SOME EFFECTS OF ESTROGEN AND ESTROGEN WITH PROGESTERONE ON RESPIRATORY GAS EXCHANGE IN CASTRATED FEMALE ALBINO RATS

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Dedicated to Uncle Dan Jordan

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CHAPTER I

INTRODUCTION

Metabolism is said to encompass all intercellular and intracellular processes. These processes may be divided into two broad categories, anabolism and catabolism. The activity of these processes can be determined at both the cellular level and grossly for the entire organism. It may be assumed that any factor affecting intercellular processes would be reflected by changes in metabolic rate.

Metabolism of the entire organism has been widely investigated since the turn of the century. The measurement of cellular metabolism is a more recent innovation as it had to await highly specialized techniques and instrumentation.

Since this investigation was confined to gross metabolism, a word of explanation is necessary. There are several methods of measuring gross metabolism but probably the least difficult of these is the general method of measuring respiratory gases, of which there are three basic techniques. The first of these is the determination of the respiratory quotient (RQ) or some modification thereof. In this method, which was used by Haldane (1892) in the operation of his original apparatus, the amount of carbon dioxide and oxygen is measured. The volume of carbon dioxide produced is then divided by the volume of oxygen consumed. The basal metabolic rate (BMR) is measured in the second of the three methods. This method consists of measuring the oxygen consumption of an individual at the basal level of activity. The subject is considered to be at the basal level of activity when he has not eaten within 12 hours, has had sufficient and restful sleep, has performed no strenuous exercise after

the sleep, is not under stress, and is in a comfortable environment. The last of the basic three methods is that of indirect calorimetry. This method measures the liters of oxygen consumed per hour and this value is multiplied by a constant (4.825) which is the average number of calories produced by food oxidation per liter of oxygen used. The resulting value is an accurate indication of the heat produced. A method of direct calorimetry is known but is probably less accurate than the indirect calorimetric method. In the current study a modified Haldane apparatus was used for several reasons. Among these are ease of handling, simplicity, and the availability of the apparatus. The result will be reported in milliliters of carbon dioxide produced and milliliters of oxygen consumed per gram of animal weight per hour.

Diurnal variation in the metabolism of the rat was shown to exist by Horst, Mendel, and Benedict (1934). The lowest level of metabolic fluctuation was found to be between 10 A.M. and 4 P.M. A great many other investigators also report diurnal metabolic variation in mammals as well as other animals. Prosser and Brown (1961) state that all homoiothermic organisms show diurnal fluctuation with the metabolic rate being highest when the animal is most active.

Temperature is also a factor which affects metabolic rate. Oxygen consumption increases when either the high or low extreme of the temperature to which the animal is normally adapted is exceeded (Prosser and Brown, 1961). This amounts to an expense of energy by the animal in response to the extreme conditions.

Nutrition is another factor influencing the metabolism of animals. The respiratory quotient changes from 0.71 for almost total utilization

of fat to 1.00 for almost total carbohydrate utilization provided no other external factors are introduced (Langley, 1965, Prosser and Brown, 1961, Guyton, 1961).

Activity drastically changes the metabolic rate of animals. According to Langley (1965) the RQ may change from 2 to 0.5 during and after exercise, respectively, in man. Prosser and Brown (1961, p. 165) show that activity may increase oxygen consumption many-fold in numerous animals. They also state, "Metabolic changes are associated with reproductive activity in many animals and are caused indirectly by reproductive hormones." They state further that internal temperature increases directly with the activity of the animal.

Some other factors which influence metabolism and/or activity include thyroid activity (Turner 1960, Young 1961, Gorbman and Bern 1962), oxygen tension (Greisheimer 1963, Best and Taylor 1958), carbon dioxide tension (Best and Taylor 1958, Guyton 1961), and adrenal gland activity (Wiggers 1949, Mitchell 1940).

One of the best investigated causes for activity variation is associated with the reproductive cycle. Richter (1954) found that castration greatly reduced spontaneous running activity of domestic, but not wild Norway rats. Wang (1923) and Slonaker (1924) state that just before and during estrus, rats have a period of heightened voluntary activity. Zuckerman (1962) reported that this cyclic period of activity does not occur prior to puberty and disappears as a result of the suppression of the reproductive cycle by old age, pregnancy, or lactation. Slonaker (1924) shows that this cyclic activity does not

appear in males and that the level of activity is approximately one half of that of the female. Wang and Guttmacher (1927) showed by histological studies and activity measurements that normal levels of activity were maintained only when intact follicular tissue was present. Richter and Hartman (1934) later proved that estrogen increased the level of activity in male and female castrates when it was injected. Zuckerman (1962, p.395) says, "findings show that both the general level of nonspecific activity and one of its associated rhythms depend largely upon the integrity of the ovary, and that both are inhibited by procedures which modify ovarian secretion." Young (1961) reported similar findings.

With so much evidence that the spontaneous activity of cycling female rats is variable and that it follows, and is in some manner, caused by the cyclic change in the gonadal hormones, one is strongly tempted to think that metabolic fluctuations would also occur.

This investigation, therefore, is an attempt to determine more precisely the possible effect of gonadal hormones (estrogen and progesterone) on suspected, and in some instances reported, metabolic changes during the reproductive cycle.

This goal was approached by attempting to establish variations in the metabolism during the reproductive cycle of intact female rats, and to simulate any observed changes in the intact animal by replacement therapy in castrated rats with gonadal hormones at approximately physiological dosages.

CHAPTER II

MATERIALS AND METHODS

Mature female rats, Rattus norvigicus (Berkenhout), of the Holtzman strain were used in the present study. They were fed Purina Lab Chow ad lib at approximately the same time each day. For the dietary analysis, see Appendix A. It was observed that all rats ate uniformly immediately following feeding and watering. A rest period followed eating, and it was during this time that the oxygen consumption and carbon dioxide production were measured. (The lack of restriction of the amount of food, it was felt, more nearly approximated the metabolic rate under normal conditions.) Since detection of any possible changes of metabolic rate due to changes in the estrus cycle was the reason for this investigation, it was considered inadvisable to superimpose a restriction of diet as this alteration would have modified the conditions normally observed in the cycling rat.

The animals were subjected to a diurnal cycle of 14 hours light and 10 hours of darkness as well as a constant temperature of $73^{\circ} \pm 2^{\circ}F$. The regulation of temperature and light was designed to insure regular estrus cycles.

Reproductive maturity was determined by making vaginal lavages daily and recording the results. The lavages were made by placing approximately 1 cc of warm water (slightly above body temperature) in the vagina of the rat with a bulbed pipette. This was aspirated several times and then the liquid was transferred to a clean slide. The vaginal lavages so obtained were divided into 4 groups. Proestrus was characterized by many epithelial cells which were nucleated and generally round in shape. Estrus was the next stage and was characterized by cornified,

enucleated epithelial cells. Metestrus followed and contained a few leukocytes and a great many cornified epithelial cells. Diestrus was the last stage and normally had many leukocytes with only a few epithelial cells. Only animals which showed the normal 4 to 5 day cycle were used.

These stages of the estrus cycle were determined at low magnification (100x). For best observation of the cell types, the amount of light was reduced. Due to the number of daily observations made, ten different lavages were placed on each slide. In the event that two or more lavages ran together on the slide, the entire series involved was repeated. A repeat was seldom necessary.

The criteria used for metabolic rate determinations were the oxygen uptake and production of carbon dioxide. Most of the previous studies of metabolic rate have been confined to the changes in O_2, with little or no emphasis on CO_2 production. The CO_2 was absorbed without measurement by some chemical such as sodium hydroxide or potassium hydroxide. In the present study, O_2 and CO_2 volumes were determined in the following manner: The rat was placed in an unsealed vacuum dessicator for five minutes. This was done so the animal would become familiar with its surroundings and to bring the system to equilibrium. After this five minute adjustment interval, the rat was sealed in the apparatus for a period of ten minutes. At the end of this period, an air sample was removed from the sealed container and analyzed for the amount of O_2 and CO_2 present.

The sample of air from the sealed container was transferred into a modified Haldane gas analysis apparatus and the appropriate procedure was

followed. The gas volume was measured and then the CO_2 was removed from the sample volume and the volume following CO_2 removal accounted for the volume of CO_2 in the sample. The O_2 was removed by absorption with pyrogallic acid. The change in sample volume following removal of CO_2 and the volume following O_2 absorption was the amount of O_2 in the original sample volume. For a more complete description of the gas analysis procedure used in this study see Appendix B. A picture of the apparatus is shown in Appendix J. The necessary adjustments were made by taking into account the ambient temperature, barometric pressure, and animal size.

An O_2 and CO_2 analysis of the atmosphere was taken within an hour of each determination of the rat samples. The atmospheric pressure and temperature were recorded at the time the atmospheric sample was taken.

