# THE EFFECT OF DIETS CONTAINING ADDED GLYCINE UPON THE RESPIRATORY QUOTIENTS OF RATS

by

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# INTRODUCTION

In addition to dietary factors there are many environmental factors which influence the respiratory quotients of fasted animals. Among those which must be considered are muscular movements, environmental and body temperature, seasonal and diurnal variations, humidity, exposure to ultra violet light, psychogenic factors, and duration of fast. The type of food metabolized also effects the respiratory quotient, and it is for this reason that so much attention has been directed toward energy metabolism in the past. A point to be emphasized, however, is that the respiratory quotient is the same for any complete process of the utilization of a certain type of foodstuff and is independent of the intermediary metabolic processes(1). For example, the supposed conversion of fat to carbohydrate could have no effect upon the respiratory quotient unless some of the material thus formed were laid down in the body, or excreted, during the experiment.

For studying the gaseous metabolism of an animal as small as the albino rat only the chamber method for determining the respiratory quotient can be used. Respiratory quotient may be defined as the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed. The earliest form of apparatus for small animals was described by Haldane(2) in 1892. Since then this technique, with some modifications, has been employed by numerous investigators in determining the gaseous metabolism of rats. Pembry(3) was first to report experiments on rats, using a somewhat modified form of the Haldane apparatus, with which he measured only the carbon dioxide production. Later Pembry and Spriggs(4) used the same apparatus for

determining both carbon dioxide production and oxygen consumption of normal rats. After feeding rats a high carbohydrate diet these workers showed that the respiratory quotient rose from a fasting 0.68 to 0.92 within one hour following feeding, and then maintained this high level for two to three hours before it started to fall again. Kingdon et al.[5] following the respiratory quotients of rats for longer fasting periods concluded that the respiratory quotient fell rather smoothly until approximately the 9th hour of fast, and then became erratic until after the 26th hour of fast. They pointed out that the 24 hour fast used so commonly in metabolic studies on rats is a time of unstable metabolism. It is to this that they attribute many of the contradictory conclusions found in the literature on energy metabolism.

The respiratory quotient of the amino acid glycine, if converted completely to carbon dioxide, ammonia, and water, would be 1.33. Since an animal never metabolises glycine alone and just how completely glycine is metabolized is uncertain, the studies of the effect of glycine on the respiratory quotient have been done on animals fed diets containing increased amounts of this amino acid and on animals eating diets containing no added glycine. Recently Paretsky and Werkman[6] have shown the steps by which glycine is completely metabolized to carbon dioxide, ammonia and water. By using bacteria they obtained final results in which four volumes of carbon dioxide were produced to three volumes of oxygen utilized giving a respiratory quotient of 1.33. However, application of their methods would be

impossible in animals with as complex intermediary metabolic processes as rats. Lusk[7] fed glycine to phlorhizinized dogs and obtained no significant change in respiratory quotient over basal even though he demonstrated an increase in the number of calories produced. Olsen, Hemingway, and Nier[8] feeding carboxyl labeled glycine to mice recovered approximately 50 per cent of the labeled carbon in the respiratory carbon dioxide during a 16 hour fast. They also noted an immediate increase in the carbon dioxide excretion in the first two hours, which was more than could be accounted for by the metabolised glycine. However, they did not give oxygen consumption figures so the respiratory quotient could not be determined. Murlin and Lusk[9] studying the combined effects of glucose, fat, and glycine obtained respiratory quotients of 0.88 to 0.90 for the first two hours following the feeding of glycine alone, 1.01 to 1.02 for glycine and glucose, and 1.00 to 1.03 for glycine and glucose given four hours after feeding fat. These results are not surprising since they only included respiratory quotients for the first two hours following ingestion of food and complete absorption from the intestinal tract probably had not occurred. Foster and Smith[10] gave intraperitoneal injections of glycine to rats and demonstrated an increase in metabolic rate, but included no respiratory data. None of this work, therefore, gives any idea as to what effect added glycine might have on the respiratory quotients of animals with an otherwise normal and adequate diet consumed ad libitum by the animal.

Mirski[11] in 1938 described the increase in liver glycogen found in fasted rats after feeding high protein diets. This he designated as the "protein effect". Todd, Barnes and Cunningham[12] later described this enhancement of liver glycogen in rats after a fast as due to prefeeding a diet containing added glycine. Todd and Telman[13] demonstrated that rats prefed the glycine diet have in their bodies after 8 hour fast and 5 hour insulin action about six times as much carbohydrate as can be accounted for by direct conversion to carbohydrate of the extra glycine present before the action of insulin. They concluded that the extra glycine present before the insulin action could not account for the increase in stored carbohydrate. With this evidence at hand, it seemed desirable to study the effects of diets containing added glycine upon the respiratory quotient of rats in hopes that it would produce more evidence as to what type of food was being metabolized and when the effect was most predominant.

## PROBLEM

Experiments were designed to obtain the respiratory quotients for fifteen minute intervals for one hour on pairs of rats treated in similar fashion except for the diet which they consumed. Each time interval of comparison, therefore, consisted of a two hour period in which the respiratory quotient of each animal, experimental and control, was determined at alternate hours. By this method three separate determinations were made on each animal during the hour that it was in the calorimeter. The effect of glycine on the respiratory quotient at various fasting intervals was studied. At the interval of greatest difference in respiratory quotient between the control fed and the glycine fed animals the effect of adrenalectomy was also investigated.

## EXPERIMENTAL

ANIMALS:

The animals used were all albino male rats of the Sprague-Dawley strain raised in our own colony. Each animal weighed within the range of 225 to 340 grams during the experimental period. From their frequent handling and long period of inbreeding these animals were tame and inactive, which made them excellent for respiratory quotient studies as a certain degree of cooperation, even from animals, is necessary in this work.

RATIONS:

Since the rats appetency for milk is so great, it was thought that better control could be gained over the quantities of ration consumed if Borden's evaporated milk, vitamin D increased, was included in both the control and glycine rations. Squibb's brewer's yeast was added in sufficient quantity to insure a high level of vitamin B complex intake for proper protein utilization. The carbohydrate in the diet was adjusted by the addition of Karo corn syrup, while the protein content was adjusted by the addition of Essenamine, a partially hydrolyzed protein derived from lactalbumin.

| Ingredient | Control Ration gms. | Experimental Ration* gms. |
|---|---|---|
| Evaporated Milk | 100 | 100 |
| Corn Syrup | 61 | 50 |
| Essenamine | 9 | 9 |
| Brewer's yeast | 10 | 10 |
| Glycine | 0 | 10 |

*The terms experimental ration and glycine ration shall be used interchangeably throughout the remainder of this thesis.

The 10 grams of glycine is roughly calorically equivalent to 11 grams of corn syrup, so in the experimental ration, equivalent amounts of corn syrup were subtracted for the glycine added.

The evaporated milk, corn syrup, yeast, and glycine, when used, were mixed in a beaker and placed in a hot water bath until warmed. The mixture was then transferred to a Waring Blender and stirred. Following slow addition of the Essenamine, the blender was run for about three minutes. The ration was then diluted to 200 ml. with water. Table 1 indicates the grams protein, fat, and carbohydrate and calories of the two rations.

APPARATUS:

Calorimeter: For serial measurements of the respiratory quotient an apparatus was used which was described by Lester and Greenberg[14]. A schematic diagram of the apparatus is shown in Figure 1. R is a round lucite box 7-3/4 inches in diameter and 3-1/2 inches deep fitted with a lid of the same material. Three holes (X, Y, Z) were drilled in the lid to allow for flushing, sampling, and temperature measurements of the chamber gases. C and E are inlet-outlet tubes for flushing the chamber; F is a small electric fan; T is a thermometer (0° - 50° C.) calibrated in 0.1° C., and D is the sampling outlet. Tube B leads to a water manometer M, and a small test tube spirometer S, which has a volume of about 70 ml. and is counterbalanced by the weight W. The spirometer is connected through a three-way stopcock to a 50 ml. syringe A which is calibrated in 1 ml. increments. The volume of the system when the spirometer is empty is 2700 ml.

