1.063	1.063	1.071	1.067	1.065	1.059	1.047	1.045
1.061	1.059	1.073	1.069	1.067	1.059	1.047	1.047
1.063	1.059	1.073	1.069	1.067	1.061	1.049	1.045
1.061	1.060	1.074	1.068	1.067	1.063	1.049	1.045
1.063	1.059	1.073	1.069	1.067	1.060	1.048	1.045
1.061	1.057	1.073	1.068	1.072	1.060	1.047	1.047
1.061	1.061	1.073	1.067	1.067	1.059	1.051	1.046
1.061	1.061	1.073	1.068	1.075	1.065	1.047	1.047
1.061	1.059	1.075	1.068	1.071	1.061	1.049	1.045
1.063	1.059	1.073	1.067	1.065	1.063	1.049	1.047
1.065	1.063	1.077	1.065	1.067	1.064	1.049	1.047
1.063	1.059	1.073	1.070	1.069	1.060	1.047	1.047
1.063	1.061	1.073	1.069	1.067	1.057	1.048	1.047
1.063	1.057	1.075	1.067	1.071	1.065	1.048	1.049
1.065	1.061	1.075	1.069	1.067	1.060	1.047	1.047
1.065	1.061	1.074	1.067	1.066	1.064	1.048	1.047
1.067	1.059	1.075	1.070	1.071	1.065	1.049	1.047
1.063	1.061	1.077	1.071	1.069	1.063	1.048	1.045
1.067	1.061	1.073	1.064	1.067	1.063	1.047	1.047
1.062	1.055	1.070	1.065	1.065	1.059	1.048	1.046
1.063	1.057	1.077	1.067	1.071	1.063	1.049	1.046
1.064	1.059	1.075	1.059	1.065	1.061	1.047	1.047
1.064	1.059	1.077	1.069	1.069	1.061	1.048	1.045
1.062	1.061	1.075	1.067	1.067	1.061	1.048	1.047
1.061	1.060	1.075	1.067	1.067	1.067	1.047	1.045
1.061	1.061	1.077	1.069	1.070	1.061	1.051	1.046
1.063	1.061	1.077	1.067	1.070	1.061	1.047	1.045
1.065	1.061	1.075	1.069	1.065	1.057	1.048	1.047
1.067	1.061	1.075	1.065	1.075	1.065	1.048	1.046
1.063	1.061	1.075	1.069	1.071	1.061	1.047	1.047
1.067	1.059	1.079	1.066	1.067	1.065	1.049	1.045
1.065	1.060	1.076	1.071	1.067	1.063	1.047	1.045
1.065	1.061	1.079	1.067	1.069	1.065	1.047	1.045
1.064	1.061	1.075	1.069	1.065	1.065	1.047	1.045
1.065	1.059	1.077	1.069	1.069	1.061	1.049	1.047
1.065	1.059	1.078	1.074	1.069	1.065	1.047	1.045
1.065	1.061	1.078	1.067	1.068	1.063	1.047	1.045
1.062	1.065	1.077	1.073	1.073	1.067	1.048	1.045
1.064	1.062	1.075	1.066	1.068	1.067	1.047	1.047
1.067	1.059	1.079	1.067	1.066	1.061	1.047	1.046
1.066	1.059	1.076	1.069	1.065	1.061	1.047	1.045
1.059	1.057	1.073	1.070	1.069	1.064	-	-