After correction to standard temperature and pressure, the atmospheric CO_2 volume was subtracted from the CO_2 sample volume and the rat O_2 sample volume was subtracted from the atmospheric O_2 volume. Both of the above values were then divided by the animal weight giving the gas produced or consumed per g of animal weight in ml at standard temperature and pressure for each animal. Appendix C shows more completely the procedure used in standardizing the data.

The gas analysis procedure described above was used throughout the study. Oxygen uptake and CO_2 produced were analyzed for the four stages of the estrus cycle. Twenty samples were made for each of the stages (proestrus, estrus, metestrus, and diestrus). This was performed in an effort to establish the changes in metabolic rates during each of the stages of the reproductive cycle in intact animals. This trial will

hereafter be referred to as Trial I and the animals used will be referred to as the control group. Thus, the values of O_2 and CO_2 obtained from this trial were later used to determine the effects of castration and replacement therapy of estrogen alone and estrogen and progesterone used in combination. As mentioned in the introduction, it was hoped that this study would give some insight on the physiological effect of estrogen and progesterone in the intact rat. The use of the metabolic rate was utilized as the method for determining the aforementioned goal. A consistent change in respiratory gas exchange would seem to indicate corresponding changes in the synthesis of gonadal hormones. Castration followed by the use of estrogen or of estrogen together with progesterone would hopefully elucidate any importance of these hormones on metabolic rate. Should such a relationship occur, it would be reasonable to assume that the observed metabolic rates are associated with changes in the synthesis of the hormones during the reproductive cycle of the intact rat.

In the subsequent two trials, Trials II and III, castrated females were used. These animals were castrated according to the following procedure. The animal was placed in a large jar into which previously a cotton wick had been placed and saturated with ether. When the animal ceased to move or struggle, it was removed and placed on an operating board; anesthesia was continued with an ether cone. The hair was clipped from the lumbar region of both sides of the animal. An incision of 2 cm was made obliquely through the skin just posterior to the last rib. A second incision was made through the muscle layer and into the peritoneal cavity. The ovary was located directly underneath this incision, embedded

in a mass of fat. The fat was withdrawn, and the ovary was separated and ligated by a suture. The ovary was then removed and the oviduct and uterus were returned to the peritoneal cavity. The incision through the muscle was then closed with several sutures and the skin closed with several clamps. The castrate animals were initially isolated until they were no longer under the influence of the anesthesia. They were then returned to their respective cages and a recovery period of 14 days elapsed before hormone therapy was begun. This 14 day period was also necessary for the depletion of the endogenous gonadal hormones which may have been in the system at the time of castration.

At the beginning of Trial II, O_2 and CO_2 determinations were made on ten of the castrate rats. Immediately following these determinations, these animals were subcutaneously injected with 2.5 μg of estradiol benzoate, hereafter referred to as estrogen. On the following day the animals were subjected again to O_2 and CO_2 determinations and then injected with an additional 2.5 μg of estrogen. Thereafter, the rats were subjected to daily O_2 and CO_2 sample determination through the cyclic changes until all had returned to metestrus. As mentioned previously, these cyclic changes were determined by daily vaginal lavages, including lavages on the first two days of treatment.

In Trial III, ten additional castrated rats were treated similarly to those of Trial II, except that they were subcutaneously injected with 2.5 μg estrogen and 0.25 mg of progesterone each day for two consecutive days.

All of the data so obtained was averaged, graphically plotted, and

treated statistically. The statistics consisted of calculating the standard deviation and the analysis of variance.

CHAPTER III

RESULTS

Table 1 shows change in CO_2 production and O_2 consumption in ml/g body wt/hr of the control group. The amount of CO_2 produced was highest in diestrus, dropped to the lowest level in proestrus and estrus, and began to rise (toward the high of diestrus) during metestrus.

While there were no significant changes found between the various stages of the reproductive cycle of the control group in the volume of CO_2 production, as seen in Table 2, there was a rather consistent change between these stages. It will be seen in the two experimental groups that similar trends in CO_2 production were established following use of estrogen and progesterone-estrogen treatments. Thus it may be assumed that changes in the production or use of gonadal hormones do cause changes in the CO_2 production.

It was observed that the CO_2 production value for the castrate nontreated animals was similar to the diestrus values of the control group.

There has been shown to be a correlation between O_2 consumption and metabolism. Using this criterion, it was shown that the rate of metabolism was at its highest point during proestrus in the control group, 2.24 ml/g body wt/hr (Table 1). The lowest volume of O_2 consumption, 1.95 ml/g body wt/hr, was found during estrus. This was contrary to expectations since the highest level of activity has been reported to occur during late proestrus and early estrus. It is possible that since the data collected was grouped with no regard to the length

of time that the animal had shown estrus, the latter phase of estrus may have lowered the average. Possibly, the vaginal lavages indicating estrus may have lagged behind internal or gonadal changes. Thus, there might have been a latent period between internal changes and bioassay.

It was observed that there was a greater variation, as shown by the standard deviation in Table 1, in the amount of O_2 consumed than in the CO_2 produced. This possibly was due to the larger values found for the O_2 consumption. The comparable F values for O_2 consumption by stages is shown in Table 3.

The CO_2 production and O_2 consumed in ml/g body wt/hr for the progesterone-estrogen treated group of animals is shown in Table 4. CO_2 production declined from a high of 2.10 ml/g body wt/hr during diestrus to a rather uniform plateau during the remaining stages of the reproductive cycle. This plateau was not observed in either the control or estrogen treated group of animals. The addition of progesterone in the treatment of this group at the dosage administered (0.25 mg) may account for the plateau. A further word of explanation will follow in the discussion.

The O_2 consumption for this group was highest during proestrus, 2.43 ml/g body wt/hr, and returned to the diestral level during estrus and metestrus (Table 4). This is similar to, but slightly higher than the O_2 consumption observed in the control group. Since proestrus is the stage during which changes in the reproductive tract preceding fertilization are taking place, it is not surprising that the highest level of metabolism occurs at this time. The similarity between the

Table 1. A summary of statistical analysis for CO_2 production and O_2 consumption in ml/g of body wt/hr for the control group by reproductive stages.

	Diestrus		Proestrus		Estrus		Metestrus	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
Average	2.02	2.16	1.92	2.24	1.92	1.95	1.98	2.02
Standard Deviations	0.29	0.56	0.31	1.16	0.31	0.71	0.26	0.66
Replications	20	20	20	20	20	20	20	20

Table 2. The F values from statistical analysis of CO_2 production of one reproductive stage compared with another in the control condition. The values under .05F are the F values required for significance at 95% level.*

Control Group	F value	.05 F
Diestrus vs. Proestrus	1.22	4.09
Diestrus vs. Estrus	1.11	4.09
Diestrus vs. Metestrus	0.29	4.09
Proestrus vs. Estrus	0.00	4.09
Proestrus vs. Metestrus	0.50	4.09
Estrus vs. Metestrus	0.50	4.09

*The F value obtained in the experiment must be equal to or larger than the value under .05 F to be significant at the 95% level.

Table 3. The F values from statistical analysis of O_2 consumption of one reproductive stage compared with another in the control condition.

Control Group	F value	.05 F
Diestrus vs. Proestrus	0.07	4.09
Diestrus vs. Estrus	1.07	4.09
Diestrus vs. Metestrus	0.51	4.09
Proestrus vs. Estrus	0.88	4.09
Proestrus vs. Metestrus	0.51	4.09
Estrus vs. Metestrus	0.11	4.09

Table 4. A summary of statistical analysis for CO_2 production and O_2 consumption in ml/g of body wt/hr for the progesterone-estrogen treated group of animals by reproductive stages.

	Diestrus		Proestrus		Estrus		Metestrus	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
Average	2.10	2.23	1.93	2.43	1.95	2.22	1.91	2.22
Standard Deviation	0.29	0.26	0.12	0.20	0.27	0.33	0.27	0.33
Replications	20		8		18		9	

control and progesterone-estrogen treated groups in corresponding stages of their reproductive cycles should be noted.

In all instances within this group, except the diestrus stage, the standard deviation was higher for O_2 consumption than for CO_2 production. The values and changes of the progesterone-estrogen treated group for both O_2 consumption and CO_2 production were more like that for the control group than the estrogen treated group.

In the estrogen treated group (Trial II) the CO_2 production for diestrus, proestrus, estrus, and metestrus was 2.11, 1.85, 1.98, and 2.08 ml/g body wt/hr respectively (Table 5). This again shows that the highest value of CO_2 production was observed during diestrus and the lowest values were found during proestrus and estrus. It is apparent in the estrogen treated group that the amount of exogenous estrogen administered (2.5μg) simulated to some degree the cyclic changes in the volume of CO_2 found in the control group.

The variance of CO_2 produced was less in each stage of the reproductive cycle of this group than that found in the control group.