TABLE 1

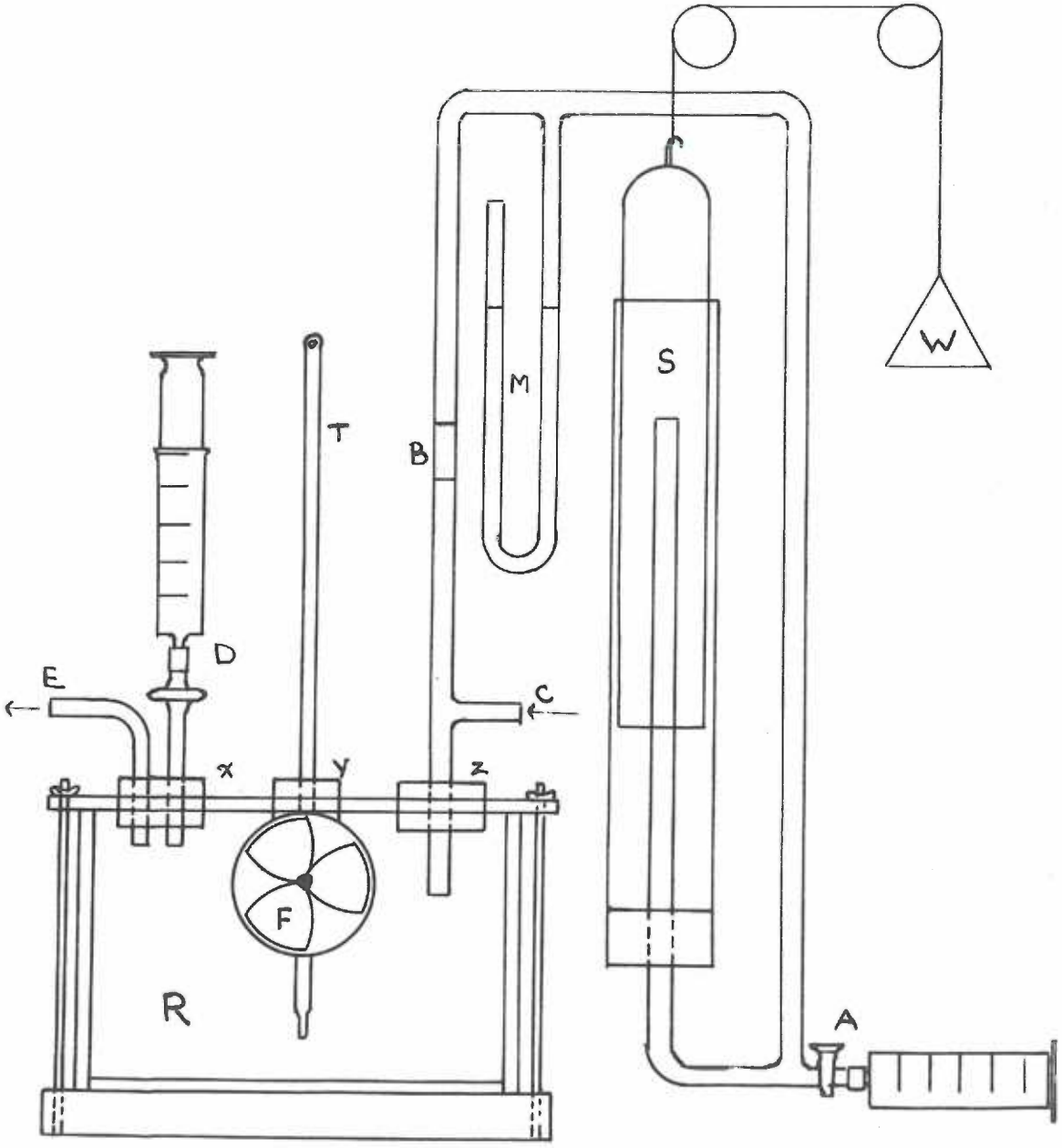
Percentage Composition on the Dry Basis of the Glycine and the Control Rations

| Substance | Control Diet | | | Glycine Diet | | |
|---|---|---|---|---|---|---|
| | Protein per cent | Fat per cent | Carbo-hydrate per cent | Protein per cent | Fat per cent | Carbo-hydrate per cent |
| Evaporated Milk | 7.0 | 8.0 | 10.0 | 7.0 | 8.0 | 10.0 |
| Karo | 0.0 | 0.0 | 52.0 | 0.0 | 0.0 | 43.6 |
| Essenamine | 9.0 | 0.0 | 0.0 | 9.0 | 0.0 | 0.0 |
| Yeast | 4.3 | 0.1 | 4.6 | 4.3 | 0.1 | 4.6 |
| Glycine | 0.0 | 0.0 | 0.0 | 10.0 | 0.0 | 0.0 |
| Totals (dry weight)* | 20.3 | 8.1 | 66.6 | 30.3 | 8.1 | 58.2 |
| Total Calories | | 420.5 | | | 426.9 | |
| Calories per ml. | | 2.10 | | | 2.13 | |

*Diluted to 200 ml. with water. For the adrenalectomized rats 20 grams of sodium chloride were added to make a one per cent solution.

FIGURE 1

Diagrammatic Sketch of the Calorimeter




Diagrammatic Sketch of Calorimeter

Figure 1

The respiratory exchange is determined by the following procedure. A rat is placed in the lucite box on a wire screen. The lid is sealed with lubricant and affixed to the box by the bolts and wing nuts present at the four corners of the cover. Enough pressure is applied to the corners to affect an air seal. After B is clamped off and A closed, air saturated with water vapor at 28° - 30° C. is blown through the chamber at a rate of about 25 liters per minute through C and exhausted at E. This rate of flushing is sufficient to prevent any significant accumulation of carbon dioxide.

Saturation of the air by water vapor was achieved by passing compressed air from the laboratory supply through a conical porous grinding stone which was attached to a 1/4 inch copper tube with "Pyseal" cement. This porous grinding stone when placed in a four liter flask partially filled with water produced small bubbles when air was forced through at the rate of about 25 liters per minute. Maintenance of saturated air was insured by having the entire system undergoing gradual cooling resulting in slight condensation of water during the time in which the calorimeter was in operation.

During the flushing the spirometer is brought to its lowest level and adjusted to atmospheric pressure with the syringe. When the spirometer indicates atmospheric pressure A is turned to room air and 50 ml. of air is admitted to the syringe. The room temperature and this volume of air is recorded. A is then turned so as to connect to the spirometer and the air in the syringe is admitted to it. At the time chosen to start the experimental period, the flushing is stopped, C and E are clamped off, the clamp at B is released, and the

temperature of the chamber and the barimetric pressure are recorded.

About 1-1/2 minutes before the end of the experimental period the electric fan is started to attain temperature equilibrium. Some 30 seconds before the end of the period the fan is stopped, the spirometer is adjusted to atmospheric pressure by the counterpoise, and B is clamped. A 50 ml. syringe is attached to D, and a 50 ml. representative sample of the gases in the chamber is obtained after the syringe plunger is pushed up and down a few times. The temperature of the chamber and the time elapsed for the experimental period are recorded. The 50 ml. sample is then removed for carbon dioxide analysis which is described below. The volume of air remaining in the spirometer is measured by the syringe, A, and this volume, the room temperature, and the barometric pressure are recorded.

Fifty milliliters of air are again admitted to the spirometer, the necessary readings are recorded, and the apparatus is started on a second determination. During the second experimental period the carbon dioxide analysis of the first is performed.