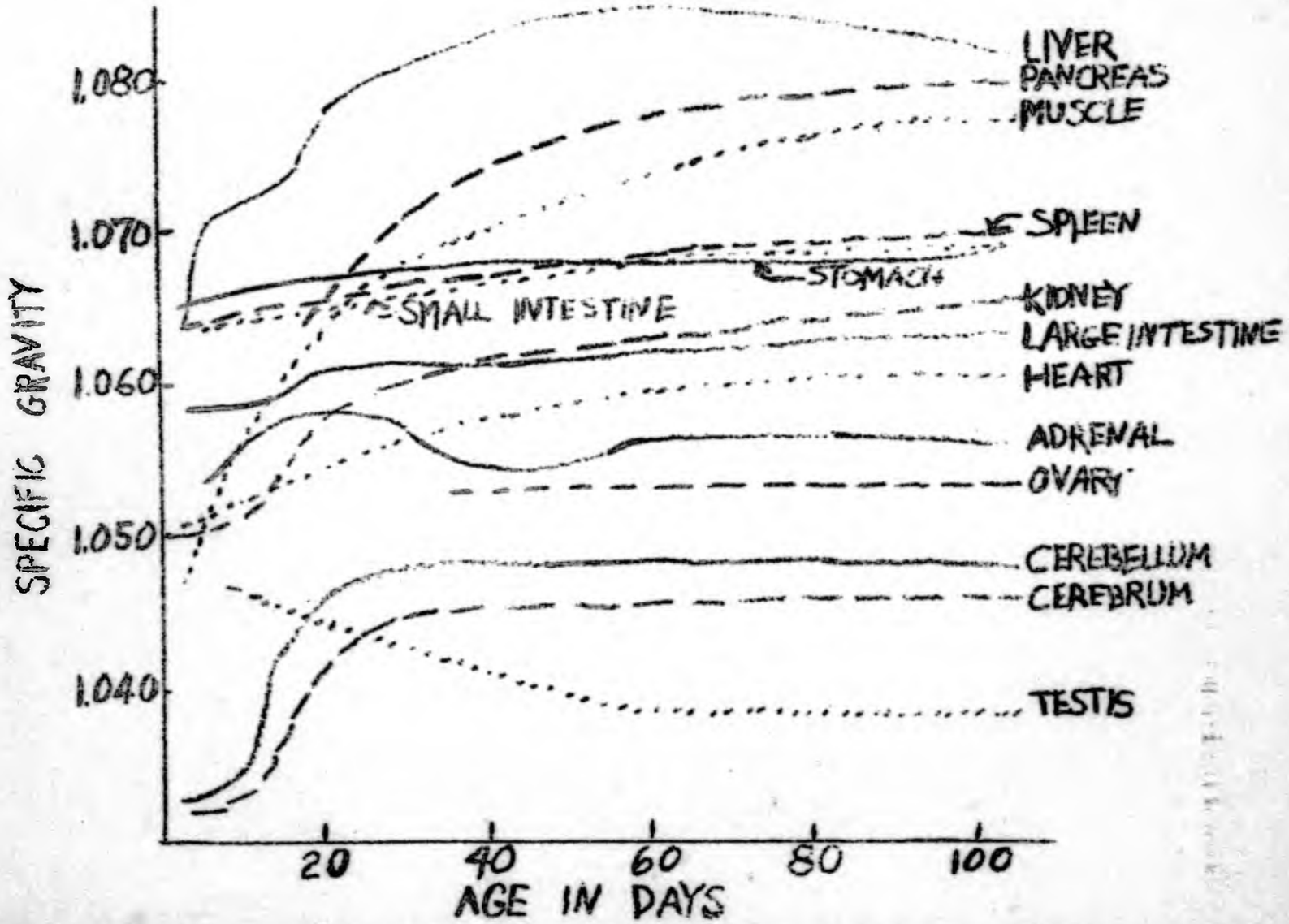

FIG 1 Specific gravities of tissues of the albino rat between birth and 110 days.