The O_2 consumed was 2.22, 2.01, 2.28, and 2.33 ml/g body wt/hr in the diestrus, proestrus, estrus, and metestrus reproductive stages respectively as shown in Table 5. It was noted that only half of the treated animals exhibited proestrus. This may have been due in part to the abruptness of the administration of the hormone in the castrate condition. A much more desirable technique would have been the use of a perfusion apparatus.

The highest values for O_2 consumption were found in estrus and

metestrus. This condition is atypical of both the control and progesterone-estrogen treated groups. A possible explanation will be offered in the discussion. The mean values and the standard deviation of O_2 consumption were higher than that of CO_2 for this group.

Table 6 presents simultaneously, the results reported in Tables 1, 4, and 5.

In order to obtain a more complete understanding of the CO_2 production and O_2 consumption in the trials in this study, the gases should be separated and a comparison made between treatments. To do this, the average values of CO_2 production and O_2 consumption by treatments and stages within treatments are plotted graphically in Figures 1 and 2.

The amount of CO_2 produced by reproductive stages for all three trials is plotted in Figure 1. It may be noted that the curve for the control group is relatively uniform. The amount of CO_2 produced was at its lowest level during proestrus, the stage at which O_2 consumption was at its highest. This could be due to the utilization of CO_2 in protein synthesis during this proliferative stage of the cycle.

The curve of the progesterone-estrogen treated group is similar to the curve of the control group. Again, it is noticed that CO_2 production is low at a time during which O_2 consumption is at its highest (proestrus).

The CO_2 production curve for the estrogen treated group seen in Figure 1 is one of marked fluctuation. This may reflect the lack of another gonadal hormone (progesterone) which may be present in the proestrus stage of the intact animal. The CO_2 production curve for this group appears to be an exaggerated example of the control condition.

Table 5. A summary of statistical analysis for CO_2 production and O_2 consumption in ml/g of body wt/hr for the estrogen treated animals by reproductive stages.

	Diestrus		Proestrus		Estrus		Metestrus	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
Average	2.11	2.22	1.85	2.01	1.98	2.28	2.08	2.33
Standard Deviation	0.19	0.36	0.21	0.27	0.26	0.35	0.22	0.23
Replications	24		5		18		13	

Table 6. The average ml/g of body wt/hr and standard deviation of CO_2 produced and O_2 consumed of control, estrogen treated, and progesterone-estrogen treated groups of animals by reproductive stages.

	Control				Estrog.				Progesterone and Estrogen			
	CO_2		O_2		CO_2		O_2		CO_2		O_2	
	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
Proestrus	1.92	0.31	2.24	1.16	1.85	0.21	2.01	0.27	1.93	0.12	2.43	0.20
Estrus	1.92	0.31	1.95	0.71	1.98	0.26	2.28	0.35	1.95	0.27	2.22	0.33
Metestrus	1.98	0.26	2.02	0.66	2.08	0.22	2.33	0.23	1.91	0.27	2.22	0.33
Diestrus	2.02	0.29	2.16	0.56	2.11	0.19	2.22	0.36	2.10	0.29	2.23	0.26

A comparison of O_2 consumption during the stages of the reproductive cycles by treatments is shown in Figure 2. This figure shows graphically the O_2 consumption mentioned earlier in Tables 1, 4, 5, and 6. In the control condition the greatest change is shown by a decline in O_2 consumption between proestrus and estrus. Following estrus there seems to be a gradual increase in O_2 consumption as the animal approaches the diestrus stage.

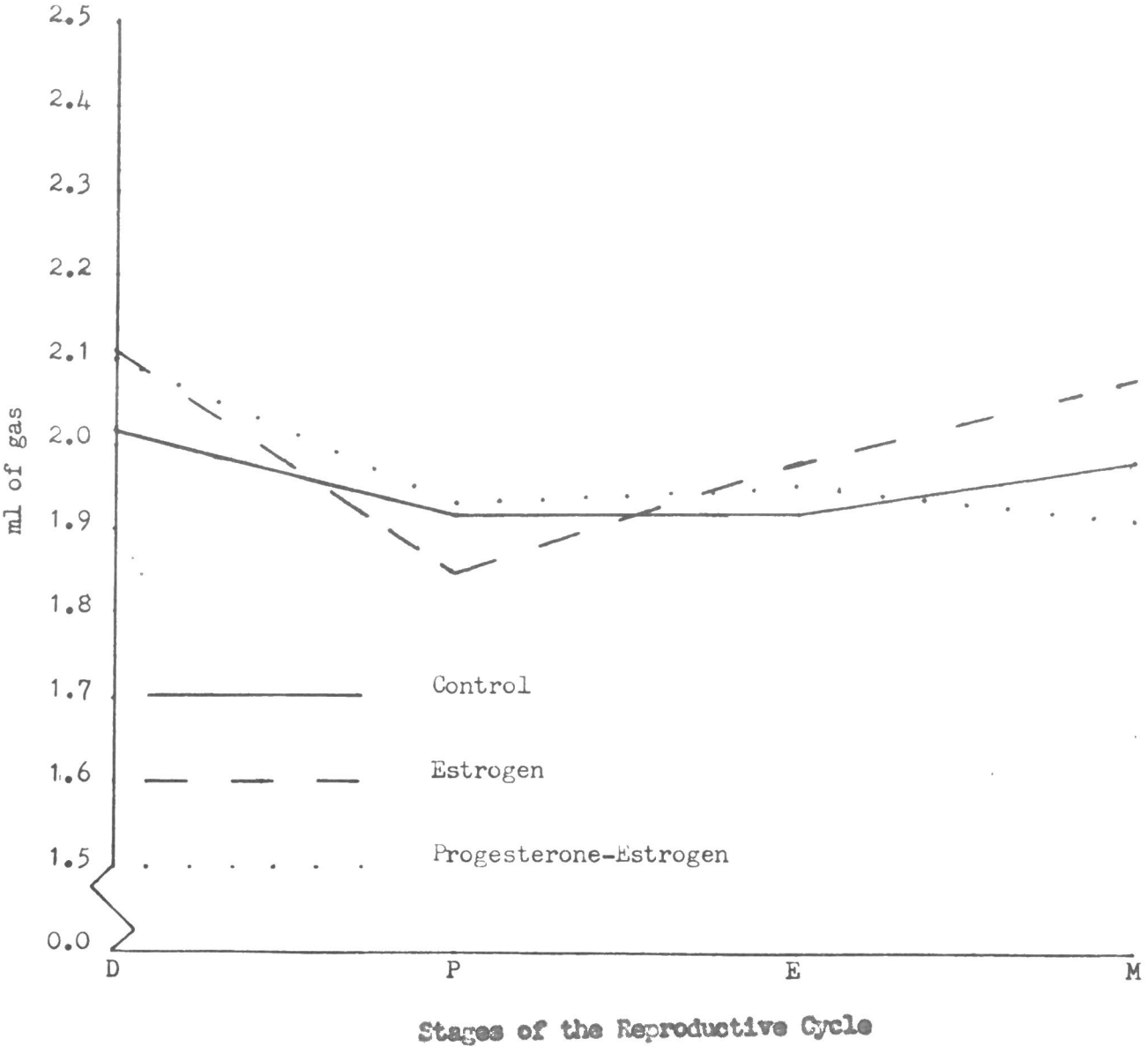
The progesterone-estrogen treated group shows a trend similar to the control group. It is easily observed that there is a greater O_2 consumption, although not statistically significant, in the progesterone-estrogen treated group than in the control group. This increase in O_2 consumption of the progesterone-estrogen treated animals may be caused by one of two factors. There is a possibility that the amount of hormone administered was in excess of the physiological amount present in the intact, cycling animal, or that the castrate animal may be very sensitive to hormone treatment.

The effect of estrogen on O_2 consumption in the estrogen treated group was to lower the O_2 consumption in the proestrus stage and to increase its consumption during estrus and metestrus. This is contrary to results in both the control and progesterone-estrogen treated groups. This may by explained in part if one assumes that there normally is some progesterone present as early as proestrus in the intact animal. Thus, it appears that in the castrate animal estrogen alone will not simulate the O_2 consumption of the intact animal.

Table 7 shows that there was no statistical difference in the CO_2

Figure 1. The average number of ml of CO_2 produced per g of body wt per hr by reproductive stages and treatments. D is diestrus, P is proestrus, E is estrus, and M is metestrus.