With the room temperature at approximately 24° C. the temperature of the chamber is maintained close to the critical temperature of the rat, 28° C. The critical temperature is defined by Benedict and McLeod (15, 16) as that environmental temperature which will have the least effect upon the resting metabolism of a rat. Any significant deviation above or below 28° C. will cause an increase in the resting metabolism of rats.

During an experimental period the temperature of the chamber changes no more than 0.8° C. The data obtained are recorded as

follows: b.p., barometric pressure; Vch, free volume of the chamber; $Tch_1$ and $Tch_2$, initial and final temperature in the chamber; $Trm_1$ and $Trm_2$, initial and final temperatures in the room; $Vs_1$ and $Vs_2$, initial and final volumes of the syringe; $Wch_1$, $Wch_2$, $Ws_1$, and $Ws_2$, initial and final vapor pressure for water in the chamber and the spirometer at the observed temperatures. The dry gas volume at standard conditions initially $V_1$ is:

$$V_1 = \left[\frac{b.p. - Wch_1}{760} \times \frac{273}{Tch_1 + 273} \times Vch\right] + \left[\frac{b.p. - Ws_1}{760} \times \frac{273}{Trm_1 + 273} \times Vs_1\right]$$

$V_2$ is similarly calculated. The per cent carbon dioxide determined (ignoring the atmospheric carbon dioxide) is always calculated to a constant time period. If the period is 15 minutes and the gas sample from the chamber is taken at 15 minutes and 30 seconds, the per cent carbon dioxide produced in 15 minutes is then 15/15.5 of that actually found by analysis. The amount of carbon dioxide produced is then ml. $CO_2$ = % $CO_2$ x $V_2$ and the amount of oxygen consumed is ml. $O_2$ = ml. $CO_2$ + $(V_1 - V_2)$. If the respiratory quotient is unity there will be no change in volume of the system; since equal amounts of carbon dioxide are produced and oxygen consumed, and $V_1$ will equal $V_2$. If the respiratory quotient is less than unity, $V_2$ will be less than $V_1$ by an amount equal to the difference between the oxygen consumed and the carbon dioxide produced.

CARBON DIOXIDE DETERMINATION:

For the determination of the carbon dioxide a modification of the methods of West et al.[17] was used. The basis of this method is

the absorption of carbon dioxide by excess standard barium hydroxide and the back titration of the excess barium hydroxide by standard hydrochloric acid using thymol blue indicator for the end point.

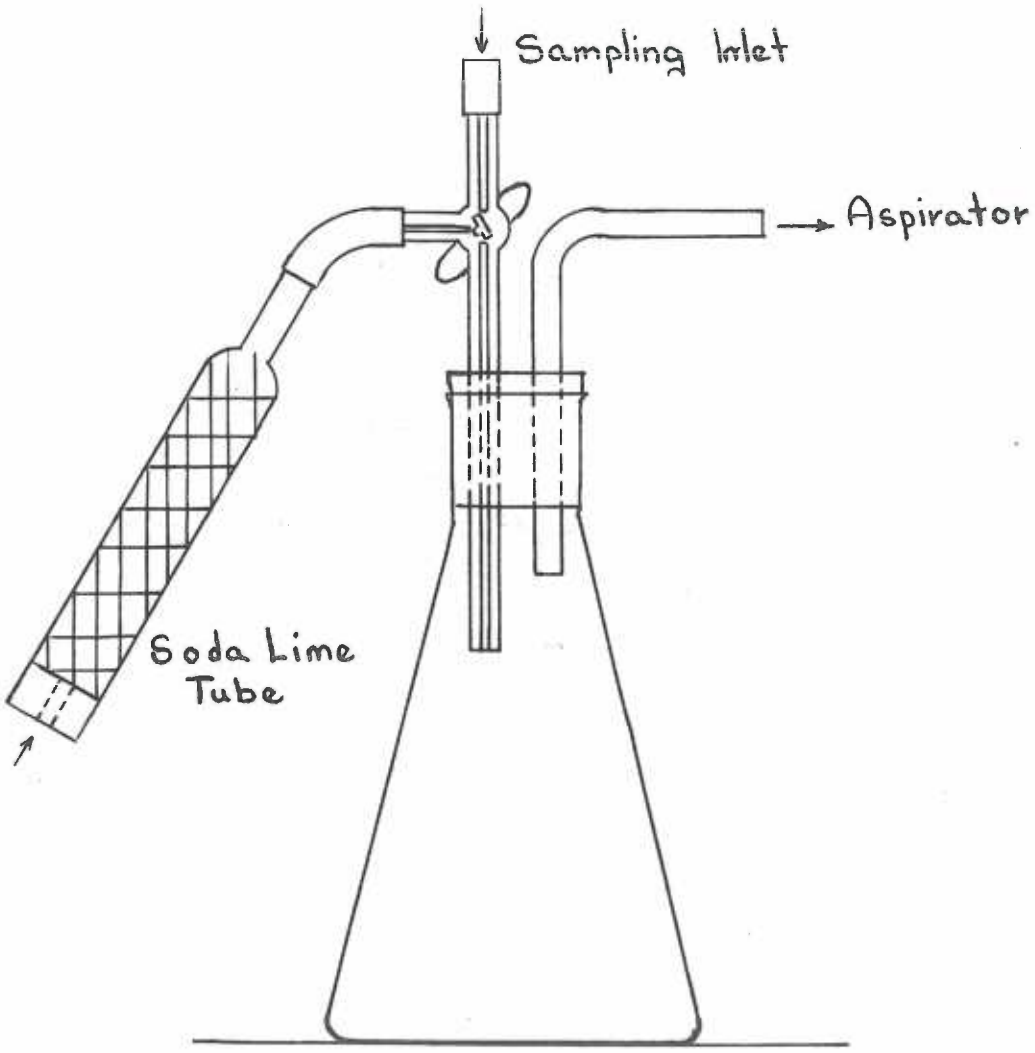
A 250 ml. Erlenmeyer flask was fitted with a three-way stopcock (Figure 2). To one arm was attached a soda lime tube for the absorption of atmospheric carbon dioxide. One arm was attached to a water aspirator. The remaining arm was left free for the attachment of the 50 ml. syringe containing the sample of chamber gases.

During the experimental interval the 250 ml. flask was washed, and then five drops of thymol blue indicator and 2 ml. of acetone were added. The stopper was then put in place and room air was drawn through the soda lime tube by the aspirator to flush out any carbon dioxide in the flask. The stopper was then removed from the flask and a piece of rubber damming was held tightly on the flask by a rubber band. The tip of an automatic pipette was passed through the dam to deliver 5 ml. of the standard barium hydroxide. The stopper was then replaced in the flask until the time for sampling the chamber gases occurred. When the 50 ml. syringe was transferred from the chamber sampling outlet to the arm of the three-way stopcock, the aspirator was turned on with the stopcock in the position which shut off all three arms. After a partial vacuum was attained in the flask the aspirator was shut off and the stopcock turned in such a position as to draw in the sample from the syringe.

After vigorous shaking, the stopcock was then turned back to the position of the soda lime tube. Intermittent shaking was done for a

FIGURE 2

Carbon Dioxide Absorption Flask




Carbon Dioxide Absorption Flask
Figure 2

period of five minutes which allowed ample time for carbon dioxide absorption. The stopper was removed a second time followed by replacing the rubber dam, and then the solution of barium hydroxide and barium carbonate was back titrated to the thymol blue end point with standard hydrochloric acid.

REAGENTS:

Hydrochloric Acid: The 0.03 N hydrochloric acid was standardized against fresh 0.03 N sodium carbonate solution prepared from analytical anhydrous sodium carbonate by the method given by Kolthoff and Sandell[18].