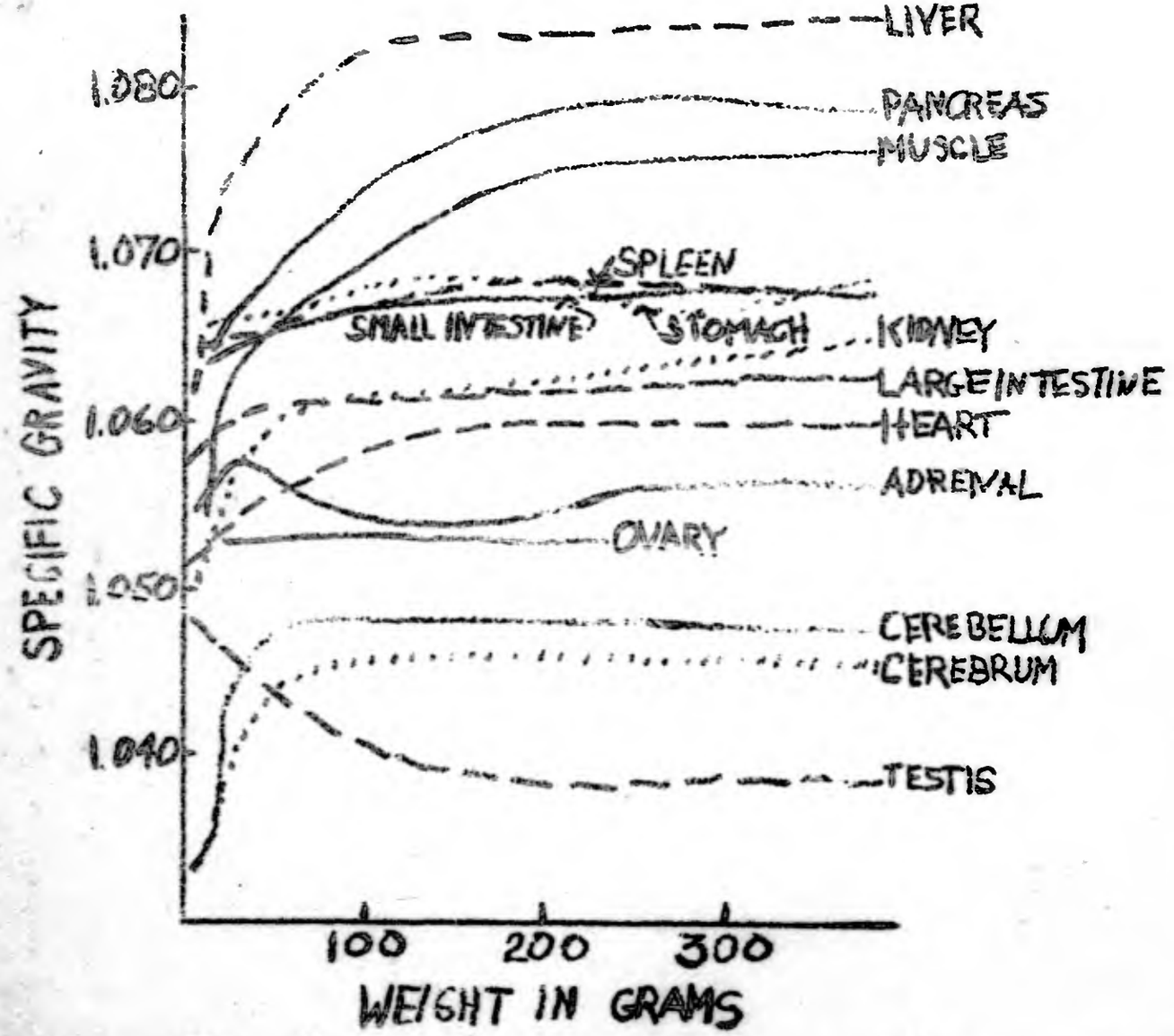

FIG. 2 The specific gravities of the tissues of the albino rat at various body weights

drawn (Figure I). In most cases, the nature of the curve was apparent by inspection. If there was any doubt, however, the standard deviation for each point was calculated. Figure II was compiled in the same manner to indicate the specific gravity-weight relationship.

The number of animals studied in each group was as follows:

Class number	Age Range	Number in Class
1	0 - 5	6
2	6 - 10	6
3	11 - 15	5
4	16 - 20	9
5	21 - 25	6
6	26 - 30	6
7	31 - 40	16
8	41 - 50	13
9	51 - 60	13
10	61 - 70	10
11	71 - 80	8
12	81 - 90	6
13	91 - 100	4
14	101 - 110	4

It was impossible to obtain all tissues, especially the ovary and small intestine, from the animals younger than fifteen days. Moreover, since the group consisted of approximately half females and half males, the number of samples of each gonad was approximately one-half the frequency for each mixed-age group. With few exceptions, all other tissues were taken from all animals.

DISCUSSION

The Method

Several workers have determined the specific gravity of single cells - their primary interest usually being the viscosity or some other characteristic of protoplasm in which the measurement of specific gravity was a factor. Earlier biologists did no more than compare the specific gravities of the cells with those of sugar solutions of known densities. From this method has evolved the present "floatation equilibrium" technique in which a graded series of solutions of known density is used to measure specific gravity. Working on the viscosity of protoplasm, Heilbrunn (6) applying Stoke's Law, determined the specific gravity of the granules in sea urchin eggs by noting the rate of their movement during centrifugation in sugar solutions of various concentrations. In 1939, Sawitz, et al (14) employed zinc sulphate solutions in a graded series of .005 specific gravity units for the measurement of the specific gravity of hook worm eggs. The use of zinc sulphate is essentially the same in principle as the copper sulphate of Phillips, et al (13) used in this investigation.