Figure 1.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2.4
2.3
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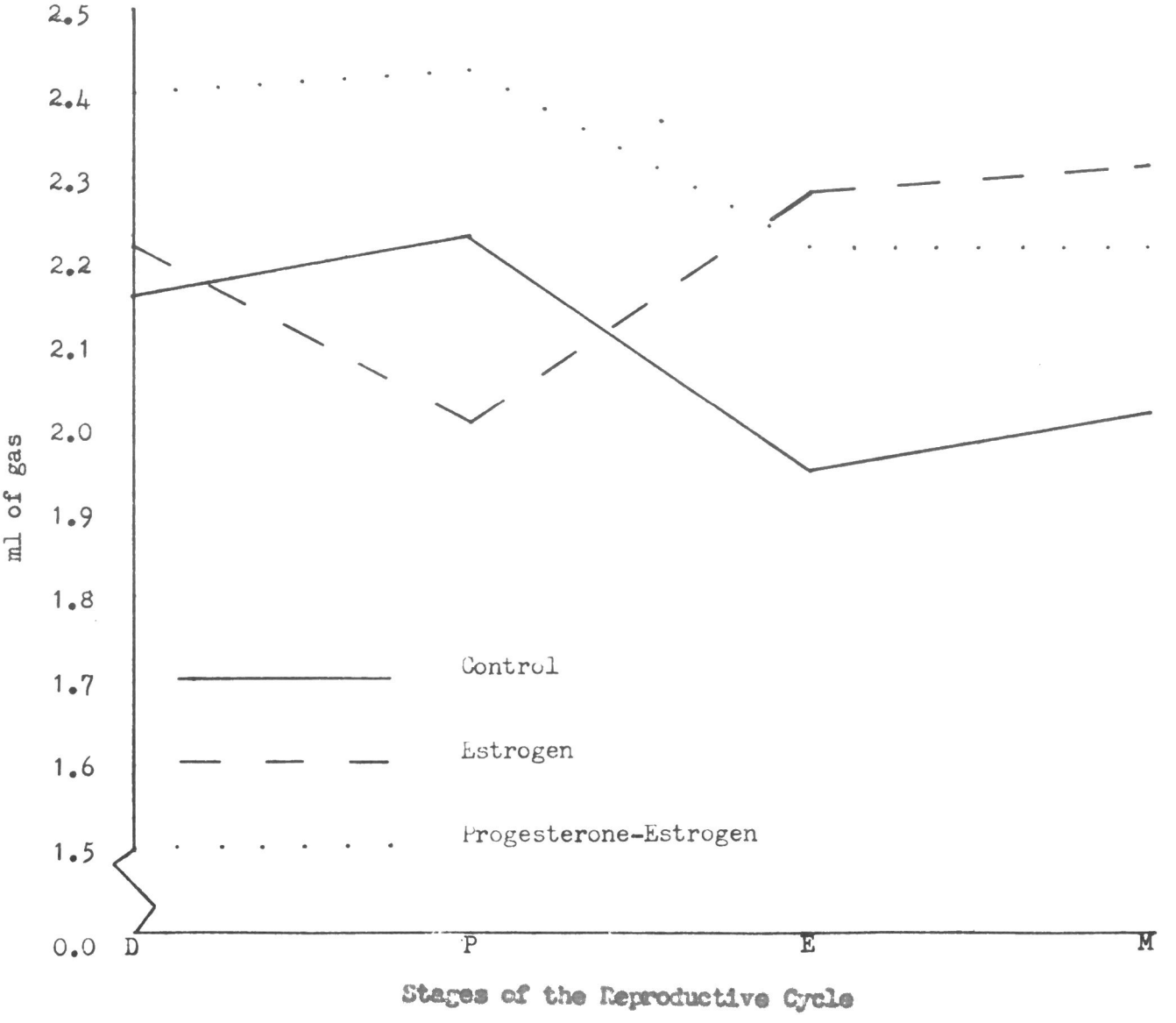
2.1
2.0
1.9
1.8
1.7
1.6
1.5
0.0
ml of gas
Control
Estrogen
Progesterone-Estrogen
D
P
E
M
Stages of the Reproductive Cycle

Figure 2. The average number of ml of O_2 consumed per g per hr by reproductive stages and treatments. D is diestrus, P, proestrus, E, estrus, and M is metestrus.

Figure 2.


2.5
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Control
Estrogen
Progesterone-Estrogen
D
P
E
M
Stages of the Reproductive Cycle

production between the control group and the estrogen treated group in comparable stages of the reproductive cycle. This indicates that estrogen treatment did simulate, to some degree, the normal, intact condition. Table 8, in which the F values were determined for the control as compared to the progesterone-estrogen treated group, shows an even closer agreement. This would seem to indicate the requirement for progesterone in combination with estrogen during the reproductive cycle.

In Tables 9 and 10 the F values for the O_2 consumption of the control group as compared with the estrogen and progesterone-estrogen treated groups respectively is shown. In general the F values of the progesterone-estrogen were less than those of the estrogen treated group when both were compared to the intact animals (control). The F values offer additional evidence for the fact that in the castrate animal, estrogen alone does not simulate the CO_2 production and O_2 consumption of the intact animal as closely as progesterone and estrogen.

There was a highly significant difference ($P > .01$) in both CO_2 production and O_2 consumption between estrogen and progesterone-estrogen treated groups during metestrus.

For a more complete summary of the statistical analysis see Appendixes D, E, and F. See Appendixes G, H, and I for figures showing both CO_2 production and O_2 consumption for each treatment by reproductive stages.

Table 7. The F values from statistical analysis of CO_2 production by corresponding reproductive stages for a comparison of the control with the estrogen treated group.

Control Group		Estrogen Treated	F value	.05 F
Diestrus	vs.	Diestrus	1.72	4.06
Proestrus	vs.	Proestrus	0.24	4.26
Estrus	vs.	Estrus	0.50	4.10
Metestrus	vs.	Metestrus	1.33	4.15

Table 8. The F values from statistical analysis of CO_2 production by corresponding reproductive stages for a comparison of the control with the progesterone-estrogen treated group.

Control Group		Progesterone-Estrogen Treated	F value	.05 F
Diestrus	vs.	Diestrus	0.87	4.09
Proestrus	vs.	Proestrus	0.14	4.21
Estrus	vs.	Estrus	0.00	4.10
Metestrus	vs.	Metestrus	0.45	4.20

Table 9. The F values from statistical analysis of O_2 consumption by corresponding reproductive stages for a comparison of the control with the estrogen treated group.

Control Group		Estrogen Treated	F values	.05 F
Diestrus	vs.	Diestrus	0.24	4.06
Proestrus	vs.	Proestrus	0.19	4.26
Estrus	vs.	Estrus	3.03	4.10
Metestrus	vs.	Metestrus	2.47	4.15

Table 10. The F values from statistical analysis of O_2 consumption by corresponding reproductive stages for a comparison of the control with the progesterone-estrogen treated group.

Control Group		Progesterone-Estrogen Treated	F value	.05 F
Diestrus	vs.	Diestrus	0.26	4.09
Proestrus	vs.	Proestrus	0.23	4.21
Estrus	vs.	Estrus	2.03	4.10
Metestrus	vs.	Metestrus	0.76	4.20

CHAPTER IV

DISCUSSION

The fact that no statistical difference could be found between the various stages of the reproductive cycle of the control group in either O_2 consumption or CO_2 production is surprising. It should be noted that the largest F value for O_2 consumption was between diestrus and estrus. The largest F values for CO_2 production were for the diestrus versus proestrus and diestrus versus estrus. Although these F values did not approach significance, they are large in comparison to the other F values with the control group. It is suggested that since the changes in the respiratory gases from one stage of the reproductive cycle to another are so small, more replications would have given statistically significant results. It should be remembered that a highly statistical difference was found, but not included, between the treated groups in metestrus for O_2 consumption and CO_2 production. It would appear therefore, that only slightly larger differences in the gas volumes measured or as stated previously, the including of more measurements would have shown significance.

There is evidence to support the above assumption, both in the current study and in earlier work. Hemmingsen (1934) came to the conclusion, after measuring respiratory gases in both intact and castrate female rats, that if there is any difference in respiratory exchange between intact and castrate female rats it is extremely small, probably about one per cent lower in the latter group. If then, it may be assumed that there are some gonadal hormones present throughout the reproductive cycle in intact rats, and that these hormones do have some influence on

the respiratory gases, then any differences between the stages insofar as respiratory gases are concerned would be even more difficult to detect in intact, cycling rats.

Earlier, however, Lee (1928) had shown by means of indirect calorimetry that the heat production per square meter of body surface increases in female rats during late diestrus and early proestrus but he reported no statistical difference. The present study agrees with those results in both the control and progesterone-estrogen treated groups but not with the estrogen treated group.

Other supportive evidence for metabolic fluctuations corresponding with the reproductive cycle includes Nalbandov (1958, p. 94) who said, "An estimate of the different rates of thyroid activity during the estrus cycle of rats and mice shows that the thyroid of female rats is most active during heat (estrus) and that that of female mice is most active during proestrus." The effects of the thyroid hormone (thyroxine) are well known. Turner (1960) states that the primary effect of thyroxine in homoiotherms is an increase in O_2 uptake and heat production. It would be expected then that an increase in the metabolic rate would occur in cyclic fashion relative to heightened thyroid activity. Velardo (1958) states that thyroid secretions have long been known to affect the reproductive system. This may indicate an interaction between the reproductive and thyroidal hormones.