Barium Hydroxide: Approximately 0.06 N barium hydroxide was standardized against the standard hydrochloric acid. The barium hydroxide was stored in two liter glass bottles protected from atmospheric air by soda lime tubes. A syphon was attached to an automatic 5 ml. pipette for delivery of 5 ml. samples of barium hydroxide. Because the barium hydroxide was stored over periods of several months it was checked at six week intervals to be certain that atmospheric carbon dioxide was not changing its normality.

PLAN OF A TYPICAL EXPERIMENT:

Adult male albino rats were used ranging from 225 to 340 grams in weight. For individual experiments an attempt was made to choose animals of similar weight. Each animal was placed in an individual 7-1/2 x 9 inch cage equipped with solid metal sides and 1/2 inch wire mesh bottom. This allowed for a quiet environment and seclusion for each animal. The wire mesh bottom was adequately large to allow feces to drop through and prevent coprophagy.

The animals, which were maintained on Purina Laboratory chow, were removed from the stock colony in the morning and placed in their separate cages with water but without food until afternoon, at which time their first feeding occurred. At this time, and for four subsequent feedings, both the control and experimental animals were given 25 ml. of the control ration in a test tube affixed with a drinking tube. The animals were fed twice a day, morning and afternoon. This preliminary period of feeding control ration was found to be advisable to accustom the animals to the new diet and to train them to eat when the food was presented to them. At the sixth feeding one of the animals was changed to the experimental ration, while the other was continued on the control ration. The animals received five feedings of experimental and control rations over a 48 hour period. At this time they were fasted the desired length of time before being placed in the calorimeter. Each pair of animals was allowed an approximately equal amount of ration. This was accomplished by removing the food from one if he seemed to be getting ahead of the other. After the preliminary feeding period the rats were trained well enough to eat their fill within one-half hour period. This decreased the problem of maintaining approximately equal intakes. Spillage was estimated at the time of feeding and subtracted from the total amount consumed. About 20 to 30 ml. of ration were eaten per day depending upon the size of the animal.

Table 2 illustrates that food consumption in most instances was quite comparable in the two rations. This is especially evident in regard to the last feeding which is possibly a critical factor.

TABLE 2

Food Consumption and Respiratory Quotients of Glycine Fed and Control Fed Animals

| Control Ration | | | | Glycine Ration | | | |
|---|---|---|---|---|---|---|---|
| Rat Wt. gms. | R.Q. | Last Meal ml. | Total Fed ml. | Rat Wt. gms. | R.Q. | Last Meal ml. | Total Fed ml. |
| **Normal Rats 7 to 9 Hour Fasting Interval** | | | | | | | |
| 285 | 1.026 | 18 | 78 | 265 | 0.761 | 17 | 77 |
| 290 | 0.951 | 18 | 87 | 255 | 0.745 | 18 | 90 |
| 285 | 0.954 | 24 | 95 | 295 | 0.843 | 22 | 96 |
| 281 | 0.934 | 25 | 93 | 292 | 0.886 | 25 | 84 |
| 274 | 0.972 | 25 | 97 | 258 | 0.837 | 18 | 73 |
| 295 | 0.944 | 18 | 91 | 292 | 0.735 | 18 | 96 |
| 340 | 0.926 | 18 | 93 | 326 | 0.874 | 17 | 104 |
| 314 | 0.989 | 24 | 104 | 310 | 0.747 | 20 | 88 |
| 235 | 0.935 | 20 | 90 | 281 | 0.779 | 19 | 92 |
| 340 | 1.083 | 23 | 103 | | | | |
| 252 | 0.998 | 11 | 64 | | | | |
| 281 | 0.934 | 18 | 70 | | | | |
| 267 | 0.998 | 18 | 87 | | | | |
| 286 | 0.823 | 15 | 102 | | | | |
| Average | 0.962 | 19.6 | 89.9 | Average | 0.801 | 19.9 | 88.9 |
| **Adrenalectomized Rats 7 to 9 Hour Fasting Interval** | | | | | | | |
| 284 | 0.934 | 18 | 72 | 269 | 0.856 | 16 | 62 |
| 288 | 0.887 | 16 | 83 | 254 | 0.769 | 18 | 83 |
| 250 | 0.853 | 19 | 79 | 238 | 0.861 | 18 | 77 |
| 280 | 0.848 | 20 | 82 | 252 | 0.740 | 18 | 75 |
| 340 | 0.800 | 17 | 86 | 302 | 0.768 | 19 | 71 |
| | | | | 279 | 0.731 | 17 | 55 |
| Average | 0.865 | 18 | 80.4 | Average | 0.788 | 17.5 | 70.5 |

It was found that a preliminary training period in the calorimeter to accustom the rats to the apparatus was not necessary to achieve consistent results. Repeated determinations on the same animals did not alter the respiratory quotient significantly from the first results determined. The animals were watched closely for activity and if the animals sat quietly or just cleaned their faces with their paws during the experimental period the results were accepted. However, any violent activity or continual shifting of the body was cause for invalidating the results. Because of the unpredictability of a rat's actions these criteria, based on muscular activity, were found to be the best method for accepting or rejecting any one set of results.

All experiments were done during the summer months between 8:00 a.m. and 12:00 noon. It is felt that this controlled any seasonal or diurnal variations in respiratory quotient.

## RESULTS

CHECK OF CALORIMETER:

To test the accuracy of the calorimeter, determinations of the respiratory quotients on pure organic compounds were performed. A small lamp with the approximate volume of 1 ml. was made from 9 mm. Pyrex glass tubing and fitted with an asbestos wick.

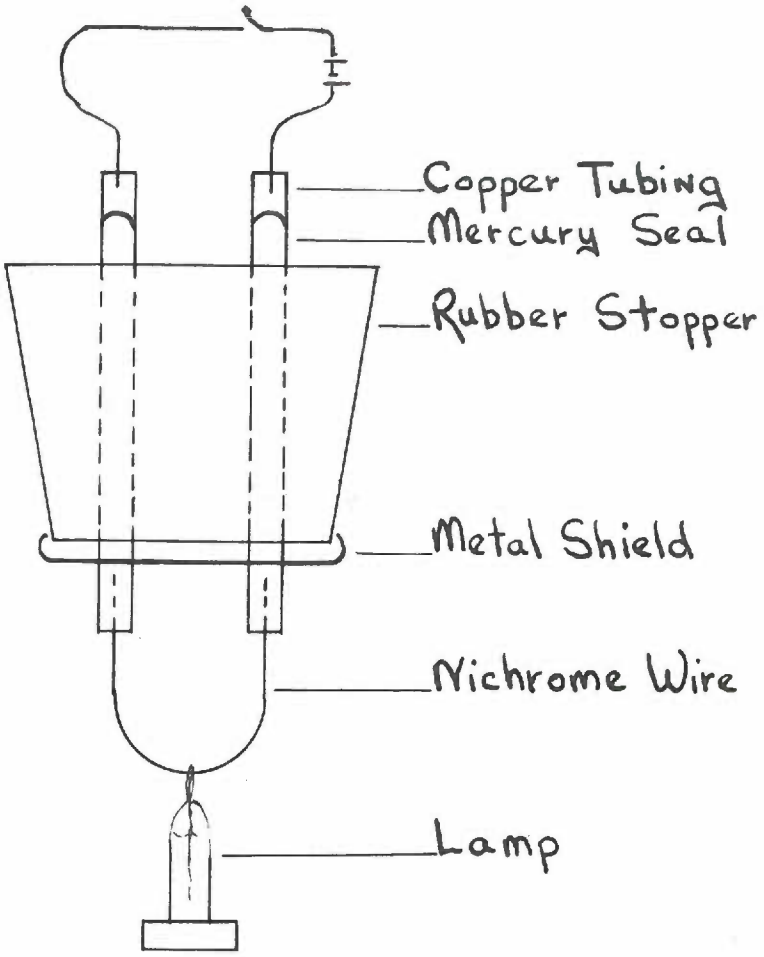
Through a rubber stopper were passed two 6 mm. copper tubes in the ends of which was clamped a piece of nichrome wire. The copper tubes were then filled with mercury to insure against air leaks. These electrodes were attached to two 1.5 volt dry cell batteries with a switch wired into the system. When the switch was closed the nichrome wire immediately became hot enough to glow (Figure 3).