Most of the data from the earlier work on tissue density are limited and inconclusive. Krause and Fisher (8) in 1866 made an extensive study of the specific gravities of human post-mortem tissues. They used for their method the standard procedure of weighing the tissue in air and then in water. Although they studied different tissues, their number of determinations was limited (never above four for a given tissue), and therefore, they established no norms. In 1880, Smidt (16), applying the same method, investigated the specific gravities of the human liver and spleen in healthy and ill subjects and in individuals of various ages. He found no definite correlation between either age or pathological conditions and the specific gravities of tissues. About the same time, Danilewezsky (2), in a quantitative determination of the gray and white substances of the brain, used the pycnometer to determine the density of the human cerebrum. This technique enabled him to measure specific gravity to the fifth decimal place, but the data showed wide variation.

More recent investigations include that of Bohnenkamp and Schmah (1), who employed the principle of Paalzow to measure the specific gravity of human autopsy tissues to the third decimal place. The volume of the tissue was determined by placing it in an air-tight chamber, adding to this a known volume of oxygen, and determining the increase in pressure. The increase in pressure on the addition of gas is a function of the volume of gas to which it is added which is the volume of the chamber less than that of the tissue.

In 1939, Swinyard (17), measured the density of fresh endocrine glands as a means of determining the volume of the glands. He placed the tissue at the center of a solution of glycerol and water of such density that it remained suspended. He used standard solutions graded in a series of .002 specific gravity units. The same pieces of tissue he used repeatedly while locating the correct matching standard, which procedure doubtless introduced some error.

Only two recent investigations have been made purely to determine the specific gravity of tissue. Tsai and Lin (18), in 1939, determined the densities of frog, rabbit, and cat tissues by weighing them in air and then in saline. After dissection of the tissues, they washed them in saline, and placed them in a moisture chamber before weighing them. The error introduced by this practice cannot be definitely ascertained, but it places their results in some doubt. They obtained specific gravity values to the fourth decimal place, with the degree of variation depending upon the particular tissue being measured. These workers not only studied various normal tissues, but also they measured the effects of muscular activity, starvation, and diet on the specific gravities of tissues. Their results showed definite trends; but since statistically too few animals were used in some phases of the work, the significance of their norms is questionable. In 1950, Siguira and Arkin (15), comparing the densities of normal and tumorous tissues of the albino mouse, used sodium chloride solutions ranging from 6% to 13% in a series with gradations of 1%. For the desired comparison the method proved adequate but otherwise lacked precision. No attempt was made to convert the results into specific gravity units.

The method of Phillips, et al (13), was selected for this study and hereafter will be referred to as "the standard copper sulphate method".

Two other workers have made studies of tissue density using this method, both in connection with other problems. Gersh, et al (4), employed it to measure changes in tissue density as a means of determining the origin of tissue bubbles after decompression from high pressures. Those workers used aqueous copper sulphate solutions and methyl alcohol solutions over a range of 0.088 to 1.200 specific gravity units to measure the specific gravity of the fat, skeletal muscle, nerve, tendon, adrenal gland, and liver of the guinea pig. Later, in 1945, Morales, et al (11), also working with guinea pigs in a study involving theoretical considerations regarding the major body tissue components, used this method to determine the specific gravity of muscle.

The standard copper sulphate method has several advantages over other techniques. Since a copper proteinate membrane forms around the tissue upon contact with the solution, the exchange of water and electrolytes is minimized and retarded. In most of the methods of other workers mentioned above, no precautions were taken to eliminate this variable. Even the interchange of water and electrolytes between the tissue and physiological saline doubtless, in time, alters the density of the tissue being measured. For this reason, the standard copper sulphate method seemed more appropriate for a study of water deprivation, which involved fluid balance. A second reason for preferring this method is its simplicity. No costly or elaborate apparatus is involved, no standard sized piece of tissue is required, and under ordinary conditions no correction is needed for temperature variations. The procedure usually requires, however, three or four pieces of tissue for each determination, which are not always easily obtained especially in small organs.