Working with human endometrium and placenta slices, Ville (1957) demonstrated that small amounts of estradiol increased O_2 consumption and pyruvate utilization. He was unable to show that progesterone and estradiol

were more effective than estradiol alone; though he did demonstrate that endometrial slices from the late proliferative phase of the cylce had higher O_2 and pyruvate utilization rates than those from the early portion of the phase and therefore more available energy due to acceleration of the Kreb's Cycle. Even though he was unable to report a synergestic action between progesterone and estrogen, it seems that since the slices from the late proliferative stages showed increased O_2 consumption, the influence of progesterone would be indicated.

Many other workers have reported various effects of estrogen on the reproductive tract of mammals in an in vitro system. Szego (1953) showed that estrogen increased O_2 consumption of the castrate rat uterus in vitro. Bullough (1955) states that one of the more fundamental actions of estrogen is the indirect stimulation of mitosis. After injecting castrate rats with estradiol 17-B, Roberts (1953) showed that after 4 hours there was a marked increase in aerobic and anaerobic glycolysis of uterine tissue. After 20 hours he demonstrated a substantial increase in respiration and glucose utilization. A 2-fold increase in active phosphorylase in 48 hours after estrogen administration in castrate female rats was found by Leonard (1958). Dirschel (1955) found a 5-fold increase in the activity of aldolase in the uterus of the castrate rat. The above reports indicate that there is an increase in energy production, a utilization of stored reserves, and a synthesis of new cellular material as a result of estrogen administration.

Though this study was not designed to relate biochemical reasons for the increase in O_2 consumption, when CO_2 production was lowest, a brief

explanation is offered. It is believed that the Kreb's Cycle provides at least some of the chemical energy utilized in mitosis (Geise, 1962). Since estrogen does increase mitosis, spontaneous running activity, and *in vitro* O_2 consumption, it is reasonable to assume that estrogen treatment or increased estrogen concentrations in the intact animal would increase the metabolic rate and therefore the O_2 consumption on the gross level. Then if the CO_2 produced as a result of the metabolism were to be fixed in amino acid metabolism during protein synthesis, the apparent paradox, (increased O_2 consumption simultaneously with decreased CO_2 production), may be explained.

In order to explain the plateau of the production of CO_2 in the progesterone-estrogen treated group, it could be assumed that progesterone enhanced the effect of estrogen and therefore protein synthesis was carried on for a longer period of time, hence the continued low CO_2 production.

The atypical response of the O_2 consumption and CO_2 production in the estrogen treated group may be explained at least in part by a latent period in the response to the hormone. Other workers have shown that there is a lag in the effect of estrogen (Leonard, 1958, Roberts, 1953). There did seem to be a lag in O_2 consumption in the present study. The decrease in O_2 consumption of the estrogen treated group is difficult to explain and awaits further investigation. In conclusion, it can be said that progesterone and estrogen are both necessary in the castrate animal to simulate the cyclic changes in the metabolic rate observed in the intact animal.

CHAPTER V

ABSTRACT

The primary objective of this work was an attempt to establish and correlate changes in the metabolic rate with the four stages of the reproductive cycle in female albino rats as measured by O_2 uptake and CO_2 production, and to duplicate the changes found in the control condition by the addition of gonadal hormones to castrate female rats. A total of twenty replications were made in each of the four stages of the estrus cycle. That is, twenty measurements of the respiratory gases were made in diestrus, proestrus, estrus, and metestrus, which gave a total of 80 measurements. All of the above measurements were made on intact animals. Following this the animals were castrated and seperated into two groups. One group was treated with 2.5 µg of estrogen which was injected subcutaneously. The injections were given on two consecutive days so that the animal received a total of 5 µg of estrogen. The other group was similarly except that they received two 2.5 mg dosages of progesterone in addition to the estrogen. The respiratory gases were measured daily in the treated groups and vaginal lavages were taken also in order to determine the stage of the reproductive cycle which the animals were exhibiting.

All of the measurements made were corrected to standard temperature and pressure and reported in ml/g of body weight/hr. The results were subjected to statistical analysis and plotted graphically.

It was noted that only one half of the estrogen treated animals showed proestrus whereas the progesterone-estrogen treated animals

showed proestrus in 80% of the instances. Statistical significance was not found for either O_2 consumption or CO_2 production in the comparisons between the control and the two treated groups in corresponding stages of the reproductive cycle. This means that there was very little variation from the control condition by the treated animals. It was apparent that the progesterone-estrogen treated animals did approach the control conditions more consistently than did the estrogen treated animals.

APPENDIX A

The rats were fed Purina Laboratory Chow which was manufactured by The Ralston Purina Company. The food contained 23.0% protein, 4.5% fat, 6.4% fiber, and was made from such substances as meat, bone meal, dried skimmed milk, wheat germ meal, fish meal, animal liver meal, various grains, and a rich vitamin supplement.

APPENDIX B

Three samples were withdrawn from the dessicator on each trial, but only the last was retained for use in the analysis in order to reduce any possible contamination there may have been in the first two samples. The discarding of the first two samples was done by means of a three way valve. (Appendix J). The air sample was passed through sulphuric acid in order to remove any moisture.

The preparation of the modified Haldane Gas Analysis Apparatus for use is as follows: An adequate quantity of mercury was poured into the apparatus through the leveling bulb (enough so that the surface will reach the valve on the burette when the leveling bulb is in the up position), and onto both surfaces of the mercury was placed a small amount of concentrated sulphuric acid. The acid prevented reaction between the atmosphere and the mercury and eliminated toxic fumes from the mercury. Actually the acid which was on the inside of the burette should have been poured into the leveling bulb prior to the addition of the mercury. (It seems to be easier that way.)

The CO_2 absorption pipette contained a 10% NaOH solution. The O_2 absorption pipette contained 10% pyrogallic acid in 20% NaOH. The latter solution was made at least one week prior to use.

CLEARING THE APPARATUS OF CO_2 AND O_2

INITIAL REMOVAL OF O_2

All stopcocks were closed. Stopcock A was opened to free the air. All of the air was removed from the burette by raising the bulb above

stopcock A. Into the burette was drained 38 ml of air by lowering the bulb and slowly closing stopcock A and raising the bulb to about 10 cm above the level of the solution in the burette (in order to obtain a positive pressure) and the bulb was held at this level.

Stopcock A was opened to the manifold line, and Stopcock C was opened to the O_2 pipette. The bulb was slowly raised, thus forcing all of the air into the O_2 pipette. The bulb was slowly lowered and the solution level in the O_2 pipette was watched closely. As soon as it reached the mark on the pipette (never past this mark), the bulb was again raised. This was repeated twenty times. The solution of the O_2 pipette was stopped at the mark on the pipette. Stopcock C was closed above the O_2 pipette.

REMOVAL OF CO_2

With stopcock A still opened to the manifold, the bulb was raised 10 cm above the solution level in the burette. It was held there, then stopcock B was opened to the CO_2 pipette. The bulb was slowly raised forcing the air into the CO_2 pipette. The bulb was slowly lowered and the solution level in the CO_2 pipette was watched closely. As soon as it reached the mark (never past this mark) on the pipette, the bulb was raised again. This was repeated three times. The absorption solution was stopped in the CO_2 pipette when the level reached the mark on the pipette. The stopcock above it was closed.

ADDITIONAL REMOVAL OF O_2

The bulb was raised 10 cm above the solution in the burette. Stopcock C was opened to the O_2 pipette. The bulb was slowly raised forcing

all of the air into the O_2 pipette. The bulb was slowly lowered while watching closely the solution level in the O_2 pipette. When it reached the mark on the pipette the bulb was raised again. This was repeated twenty times. The solution of the O_2 pipette was stopped at the mark on the pipette. Stopcock C was closed above it. (This step was a safety measure to be certain that all of the O_2 and CO_2 in the system had been removed.)

The surface of the mercury in the leveling bulb was made level with the mercury surface in the burette. (This brought the system to atmospheric pressure.)

The apparatus was then free of CO_2 and O_2, and was ready for the analysis of a sample.

The CO_2 analysis was always run first, since the O_2 pipette absorbed CO_2 also.

THE ANALYSIS OF A SAMPLE

Stopcock A was opened to free the air. The bulb was raised until the acid solution appeared at the top of the burette. Stopcock A was closed.

The sample to be analyzed was obtained by opening stopcock A after lowering the bulb slightly first. The bulb was then lowered slowly and a sample of approximately 40 ml was withdrawn from the dessicator. (This was done three times but only the last sample was used in the analysis for the reason stated previously.) Stopcock A was closed while the rat was removed from the dessicator. Stopcock A, was then opened

to the atmosphere and the burette volume was adjusted to 38 ml by holding the bulb against the burette until the two surfaces of the mercury were on the same plane, at 38 ml. A mirror was installed behind the burette to increase the accuracy of the measurements. After adjustment to 38 ml stopcock A was closed.