The material to be tested was placed in the vial, and the wick was placed in contact with the nichrome wire. The switch was closed and the material ignited immediately. The material was allowed to burn until the oxygen supply was depleted. To allow for the expansion of gases due to the increase in temperature the spirometer was empty at the beginning of the experiment and allowed to rise with the expanding gases. As the chamber cooled and as the oxygen was consumed 50 ml. of air from the loading syringe was slowly added to the spirometer. After 15 minutes, when temperature equilibrium had occurred, the difference in spirometer volumes was measured by emptying the remaining air with the syringe.

The materials used for testing were absolute ethyl alcohol and B B' dihydroxyethylether, which have theoretical respiratory quotients

FIGURE 3

Apparatus for the Ignition of the Lamp for Making the Alcohol Check on the Calorimeter




Ignition Apparatus for Alcohol Check

Figure 3

of 0.667 and 0.800 respectively. The results are shown below.

| Ethyl Alcohol | B B' Dihydroxyethylether |
|---|---|
| 0.67 | 0.79 |
| 0.70 | 0.82 |
| 0.68 | 0.83 |
| | 0.82 |
| 0.683 av. 2.4% error | 0.815 av. 2.0% error |

Since many workers[16, 17] have had trouble making alcohol checks on small volume calorimeters, these checks were considered accurate enough for the purpose for which the calorimeter was planned.

STUDIES ON CONTROL FED AND GLYCINE FED ANIMALS:

The results of these respiratory quotients are summarized in Graph 1 and Table 2.

It may be easily seen that the respiratory quotient of the rats maintained on the control ration remained high, above 0.962 until the 11 to 13 hour fasting interval at which time it fell rather rapidly and approached the level obtained when the experimental diet was fed. On the other hand, the respiratory quotient of rats with added glycine in their diet fell in a step-wise fashion from the beginning of the fasting period. At the 7 to 9 hour fasting interval the difference in the respiratory quotient between the control fed and the glycine fed animals was the greatest. It is felt that this difference is highly significant in that the $T_{21df}$ value was 5.62*. Because of this large

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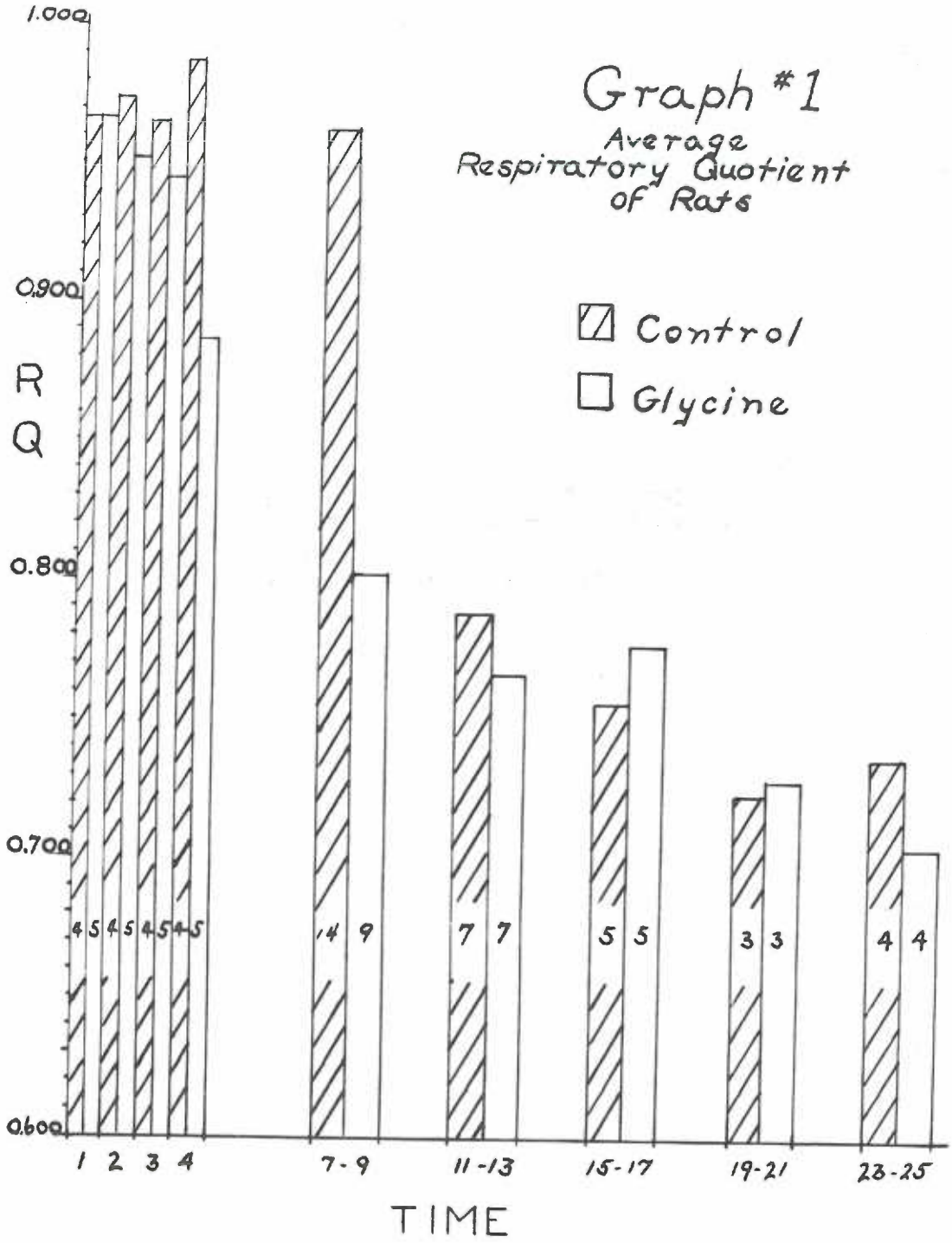
*$T_{21df}$ is a statistical expression denoting the degree of significance that may exist between two populations[20].

GRAPH 1

Average Respiratory Quotients of Rats Fed Control and Glycine Rations and Fasted for Various Hours

The Number in Each Column Indicates the Number of Animals Used


Graph #1
Average
Respiratory Quotient
of Rats
Control
Glycine
1.000
0.900
0.800
0.700
0.600
R
Q
4 5 4 5 4 5 4 5
14 9
7 7
5 5
3 3
4 4
1 2 3 4
7-9
11-13
15-17
19-21
23-25
TIME

difference the 7 to 9 hour fasting interval was selected for further studies.