The data have been presented in specific gravity units. These values, although assumed to be the true values, may be in slight error to one side or the other of the true specific gravities. Since this method will probably be used for comparative studies, and since, considering that there is variation in the specific gravities of individual tissues, its use as a comparative method is of more importance than a knowledge of an absolute measurement.

Several variables may have shifted the measurements away from the true value. The coefficient of expansion of blood and plasma was found by Phillips, et al (13), to be almost the same as that of the aqueous standard solutions of copper sulphate, and therefore no temperature correction is necessary when working with blood. This has been assumed to be true for tissue also. The effect of death upon tissue density has not been determined. For a given tissue, however, the time which elapsed between death and the determination of the density, was fairly constant because the tissues were removed in the same order during each dissection. This time ranged from about five minutes for the spleen, the first tissue to be measured to sixty or eighty minutes for the cerebrum, the last. It is not known whether the density of an organ varies from region to region. In this study, the tissue was removed from the same region of a given organ each time. Smidt (16) in his work on the specific gravity of the spleen, measured the density of the whole organ, then each half. He found no significant difference in the densities of the two halves.

The study of the specific gravities of tissues shows promise of being a means of detection and investigation of diseased tissues. One 105 day old rat was found to have bilateral ureter and bladder stones and degenerated kidneys. The specific gravity of the diseased kidney was 1.059, while the average for that age group was 1.065 with none of the individuals in that age group falling below 1.061. Siguira and Arkin (15) found that in both mouse and man, tumorous tissues were less dense than heart, kidney, spleen, muscle, or liver tissues. Krause and Fisher (8) investigated the specific gravities of pathologically altered human tissues, and with the exception of the spleen, found no measurable differences in density from normal specimens. Although very little evidence had been collected as yet, these preliminary studies indicate that the standard copper sulphate method of determining tissue density might find application in the study of pathological tissues.

This method is doubtless of value as a tool in physiological research. This is indicated by the results from the study on water deprivation in which measurable changes in the specific gravities were induced experimentally, and from the study of age in which normal physiological changes in the specific gravities of tissues were followed. Such factors as growth, diet, exercise, disease, etc., seen to produce measurable changes in tissue density. Tsai and Lin (18) reported that repeated stimulation and contraction of the frog gastrocnemius produced a decrease in the specific gravity of that muscle, that high protein feeding brought about an increase in the density of the liver, and that starvation was followed by a decrease in the densities of muscle and liver tissues of the rabbit.

The value of the standard copper sulphate method as a measurement of the relative specific gravity of tissue is demonstrated by the following facts: (1) The specific gravity of each tissue fell within definite limits so that a characteristic density for a given tissue could be ascertained: (2) studies of tissue density and age and water deprivation produced significant results: (3) these results were measurable to the third decimal place.

Norms of the Specific Gravities

Albino Mouse and Rate Tissues

Obviously, the specific gravity of a given tissue falls within definite limits so that a characteristic density of each tissue can be determined. The values listed in the summary, Table III, for the specific gravities of normal albino mouse tissues represent the first significant study to determine the normal range for the densities of given tissues. On this basis, predictions may be made and comparisons between abnormal and normal tissues may be drawn. By establishing the specific gravity ranges of normal tissues this study has laid the foundation for further investigation.

It is evident that in both the mouse and rat, the brain is the least dense tissue and the liver the most dense, with the other tissues

ranging between. While this general pattern holds true for most other species, it is not true for all. Table V is a summary of the tissue densities of various animals as given in the literature. These values are by no means established norms, since they are based upon insufficient quantities of data.

It is not within the scope of this work to discuss the reasons for the differences in the specific gravities of various tissues. It is assumed, however, that the characteristic density of a given tissue is dependent primarily upon fat, water, and protein content, and to a less extent upon carbohydrate and salt content. That the brain is the least dense of the body tissues might be predicted from the data of Laramore and Grollman (9) whose work with rats indicated that the brain has the highest fat and water content (8.4% and 77.9% respectively) of any tissue studies. Since these tissue components vary with conditions of nutrition, age, activity, etc., no absolute point can be given as the normal specific gravity of a tissue, but rather a normal range.

The degree of individual variation as indicated by the standard deviation in Table III seems to be a characteristic of the tissue, the brain being the least variable, the stomach the most. The wide variability in the values of the specific gravity of the stomach may be due in part to the difficulty of completely emptying the contents of this organ. Variation might be produced by age differences, as the discussion below will indicate. However, the mice in these studies were nearly the same age.