CO_2 ANALYSIS

The meniscus was checked on the CO_2 pipette. The bulb was raised slightly above the solution level in the burette. Stopcock A was opened to the manifold line. Stopcock B was opened and the sample gas was slowly transferred into and out of the pipette five times. The liquid level in the pipette was stopped at the mark on the pipette. Stopcock B was closed. The bulb was held against the burette and the position adjusted until the two liquid surfaces were level and this volume was recorded. This volume was subtracted from the original volume to obtain the ml's of CO_2 absorbed.

O_2 ANALYSIS

The bulb was raised slightly above the solution in the burette. Stopcock C was opened. The sample gas was slowly transferred into and out of the O_2 pipette twenty times. The absorption liquid level was stopped at the mark on the pipette. Stopcock C was closed. The bulb was held against the burette and the position adjusted until the two surfaces of the mercury were on the same plane and this volume was recorded. This volume was subtracted from the value obtained after the CO_2 volume was subtracted from the original volume. The volume obtained was the amount of O_2 present in the original sample.

Two modifications which were found to be very useful were the addition of two pulleys connected by a cord to which was attached the leveling bulb and the addition of a mirror which was placed behind the burette. These arrangements greatly facilitated making the precise movement required and aided tremendously in improving accuracy of the readings.

APPENDIX C

The volumes of CO_2 and O_2 measured were corrected to standard temperature and pressure (STP) through use of the general gas law, $\frac{PV}{T} = \frac{P'V'}{T'}$. After correction to STP, the volumes were divided by the weight of the rat to obtain the ml of gas per g of animal weight. Since the animals remained in the closed container for 10 minutes, the values in ml/g were multiplied by 6 to obtain the ml/g/hr. The values were then multiplied by 100 since the volume of the dessicator unoccupied by the rat was approximately 3800 ml. (The dessicator volume was about 4140 ml and the average rat volume was in excess of 300 ml. Subtracting the rat volume from the dessicator volume leaves approximately 3800 ml which is 100 times larger than the volume of the sample measured.)

APPENDIX D

Diestrus			Proestrus			Estrus			Metestrus		
An. No.	CO_2	O_2	An. No.	CO_2	O_2	An. No.	CO_2	O_2	An. No.	CO_2	O_2
18	2.12	2.05	18	2.17	2.93	14	1.64	1.26	13	1.72	1.64
1	1.59	2.30	22	1.73	2.14	20	2.10	2.03	15	2.12	2.50
3	1.90	2.59	21	2.07	1.51	1	2.09	2.72	1	2.35	3.47
21	2.09	2.48	2	2.20	2.36	12	1.99	1.21	2	1.86	1.00
22	2.30	2.79	2	1.64	1.35	2	1.49	1.84	18	1.96	0.59
18	2.41	2.79	18	1.94	1.91	23	1.83	2.28	3	1.87	1.76
3	1.54	1.81	21	1.88	2.24	9	1.19	3.82	3	2.16	1.81
9	1.80	2.04	22	2.67	2.98	18	2.18	1.19	1	1.56	1.45
10	1.92	2.33	1	1.48	1.58	3	1.69	0.65	1	1.93	2.44
18	2.66	3.03	2	1.76	1.97	1	1.54	1.68	2	1.97	2.27
2	1.87	2.24	3	1.58	1.81	21	2.18	2.14	3	2.24	2.79
1	1.89	1.09	4	1.97	2.09	22	2.14	2.35	22	2.44	2.64
2	1.94	0.80	9	2.17	2.20	1	1.73	1.99	21	1.96	1.85
2	1.91	2.05	1	1.64	1.23	3	1.97	1.25	18	2.34	2.33
1	1.99	2.35	3	1.70	6.68	21	1.84	1.33	1	1.64	1.39
3	2.32	2.27	22	1.84	1.57	22	2.21	2.41	20	1.48	1.61
2	1.81	1.64	16	1.66	1.58	21	2.50	2.80	16	2.17	2.58
2	2.40	2.60	16	1.79	2.06	13	2.20	2.21	6	1.91	2.07
3	2.11	2.33	22	2.54	2.81	14	1.94	1.88	20	1.94	2.09
2	1.78	1.61	6	1.90	1.72	17	1.91	1.97	22	2.00	2.18
$\bar{X}$	2.02	2.16		1.92	2.24		1.92	1.95		1.98	2.02
Σx	40.35	43.19		38.33	44.72		38.36	39.01		39.62	40.46
Σx^2	82.95	99.16		75.30	125.44		75.37	85.75		79.77	90.10
$(\Sigma x)^2/N$	81.41	93.27		73.46	99.99		73.57	76.09		78.49	81.85
Σx	1.54	5.89		1.84	25.45		1.80	9.66		1.28	8.25
σ	0.29	0.56		0.31	1.16		0.31	0.71		0.26	0.66
Replications	20			20			20			20	

The results of the statistical analysis of the CO_2 and O_2 measurements of the control group by stages of the reproductive cycle. The values are reported in ml/g of body weight/hour.

APPENDIX E

Diestrus			Proestrus			Estrus			Metestrus		
An. No.	CO_2	O_2	An. No.	CO_2	O_2	An. No.	CO_2	O_2	An. No.	CO_2	O_2
11	2.07	1.51	4	1.73	1.73	11	1.76	2.02	4	1.81	2.48
13	2.28	1.39	9	1.88	2.04	13	1.93	1.93	6	1.77	2.22
14	2.17	1.48	10	1.63	1.78	14	2.08	2.12	11	1.86	1.86
15	2.02	2.11	5	2.19	2.39	15	2.30	2.54	14	2.06	2.10
23	2.33	2.42	6	1.84	2.11	23	2.03	1.54	15	2.27	2.47
9	2.11	2.19				9	1.80	2.29	23	2.09	2.33
10	2.02	2.69				10	1.21	1.81	5	2.04	2.34
4	1.95	2.23				4	1.83	2.17	13	2.29	2.31
5	2.22	2.14				5	2.12	2.37	4	2.00	2.38
6	1.85	2.18				6	1.72	2.20	6	2.09	2.38
11	2.21	2.24				11	1.85	2.18	11	2.00	2.26
13	2.03	2.21				13	2.07	2.30	14	2.57	2.30
14	2.24	2.45				14	2.17	2.44	15	2.16	2.87
15	2.23	2.71				15	2.27	2.57			
23	2.17	2.57				23	2.16	2.32			
9	2.01	2.48				9	2.21	2.98			
10	1.87	2.35				10	2.02	2.87			
5	2.71	2.51				5	2.15	2.45			
6	1.83	1.88									
11	2.08	2.39									
13	2.27	2.54									
14	2.15	2.57									
15	1.86	1.95									
23	2.00	2.15									
$\bar{X}$	2.11	2.22		1.85	2.01		1.98	2.28		2.08	2.33
Σx	50.68	53.34		9.27	10.05		35.68	41.10		27.01	30.30
Σx^2	107.88	121.60		17.37	20.49		71.89	95.91		56.68	71.25
$(\Sigma x)^2/n$	107.02	118.55		17.19	20.20		70.73	93.85		56.12	70.62
Σx	0.86	3.05		0.18	0.29		1.16	2.06		0.56	0.63
σ	0.19	0.36		0.21	0.27		0.26	0.35		0.22	0.23
Replications	24	24		5	5		18	18		13	13

The result of the statistical analysis of the CO_2 and O_2 measurements of the estrogen treated group by stages of the reproductive cycle. The values are reported in ml/g of body weight/hour.