STUDIES ON THE CONTROL FED AND GLYCINE FED ADRENALECTOMIZED ANIMALS:

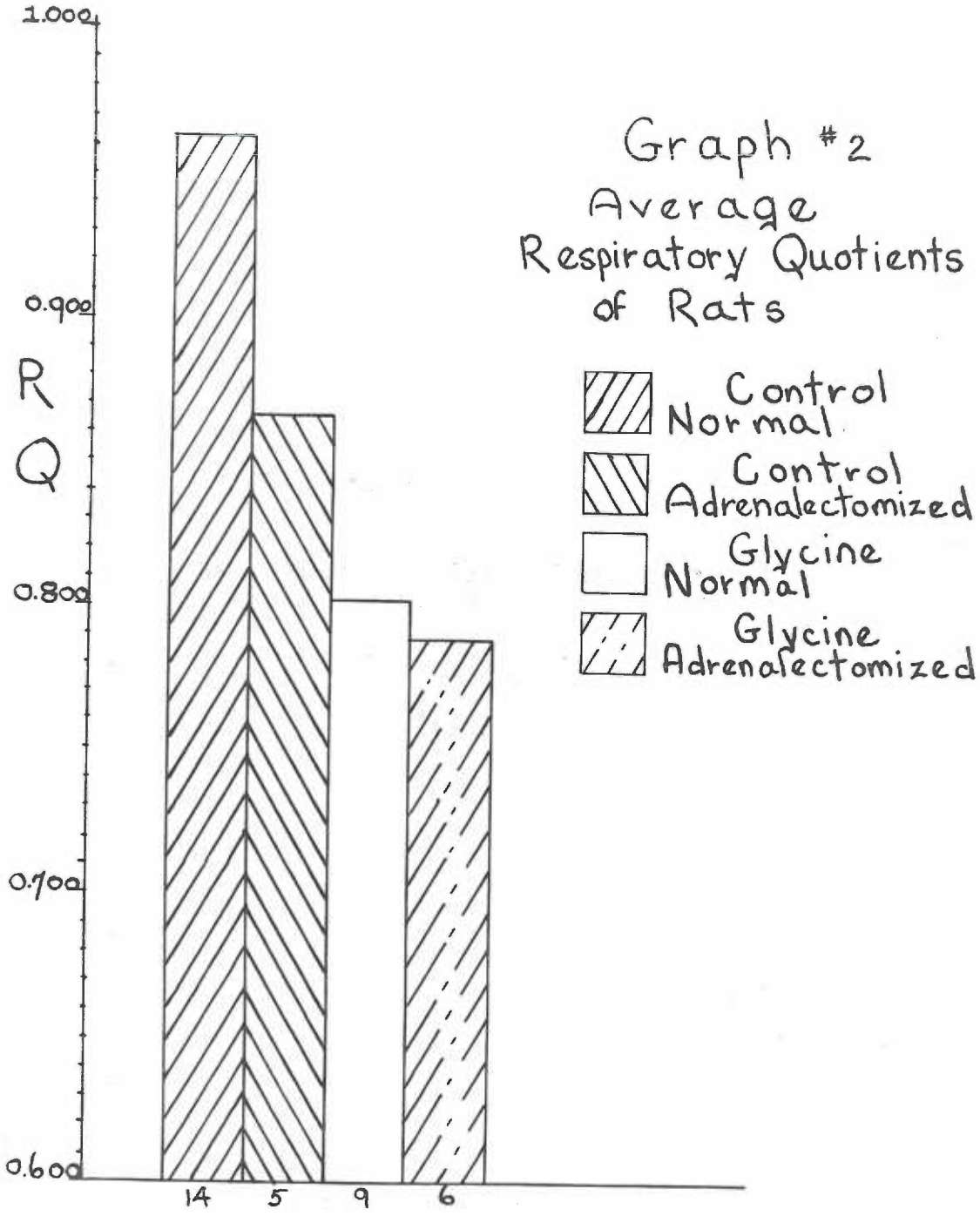
The results from studies on the respiratory quotients of adrenalectomized rats fed control and added glycine diets are shown in Graph 2 and Table 2.

It is of interest to note that by the exterpation of the adrenal glands the respiratory quotient of the control fed animals was lowered to a value of 0.865 compared to a value of 0.962 for normal animals. In the glycine fed animals it fell only from an average value of 0.801 in the intact animal to 0.788 in the adrenalectomized animal. In other words, the respiratory quotient in the control fed adrenalectomized animal decreased 7.4 times as much as in the glycine fed adrenalectomized animal. The significance of this is touched upon in the discussion.

GRAPH 2

Average Respiratory Quotients of Normal and Adrenalectomized Rats Fed Control and Glycine Rations and Fasted 7 to 9 Hours

The Number Below Each Column Indicates the Number of Animals Used


1.000
0.900
R
Q
0.800
0.700
0.600
14
5
9
6
Graph #2
Average
Respiratory Quotients
of Rats
Control
Normal
Control
Adrenalectomized
Glycine
Normal
Glycine
Adrenalectomized
Seven to Nine Hour Fast

## DISCUSSION

It is well to remember that the respiratory quotient does not measure intermediary metabolism, only the ratio of the carbon dioxide produced to the oxygen consumed. The ratio is high when a preponderance of carbohydrate is being metabolized to carbon dioxide and water, due to the fact that that molecule contains a high proportion of oxygen, and that little more oxygen is required to complete the reactions to carbon dioxide and water. The respiratory quotient is also high when metabolites containing oxygen in their structure are converted to metabolites with less oxygen in their structure, i.e. the conversion of carbohydrate to fat. The respiratory quotient may exceed unity if at the time it is measured, this conversion is a predominant metabolic reaction taking place in the body. However, when fat is metabolized to carbon dioxide and water, and this is a predominant metabolic reaction, the respiratory quotient is low. Lusk[21] states that when fat alone is metabolized the quotient will be 0.707 and when carbohydrate alone is oxidized it will be 1.00. These situations, of course, are never attained during life.

The respiratory quotient of protein presents a much different picture. Unlike the other two major foodstuffs, protein is not completely oxidized in the animal body. The portions of the protein molecule which contain nitrogen are eliminated in the urine in the form of urea, ammonia, uric acid, etc. It can be calculated[1] that 1.0 gram of protein contains approximately 0.16 grams of nitrogen. In other words, for every gram of nitrogen found in the urine 6.25 grams of protein must have been deaminated. Assuming that the deaminated residues of

the amino acids are completely oxidized to carbon dioxide and water during the period of observation, each gram of urinary nitrogen represents 6.25 grams of protein completely metabolized. This would require 6.044 liters of oxygen and produce 4.845 liters of carbon dioxide(21).

Protein free respiratory quotients, even though desirable, were not attempted because of the difficulty in obtaining 15 minute urine samples from the rats without causing loss of time and increase in activity on the part of the rats, which would necessarily be reflected in the values determined. The apparatus may be criticized in that it did not allow for carbon dioxide absorption. Thus with increasing time the animal's environment contained decreasing amounts of oxygen and increasing amounts of carbon dioxide. At the end of a 15 minute period the carbon dioxide content of the chamber was calculated to be approximately 2 to 3 per cent. According to Fulton(22) this would cause an increase in the alveolar carbon dioxide pressure by about 2.5 mm. which is sufficient to double the respiratory rate. It may be reasoned from this that doubling the respiratory rate would increase muscular exercise, thereby increasing carbon dioxide output by hyperventilation and causing an increase in the respiratory quotient. In most respiratory quotient work the time interval for determining the respiratory quotient is much longer than fifteen minutes, and unless a method for the removal of the carbon dioxide is devised a concentration would soon exist that would be incompatible with life. However, because the increased carbon dioxide tension was imposed both upon

the experimental and control animals any effect would be constant for both types of animals. Since the consecutive respiratory quotients on the same rat remained quite constant the increase in the carbon dioxide tension becomes even less significant.

From Graph 1 it may be seen that the respiratory quotient of the control animals maintained a high level (0.962) past the 7 to 9 hour interval. This may be taken as an indication that these animals were metabolizing chiefly carbohydrates or depositing fat in their body stores(1). Within the next 4 hour interval of fast the respiratory quotient dropped rapidly to 0.787 which was taken to indicate that these animals had used up their carbohydrate reserves to such an extent that their metabolism had changed to one which was chiefly utilizing fat and protein. In contrast, the glycine fed animals' respiratory quotients fell continually as the fasting period increased until the 11 to 13 hour interval was reached. It cannot be said from these results that these animals were actively storing glycogen, but when compared to the control group it is easily seen that they were not utilizing as much carbohydrate in their metabolism. If, however, the deposition of glycogen was going on at this time, it was coming from a material which contained less intrinsic oxygen in the molecule, possibly protein, than the carbohydrate to which it was being transformed. Thus with relatively little carbon dioxide production and relatively high oxygen utilization a lower respiratory quotient would be obtained.