The Effect of Water Deprivation on Tissue Densities

Since various solids differ in specific gravity from water, the measurement of tissue density may be used to detect alterations in solid and water content of tissues. As seen in Table III, there was an increase in the mean of the densities of every tissue after a period of 48 hours of water deprivation. Statistically significant differences were found for stomach, muscle, and small intestine tissues. This indicates that under conditions of water deprivation the loss of water is relatively more rapid in smooth and skeletal muscle than in glandular, nervous, and cardiac tissues.

During the first phase of this investigation, norms were established for each tissue of the albino mouse. In order to ascertain whether these values could be changed experimentally, this study of water deprivation was undertaken. Again, it is not within the scope of the present work to interpret the changes found.

The Relationship of Age and Tissue Density

The age range investigated (from birth to 110 days) is the period of most active growth for the rat. Figure I indicates that the majority of tissues studied increased in density with age. The notable exceptions to this were the ovary which remained of constant density,

TABLE V

The Densities of Various Animal Tissues

REFERENCES	ANIMAL	METHOD	SPLEEN	PANCREAS
Bohnenkamp & Schmah (2)	Human	Principle of Paalzow		
Danilwesky (3)	Dog	Pycnometer		
Danilwesky (3)	Human	Pycnometer		
Gersh, et al (5)	Guinea pig	Standard Copper Sulfate		
Krause & Fisher (9)	Human	Weighed in air and in H_2O	1.058	1.047
Morales, et al (13)	Guinea pig	Standard Copper Sulfate		
Smidt (18)	Human	Weighed in air and in H_2O	1.063	
Smith	Mouse	Standard Copper Sulfate	1.068	
Smith	Rat (100 day)	Standard Copper Sulfate	1.070	1.079
Swinyard (19)	Rat	Floatation equilibrium in graded series of glycerol and water		
Tsai & Lin (20)	Cat	Weighed in air in and saline	1.067	
Tsai & Lin (20)	Frog	Weighed in air and in saline		
Tsai & Lin (20)	Rabbit	Weighed in air and in saline		

*Cerebrum
**Cerebellum

Summarized from the Literature

ADRENAL	OVARY	TESTIS	LIVER	KIDNEY	HEART	MUSCLES	STOMACH	SMALL INTESTINE	LARGE INTESTINE	BRAIN
			1.040 1.089	1.041 1.046	med. cort.	1.039				1.040
					1.036					
					1.040					
						1.065				
1.054	1.045	1.045	1.057	1.044 1.049	med. - cort.	1.041 1.066	-	-	-	1.032
			1.050	1.049	1.054					1.047*
	1.054			1.061	1.063	1.070	1.067	1.067	1.066	1.048**
1.055	1.052	1.041	1.083	1.065	1.060	1.077	1.070	1.068	1.064	1.045* 1.047**
1.039										
		1.051	1.067	1.051	1.055	1.070	1.060	1.066		1.044
			1.050			1.063	1.051			
		1.043	1.071	1.062	1.056	1.077	1.058	1.052		1.043

the testis which became less dense, and the adrenal gland which fluctuated within a relatively narrow density range.

The extent of the change in tissue density with age is dependent upon the tissue in question. For instance, for the spleen, stomach, small intestine, and large intestine there was but slight increase in density during the ages studied. On the other hand, the densities of the liver, muscle, pancreas, kidney, heart, and brain increased greatly during the same period. It will be noted, that in most tissues, the slope of the curve is greater for younger tissues than for older, and that there is a tendency for the curves to level off and become nearly horizontal with advancing age. It is of particular interest that the specific gravity of the kidney shows a continued rise even at 110 days.

Since growth encompasses cell division, enlargement, and differentiation, weight and size studies of growth do not give the complete picture. The measurement of density indicates differentiation while size measurements indicate enlargement and division.

It is of interest that histochemical data indicate a decrease in water and fat content over the age range studied, and an increase in heavier protein molecules. These factors might in part account for the rise in density with age. Hurst (7) working with albino rats found that the water content of the body as a whole decreased gradually with increasing age from about 83% of the live weight at one day to about 66% at 112 days. The data in Table VI taken from Donaldson (4), indicate the decrease in the water content of various tissues over the range of this study.