APPENDIX F

Diestrus			Proestrus			Estrus			Metestrus		
An. No.	CO_2	O_2	An. No.	CO_2	O_2	An. No.	CO_2	O_2	An. No.	CO_2	O_2
1	2.23	2.02	1	1.82	2.26	23	1.93	2.31	1	1.94	2.21
3	2.25	2.01	3	1.92	2.42	1	1.95	2.30	13	1.85	1.85
13	2.19	1.83	13	1.89	2.62	3	1.66	2.18	15	2.03	2.31
14	2.33	2.01	14	1.97	2.72	13	1.67	2.08	14	2.18	2.58
15	2.74	2.15	15	1.75	2.56	14	2.14	2.43	7	1.65	1.96
11	1.70	2.17	11	2.08	2.34	15	1.54	1.81	11	2.06	2.62
23	1.57	1.85	7	1.93	2.10	7	1.98	1.98	23	1.71	1.93
7	1.93	2.16	8	2.10	2.44	11	2.10	2.19	8	2.31	2.63
22	1.59	1.86				8	2.07	2.07	22	1.48	1.93
8	1.96	2.18				22	1.77	2.08			
1	2.16	2.26				23	1.85	1.88			
3	1.99	2.32				22	1.78	1.80			
13	2.32	2.26				3	1.57	1.98			
14	2.29	2.51				14	2.14	2.17			
15	2.43	2.47				7	2.08	2.61			
7	2.00	2.50				11	2.33	2.91			
11	2.12	2.53				8	2.00	2.15			
23	1.86	2.23				13	2.57	2.99			
22	1.91	2.59									
8	2.36	2.72									
$\bar{X}$	2.10	2.23		1.93	2.43		1.95	2.22		1.91	2.22
ΣX	41.93	44.63		15.46	19.46		35.13	39.92		17.21	20.02
ΣX^2	89.55	100.87		29.98	47.62		69.77	90.46		33.48	45.37
$(\Sigma X)^2/N$	87.91	99.59		29.88	47.34		68.56	88.53		32.91	44.53
Σx	1.64	1.28		0.10	0.28		1.21	1.93		0.57	0.84
σ	0.29	0.26		0.12	0.20		0.27	0.33		0.27	0.33
Replications	20			8			18			9	

The result of the statistical analysis of the CO_2 and O_2 measurements of the estrogen-progesterone treated group by stages of the reproductive cycle. The values are reported in ml/g of body weight/hour.

Figure 3. The average volume (in milliliters) of CO_2 produced and O_2 consumed per gram of body weight per hour by reproductive stages in the control group. D is diestrus, P is proestrus, E is estrus, and M is metestrus.

APPENDIX C

Figure 3.

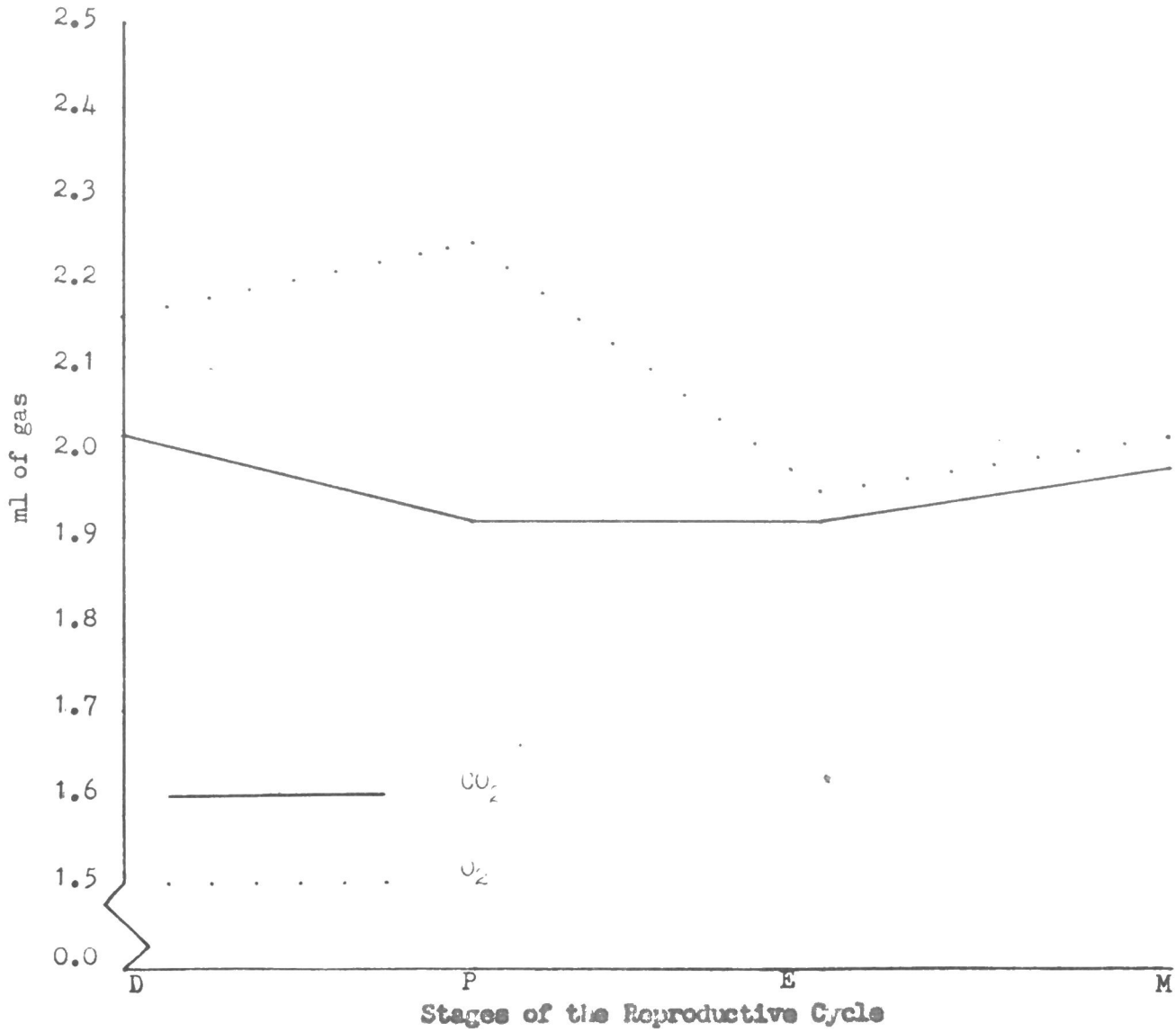


Figure 4. The average volume (in milliliters) of CO_2 produced and O_2 consumed per gram of body weight per hour by reproductive stages in the estrogen treated group. D is diestrus, P is proestrus, E is estrus, and M is metestrus.

APPENDIX H

Figure 4.