These findings are supported by the results of Todd, Barnes, and Cunningham(12) in which the carbohydrate reserves (measured as liver

glycogen) of rats fed added glycine were over one per cent at the end of a 24 hour fast, while the control diet gave liver glycogen values of about 0.3 per cent at this fast. MacKay, Wick, and Carne[23], even though their work was on stomach tube fed rats, also showed that the liver glycogen after feeding glucose (a high carbohydrate diet) was decreasing rapidly at the 6 to 15 hour fasting interval, signifying at least carbohydrate was disappearing from the body stores at this time. However, these workers demonstrated a delayed increase in liver glycogen following the administration of glycine. Beginning at the six hour fasting interval there was a rapid increase in liver glycogen which reached a maximum at about the fifteen hour fasting interval.

It is not believed that the difference in the respiratory quotient at the 7 to 9 hour interval can represent the effect of the Specific Dynamic Action of glycine upon the metabolism of the animals. SDA is defined as the unavoidable energy waste incident to food utilization[24] and has been shown to be very high for glycine[21]. It is frequently measured by the increase in oxygen consumption following the feeding of the food in question. In order to determine if this factor was present at the 7 to 9 hour fasting interval the metabolic rates were determined for both the control fed and glycine fed rats at this time of fast. This was accomplished by application of the formula $MR = \frac{C \times kW}{S}$ where MR is the metabolic rate, C is the caloric value for a liter of oxygen at the particular respiratory quotient value, S is the surface area in square meters as obtained from Diack's formula[25] $S = \frac{7.47 \times \sqrt[3]{W^2}}{10,000}$,

and 4V is four times the 15 minute oxygen consumption. The results would then be Cal./Hr./M$^2$. The metabolic rate for the control animals at the 7 to 9 hour interval was 45.3 Cal./Hr./M$^2$. This did not vary significantly from the metabolic rate of the glycine fed animals for the same period, which was 45.0 Cal./Hr./M$^2$. From this it was concluded that the SDA of glycine was not being manifested in the respiratory quotients at the 7 to 9 hour fasting interval.

The respiratory quotients of fasting intervals greater than the 11 to 13 hour interval are not considered as significant due to the few number of determinations, and also due to the fact that with the increased fasting time the animals became more restless. This was attributed to their increased hunger and the activity normally associated with any animal's search for food.

Mirski[11] after describing an increased liver glycogen in fasted rats pre-fed a high protein diet was unable to determine the cause for his "Protein Effect", but concluded that it was due to gluconeogenesis. Todd, Barnes, and Cunningham[12] demonstrated that this effect came from feeding diets containing added glycine rather than extra protein, and they postulated gluconeogenesis possibly mediated through the adrenal cortex. Other workers in this field[13, 35] likewise concluded that the "protein effect" is due to adrenal cortical activity. Most of these workers have shown that bilateral adrenalectomized rats will not show an increased fasting liver glycogen after a high protein or added glycine diet.

Bilateral adrenalectomy effects the carbohydrate metabolism of rats very markedly. It is common knowledge that adrenalectomized

animals withstand the stress of fasting very poorly. Britton and Silvette[26] demonstrated a fall in blood sugar and liver and muscle glycogen in adrenalectomized rats. Cori and Cori[27] could find only a trace of liver glycogen at the end of a 24 hour fast, but they demonstrated that adrenalectomized animals could utilize glucose to form glycogen immediately following forced feeding of glucose. They found an average respiratory quotient of 0.718 following a 22 hour fast in adrenalectomized rats which increased to 0.870 in the four hours following stomach tubing with glucose. This change in respiratory quotient is compared[28] with a rise in respiratory quotient from 0.711 to 0.844 in normal rats treated in a similar fashion. These results were interpreted to mean that adrenalectomized rats are capable of utilizing glucose in a normal manner if glucose per se is supplied in their diet. Evans[29] showed that about a 25 per cent decrease in urinary nitrogen excretion and about a 90 per cent decrease in urinary ketone excretion occurred in adrenalectomized rats fasted for 48 hours. He could not find a significant difference in respiratory quotients between adrenalectomized and normal rats. From their observations on the effects of cortical extracts on the respiratory quotients of glucose fed adrenalectomized rats, Long[30] and Russell[31] inferred that the adrenal cortical hormone may depress carbohydrate oxidation. However, after exterpation of the adrenal cortex, rats rapidly utilize their carbohydrate stores and have a decreased ability to convert protein to carbohydrate so that they rapidly deteriorate with fasting.

Graph 2 compares the respiratory quotient of the control fed adrenalectomized animals with the respiratory quotients of the control fed normal animals. The difference may be explained if one assumes that the carbohydrate reserves of the adrenalectomized animals have been depleted more rapidly. With decreased ability to utilize protein the adrenalectomised rats must rely upon the carbohydrate in their diet for readily available energy. As the length of the fast increases less and less carbohydrate remains available to the adrenalectomized animal and the respiratory quotient would be expected to fall. At the 7 to 9 hour fasting interval, it is likely that little available carbohydrate remains and the respiratory quotients found are as might be expected.

The decrease in the respiratory quotient in the glycine fed adrenalectomized rats and in the glycine fed normal rats is not considered significant in that the change is less than two per cent. However, if the difference in the respiratory quotients between the control fed normal and the glycine fed normal rats is considered to be due to the added glycine and if the exterpation of the adrenal glands blocks this glycine effect, as shown by liver glycogen studies[12], then the respiratory quotients of the control fed adrenalectomised rats and of the glycine fed adrenalectomized rats should approach each other. In Graph 2 the respiratory quotients of the adrenalectomized control and glycine fed rats may be seen to approach each other; this was brought about, however, by the reduction of the respiratory quotients of the control fed adrenalectomized rats.

It might be expected that the respiratory quotients of the glycine fed adrenalectomized rats should rise, but if the data are inspected closely, an explanation, in part, for this apparent discrepancy may be found. The control ration contains more glucose than the glycine ration by approximately 0.17 Cal. per ml. It is doubtful that this amount of glucose could be responsible for the difference in the respiratory quotients between the control fed and glycine fed adrenalectomized rats. In reducing the glucose content of the experimental ration a substance was added, namely glycine, which the adrenalectomized animal has a decreased ability to metabolize normally. This serves to reduce the overall availability of the carbohydrate reserves. It is doubtful that the difference in the respiratory quotients between the control fed and glycine fed adrenalectomized animals is due to the decreased rate of absorption of glucose from the intestinal tract in adrenalectomized animals as postulated by Verzar and McDougall[32]. Duel et al.[33] claim that adequate sodium ion intake in adrenalectomized rats would allow the intestinal absorption of glucose to proceed at a normal rate. The adrenalectomized rats used in the above experiments were maintained on one per cent sodium chloride as drinking water for five days following operation, after which one per cent sodium chloride was also added to their liquid rations. It is believed that this constituted an adequate sodium ion intake[34].

If these factors did enter into the causation of the difference in the respiratory quotients in the adrenalectomized rats, they would effect the respiratory quotients in the direction in which it was

determined. More glucose and slow absorption in the control ration would tend to give a higher respiratory quotient in the control fed adrenalectomized rats at the 7 to 9 hour fasting interval, while less glucose and an increase in a substance difficult for an adrenalectomized animal to utilize would tend to lower the respiratory quotient in the glycine fed adrenalectomized rats.