Williams, et al (19), divided the total body lipids of the rat at different ages into neutral or stored fat and essential or structural fatty compounds. They found that at birth, on a dry weight basis, the essential fat made up 9.54% of the dry weight, while at 70 days it was only 5.22%. Moulton (12), in a study of age and chemical development, defined the point in the growth of mammals at which the concentration of water, proteins, and salts becomes comparatively constant in the fat-free cell, as the point of "chemical maturity". On this basis, he fixed the point of "chemical maturity" for the rat at fifty days after conception. This point of completed chemical growth compares favorably with the point where the rise in tissue density levels off for many tissues as shown in Figure I.

TABLE VI

Changes with Age in the Water Content of Various Tissues of the Albino Rat

Tissue	Age	% Water	Age	% Water	Age	% Water
Spleen	Birth	85.7	70 days	79.4	-	-
Liver	Birth	80.6	20 days	75.7	1 yr.	74.0
Kidney	Birth	86.1	30 days	82.8	1 yr.	77.1
Heart	Birth	86.2	20 days	82.6	1 yr.	77.6
Muscle	Birth	89.3	20 days	74.4	No subsequent change	
Brain	Birth	88.0	25 days	81.0	50 days	79.0

The notable downward trend of the specific gravity of the testis is exceptional. The curve levels off, however, about the 60th day - the time of puberty for the rat. Perhaps, the age-density changes of the testis reflect some refining process prior to the production of functionally adequate spermatozoa. Wide variations from the norm were found in the specific gravities of ovarian tissues of different animals. Possibly the metabolic and funtional cyclic changes effect the density to produce this variability.

The increase in the density of muscle with age is of striking magnitude and rate. Lowry, et al (10), found that during this period of growth in the rat there is a decrease in the concentration of water, collagen, chloride, total phosphorous, and potassium in skeletal muscle. These changes were interpreted as an increase during growth in the proportion of the intracellular tissue at the expense of the extracellular compartment. Perhaps, this is one factor responsible for the increase in density.

The specific gravities of the wall of the stomach, small intestine and large intestine increase relatively little with age. Apparently, smooth muscle is rather stable, and undergoes little noticeable change after birth. Haggqvist (5) reports that he can find no observations on age change in gastrointestinal tissue.

It is of interest to note that the degree of variation of the specific gravity of a given tissue sometimes changes with age. The variations from the means of the specific gravities of the adrenal gland, spleen, and kidney tissues were small in younger animals, but increased with age. On the contrary, the specific gravity of the liver varied more in the younger animals than in the older ones. Again, it is possible only to point out this trend since no explanation can be given at present.

CONCLUSIONS

The application of the use of the standard copper sulphate method for the determination of the specific gravity of blood as outlined by Phillips, Van Slyke, Hamilton, Dole, Emerson, and Archibald was tested for use in similar specific gravity determinations with tissues, and the following observations were made:

(1) Valid norms with standard deviations for the specific gravities of the albino mouse and the albino rat were established showing that each tissue has a characteristic specific gravity.

(2) Measurable changes in the normal specific gravity for a given tissue can be induced experimentally since, following water deprivation for 48 hours, an increase in the average specific gravities of all tissues studied occurred. Statistically significant increases in density were found for stomach, muscle, and small intestine.

(3) The specific gravities of all the tissues studied increased as the age of the animal increased over the age range investigated,

with the exception of the specific gravities of the ovary which remained unchanged, of the testis which decreased, and of the adrenal gland which fluctuated.

(4) There is evidence that this method for the determination of the specific gravities of tissues may become an important tool in the investigation of pathological and experimental physiological conditions.

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THERMAL RESPIRATOR

A great percentage of the time devoted to the contract was spent on the design, testing, and development of a breathing device known as a thermal respirator. The purpose of the respirator is to conserve the heat of expired air and apply it to the warming of air to be inspired. The device consists essentially of a heat exchanger. The device was finally perfected and a patent, patent number 2610038, was granted to Norman E. Phillips and Loyal Goff. The right to manufacture and use the device was granted to the military services.

A detailed description of the device and a description of the testing is omitted since this has been earlier communicated to the Navy and working models of the apparatus were constructed by the Ohio Chemical Company. These working models are now being field tested in various cold environments and the reports of the tests are being sent to the Office of Naval Research.