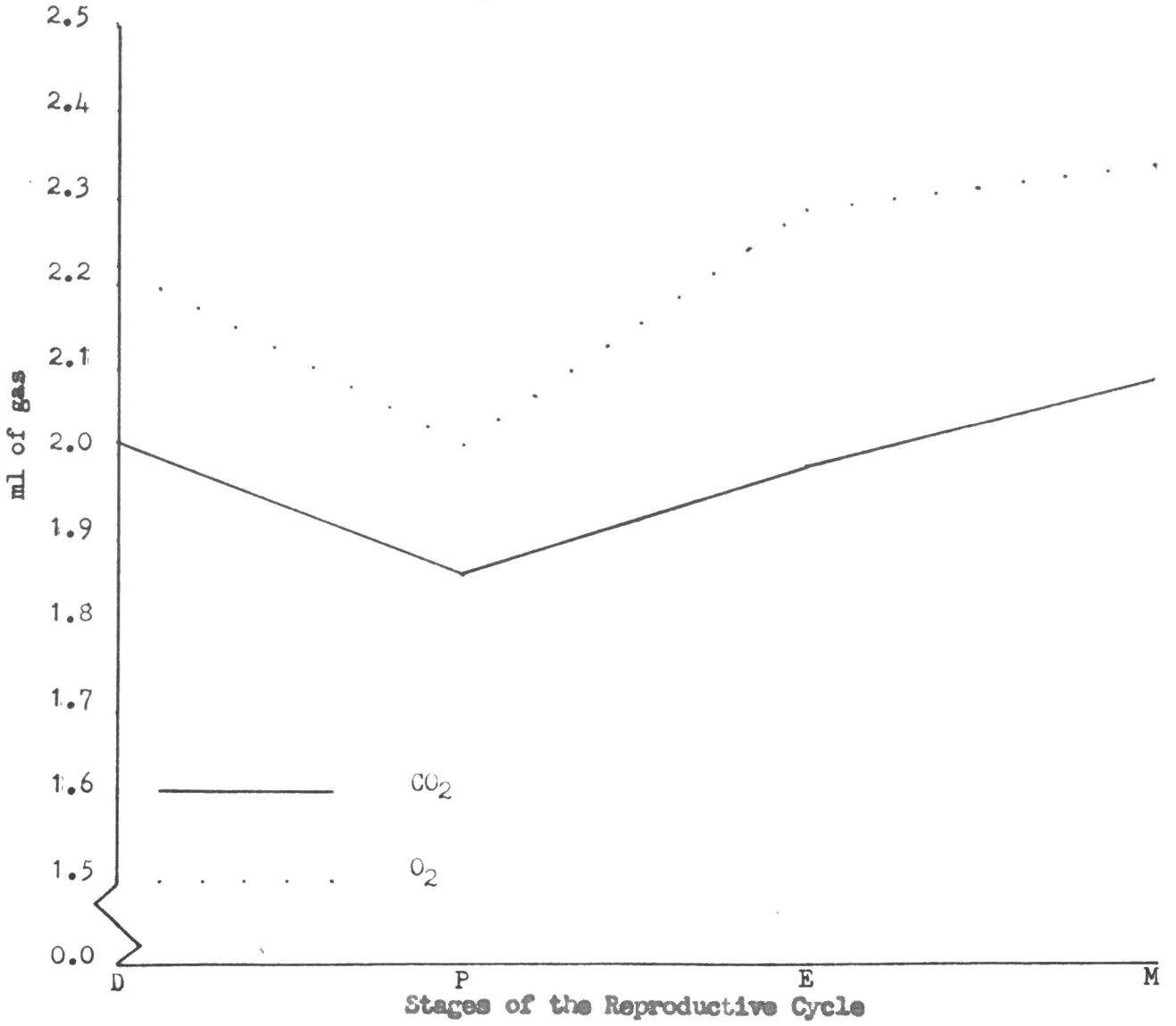
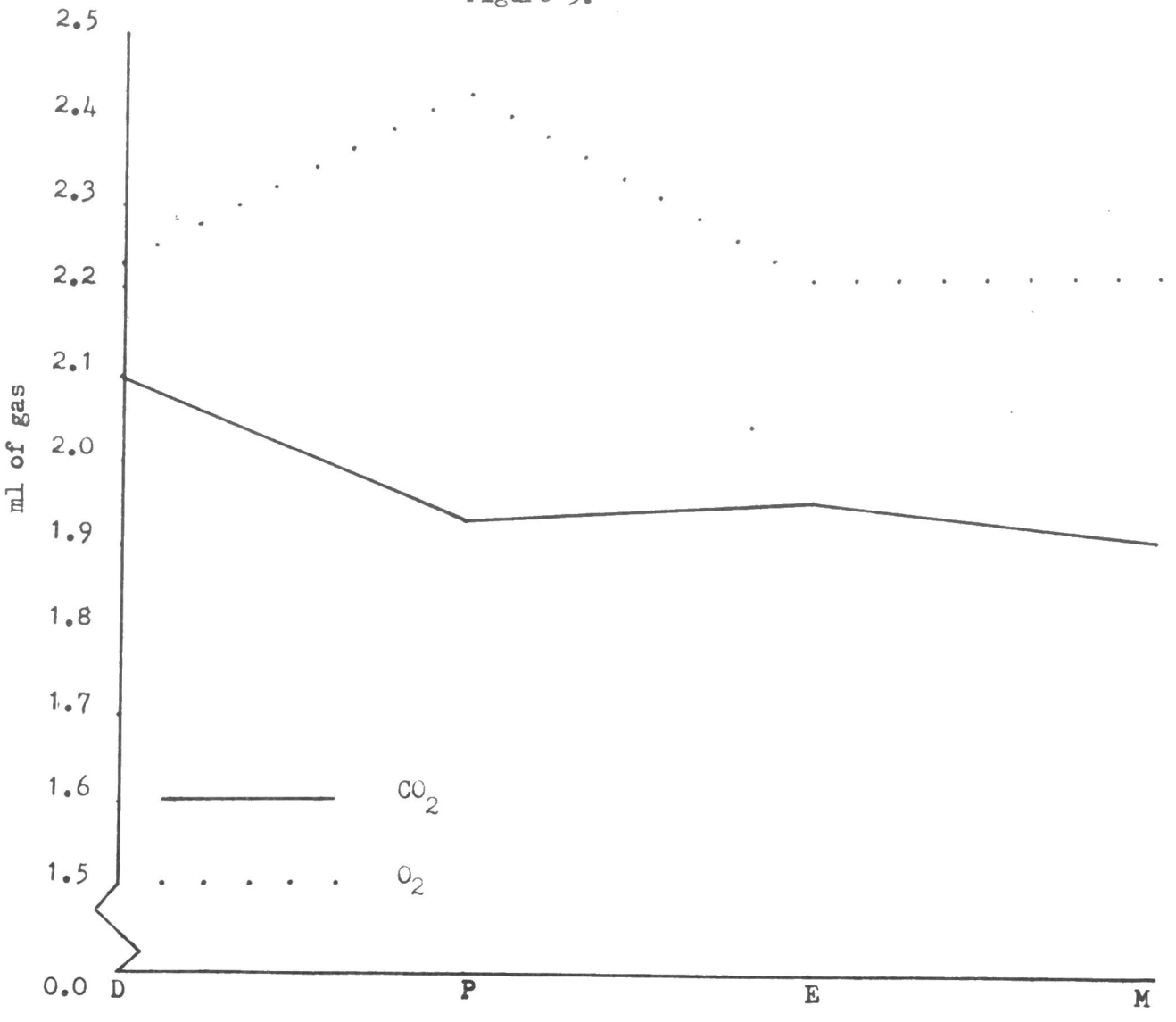


Figure 5. The average volume (in milliliters) of CO_2 produced and O_2 consumed per gram of body weight per hour by reproductive stages in the progesterone-estrogen treated group. D is diestrus, P is proestrus, E is estrus, and M is metestrus.

APPENDIX I

Figure 5.

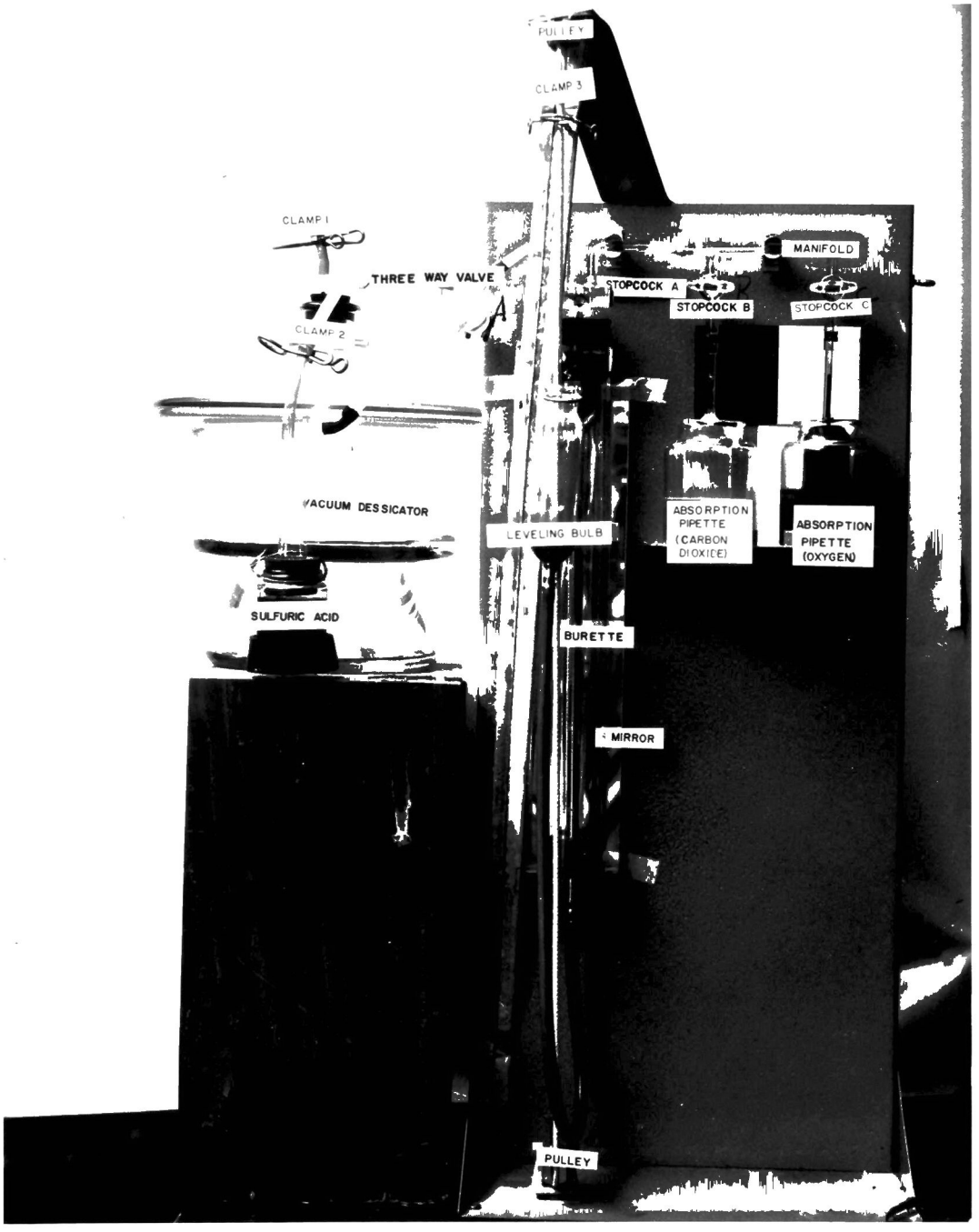


Stages of the Reproductive Cycle

Figure 6. The modified Haldane gas analysis apparatus used in this study.

APPENDIX J

Figure 6



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