It is to be pointed out again that intermediary metabolic pathways cannot be indicated from results obtained from studies on respiratory quotients. However, it is believed that the results presented here support earlier findings from prefeeding rats with glycine; namely that glycine, probably by some interaction of the adrenal cortex, allows increased liver glycogen following a fast by gluconeogenesis as manifested by lower respiratory quotients from the onset of fasting.

## SUMMARY

Respiratory quotients of rats fed a liquid milk base ration were compared with the respiratory quotients of rats fed a similar ration containing glycine replacing an equivalent amount of carbohydrate. The lower respiratory quotients in the glycine fed group from the 0 to 13 hour fast were interpreted as a decrease in carbohydrate oxidation, and as indirect evidence of either increased fat or protein oxidation. The data can also be interpreted as further evidence for an earlier assumption that glycine feeding stimulates the glyconeogenic processes. The conversion of protein to carbohydrate at an accelerated rate must be accompanied by a lowering of the respiratory quotient.

The greatest difference in respiratory quotient was found to be at the 7 to 9 hour fasting interval.

Similar studies were carried out on adrenalectomized rats fasted 7 to 9 hours. The respiratory quotients were much lower in the control fed group and changed insignificantly in the glycine fed group compared with respiratory quotients in normal animals. This was interpreted to indicate that after a 7 to 9 hour fast the operated animals were depleted of carbohydrate stores, and since they are unable to convert protein to carbohydrate in a normal manner, it must be assumed that they were oxidizing a greater than normal proportion of fat and protein.

## BIBLIOGRAPHY


1. Richardson, H. B. The respiratory quotient. Physiol. Revs., vol. 9, pp. 61-125, 1929.

2. Haldane, J. A new form of apparatus for measuring the respiratory exchange of animals. J. Physiol., vol. 13, pp. 419-430, 1892.

3. Pembry, M. S. The effect of variations in external temperature upon the output of carbonic acid and the temperature of young animals. J. Physiol., vol. 18, pp. 363-380, 1895.

4. Pembry, M. S., and Spriggs, E. I. The influence of fasting and feeding upon the respiratory and nitrogenous exchange. J. Physiol., vol. 31, pp. 320-345, 1904.

5. Kingdon, C. L., Bunnell, I. L., and Griffith, F. R. The fasting respiratory metabolism of the white rat for 36 hours following controlled feeding. Am. J. Physiol., vol. 137, pp. 114-123, 1942.

6. Paretsky, D., and Werkman, C. H. The bacterial metabolism of glycine. Arch. Biochem., vol. 25, pp. 288-298, 1950.

7. Lusk, G. An investigation in the causes of the specific dynamic action of the foodstuffs. J. Biol. Chem., vol. 20, pp. 555-619, 1915.

8. Olsen, N. S., Hemingway, A., and Mier, A. C. Metabolism of glycine. Studies with the stable isotope of carbon. J. Biol. Chem., vol. 148, pp. 611-618, 1943.

9. Murlin, J. R., and Lusk, G. Animal calorimetry. The influence of the ingestion of fat. J. Biol. Chem., vol. 22, pp. 15-42, 1915.

10. Foster, G. L., and Smith, B. E. Hypophysectomy and replacement therapy in relation to respiratory quotients and specific dynamic action in rats. J. Am. Med. Assoc., vol. 87, pp. 2151-2153, 1926.

11. Mirski, A., Rosenbaum, I., Stein, L., and Wertheimer, E. On the behavior of glycogen after diets rich in protein and in carbohydrate. J. Physiol., vol. 92, pp. 48-61, 1938.

12. Todd, W. R., Barnes, J. M., and Cunningham, L. Maintenance of liver glycogen by rats fasted after feeding individual amino acids. Arch. Biochem., vol. 13, pp. 261-264, 1947.

13. Todd, W. R., and Talman, E. On the glyconeogenetic action of fed glycine in the rat. Arch. Biochem., vol. 22, pp. 386-392, 1949.

14. Lester, D., and Greenberg, L. A. A simple apparatus for the serial measurement of the respiratory exchange in the rat. J. Biol. Chem., vol. 176, pp. 53-58, 1948.

15. Benedict, F. G., and McLeod, G. J. Heat production of the albino rat. I. Technique of activity control and the influence of fasting. II. Influence of environmental temperature, age, sex, and comparison with the basal metabolism of man. J. Nutrition, vol. 1, pp. 343-398, 1928-29.

16. Benedict, F. G. Multiple chamber respiration apparatus for rats and other small animals. J. Nutrition, vol. 3, pp. 161-176, 1930-31.

17. West, E. S., Christensen, B. E., and Rinehart, R. E. A micromethod for the determination of carbon dioxide in blood and other fluids. J. Biol. Chem., vol. 132, pp. 681-686, 1940.

18. Kolthoff, I. M., and Sandell, E. B. Textbook of Quantitative Inorganic Analysis, ed. 1, The Macmillan Co., New York, 1941.

19. Wesson, L. G. An apparatus and method for the determination of respiratory quotients of small animals. J. Biol. Chem., vol. 73, pp. 499-522, 1927.

20. Dickson, W. J., and Massey, F. J. Introduction to Statistical Analysis, ed. 1, McGraw-Hill Book Co., Inc., New York and London, 1951.

21. Lusk, G. The Elements of the Science of Nutrition, ed. 4, W. B. Saunders and Co., Philadelphia, 1928.

22. Fulton, J. F. A Textbook of Physiology, ed. 16, W. B. Saunders and Co., Philadelphia, 1949.

23. McKay, E. A., Wick, A. N., and Carne, H. O. Relative amounts of hepatic glycogen deposited by glucose, glycine, and d-l alanine. J. Biol. Chem., vol. 132, pp. 613-617, 1940.

24. Sadhu, D. P. The specific dynamic action of nutrients with special reference to the effects of vitamins and hormones. Univ. Missouri College of Agriculture Research Bull. 408.

25. Mack, S. L. Determination of the surface area of the white rat. J. Nutrition, vol. 3, pp. 289-296, 1930-31.

26. Britton, S. W., and Silvette, H. Effects of corticoadrenal extract on carbohydrate metabolism in normal animals. Am. J. Physiol., vol. 100, pp. 693-700, 1932.

27. Cori, C. F., and Cori, G. T. The carbohydrate metabolism of adrenalectomized rats and mice. J. Biol. Chem., vol. 74, pp. 473-494, 1927.

28. Cori, C. F., and Cori, G. T. The relation between sugar oxidation and glycogen formation in normal and insulinized rats during the absorption of glucose. J. Biol. Chem., vol. 70, pp. 557-575, 1926.

29. Evans, G. The adrenal cortex and endogenous carbohydrate formation. Am. J. Physiol., vol. 114, pp. 297-308, 1935-36.

30. Long, C. N. H. A discussion of the mechanism of action of adrenocortico hormone on carbohydrate and protein metabolism. Endocrinology, vol. 30, pp. 870-883, 1942.

31. Russell, J. A. The relationship of anterior pituitary and adrenalcortex in the metabolism of carbohydrate. Am. J. Physiol., vol. 128, pp. 552-561, 1940.

32. Verzar, F., and McDougall, E. J. Absorption from the Intestine, ed. 1, Longmans and Green Co., New York, 1936.

33. Duel, H. J., Hollman, L. F., Murray, S., and Samuels, L. T. The rate of absorption of glucose and of glycogen formation in normal and adrenalectomized rats. J. Biol. Chem., vol. 119, pp. 607-615, 1937.

34. Andersone, E., and Joseph, M. Urinary nitrogen and survival time of fasted adrenalectomized salt treated rats. Proc. Soc. Exptl. Biol. Med., vol. 46, pp. 321-325, 1941.

35. Samuels, L. T., Butts, J. S., Schutt, H. F., and Ball, H. H. Glycogen formation after alanine administration in adrenalectomized animals. Proc. Soc. Exptl. Biol. Med., vol. 35, pp. 538-539, 1937.