ABSTRACT

James Marion Robertson. CYCLICAL SUCCINATE AND GLUTAMATE DEHYDROGENASE ACTIVITIES IN RAT OVARIAN AND LIVER MITOCHONDRIA. (Under the direction of Everett C. Simpson and Takeru Ito) Department of Biology, June, 1971.

Ovarian mitochondria demonstrating respiratory control were isolated. This indicated that this type of mitochondria was not generally different from extensively studied liver mitochondria. No significant difference in ovarian mitochondria protein concentrations were observed during the estrous cycle.

Oxygen consumption determinations of ovarian and liver mitochondria were conducted during the diestrus, proestrus and estrus stages of the estrous cycle. Sodium succinate and sodium glutamate were used as substrates for separate determinations of all mitochondrial types isolated. Activity was expressed as the n moles of O_2 consumed per mg of mitochondrial protein per minute. Both resting state and active state activities were determined. Respiratory control ratios (RCR) were calculated from the average resting and active states recorded.

The average succinic dehydrogenase (SDH) and glutamate dehydrogenase (GDH) activity during the resting state increased slightly during diestrus in both ovarian and liver mitochondria. Ovarian resting state SDH activity was about twice

as great as liver SDH activity throughout the estrous cycle.

In the active state, both liver and ovarian SDH activity was greatest during estrus. Liver active state GDH activity was also highest during estrus. To the contrary, ovarian GDH active state activity was greatest in proestrus. Liver mitochondrial GDH activity was significantly higher than ovarian mitochondrial GDH during the active state ($p=.01$) in all three cycle phases tested.

The RCR values for both ovarian and liver mitochondria were lowest in diestrus in all cases. The greatest RCR values were during estrus except in ovarian mitochondria utilizing glutamate. In this case, the highest RCR was in proestrus.

CYCLICAL SUCCINATE AND GLUTAMATE DEHYDROGENASE ACTIVITIES IN RAT OVARIAN AND LIVER MITOCHONDRIA

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MY PARENTS,

MR. AND MRS. MARION P. ROBERTSON

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INTRODUCTION

Although a moderate amount of research has been conducted involving succinic dehydrogenase activity within the ovary, the techniques most often used involved manometric determinations of ovarian homogenates and arbitary histochemical staining. These studies most often employed prepubertal rats, and few investigations have utilized mature rats.

Manometric studies usually involved comparisons of whole ovary homogenates with that of ovaries following hormonal treatment. Histochemical studies occasionally contradicted results determined in manometric studies and other histochemical studies.

This investigation is concerned with both glutamate and succinic dehydrogenase activity in mature rat ovarian mitochondria and liver mitochondria during specific periods of the estrous cycle.

REVIEW OF LITERATURE

It is well established that mammalian ovaries undergo histological and metabolic changes concomitant to reproductive processes. It is further known that ovarian function is mediated by the following:

1. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) of the pituitary.
2. An obscure "luteolytic" factor of the uterus that exerts an inhibitory effect on the corpora lutea of the ovary during the estrous cycle.
3. A neural mechanism within the uterus that maintains corpora lutea during pregnancy.
4. The luteotrophic hormones present during pregnancy.
5. The effect of estrogens and progestogens on certain areas of the hypothalamus that, in turn, regulate the release of FSH and LH.

The regulatory mechanisms listed above are responsible for reproductive cyclic phenomenon often associated with vertebrates or with maintenance of ovarian corpora lutea during early pregnancy. An understanding of these processes is essential in interpreting results obtained in studies involving reproductive physiology.

Foreman (1969) has reviewed the results of investigations first concerned with ovarian oxygen consumption (Fujita, 1928; Lipschutz and Vesnjakov, 1928, 1929; Brouha-

Dubbison, 1938). It was determined that estrus rat ovaries exhibited greater oxygen consumption than those in the proestrus stage of the estrous cycle and that oxygen uptake of adult rat ovaries was higher than that of ovaries from immature rats. FSH has been shown to increase oxygen consumption of new born rat ovaries (Barnhart, 1954), chick ovary slices (Nalbandov and Nalbandov, 1949), and anestrous prairie dog ovaries (Foreman, 1967).

In man, the concentration of Krebs cycle intermediates throughout the menstrual cycle has been analysed (Behrman and Duboff, 1960). All intermediates were found to rapidly increase starting on the thirteenth day and to attain highest concentrations on day 14-16, the approximate time of ovulation. A gradual decrease to preovulatory concentrations followed. Postmenopausal ovaries failed to give concentrations as high as the lowest concentrations present in premenopausal ovaries. The authors postulated that "a change in biochemical architecture and environment, with release of energy, could be responsible for the rupture of the follicle".

Succinic dehydrogenase (SDH) was the first enzyme, associated with Krebs cycle, that was demonstrated in the ovary (Meyer et al., 1945). Manometric determinations of oxygen consumption of ovarian tissue homogenates in the presence of sodium succinate were made on immature rats and immature rats previously stimulated with FSH, adult rats in

diestrus, pseudopregnancy (7 days) and pregnancy (18-20 days). In the three adult groups, SDH activity of dissected lutein tissue was also determined. This study demonstrated that the activity of this enzyme was greatest within the corpora lutea of the ovary. In relation to this, it was also shown that the corpora lutea of pregnant and pseudo-pregnant rats had greater SDH activity than corpora lutea in the diestrus phase of the estrous cycle. The authors suggested that energy required for production of progesterone may be mediated through Krebs cycle.

In the same investigation, ovarian SDH activity was found to decrease after *in vivo* injections of rat FSH in 26 day old immature rats in comparison with control subjects. This result was not expected, and it was attributed to homogenate dilution following hormone treatment.

Exploring further the corpora lutea of pregnancy SDH activity, Meyer *et al.* (1947) found a marked increase from the seventh to the eleventh day of pregnancy. Activity remained constant from the eleventh to the twentieth day.

A histochemical investigation confirmed the corpora lutea as the ovarian region of highest SDH activity in man with no activity demonstrated in the corpora albicantia. Moderate activity was found in the stroma (Foraker *et al.*, 1953). Due to the fact that pathological ovaries were studied and the mean average age of the donors was 42 years, care must be taken in interpreting these results.

A thorough study employing histochemical techniques for determining the activity of SDH and other oxidative enzymes in the rat and rabbit has been conducted (Okano et al., 1966). In corpora lutea of the rat, low activity was observed during diestrus and no activity during estrus. A rapid increase was demonstrated during early pregnancy in the corpora lutea, granulosa and theca interna.

Another histochemical investigation demonstrated the greatest activity of SDH during proestrus (Pupkin et al., 1966). The corpora lutea increased in SDH activity for three cycles following formation. In fact, the activity of this enzyme was greater during the third cycle following corpora lutea formation that during the proestrus preceding corpora lutea formation (granulosa cells of developing follicles).

Eckstein (1962) utilized immature rats to show that human chorionic gonadotrophin (HCG) and serum gonadotrophin (PMS) effected an increase in SDH activity when determined spectrophotometrically. PMS was more effective before 50 days of age and HCG afterwards. The author speculated that the mode of action of HCG involved the maintenance of corpora lutea by specifically stimulating activity of Krebs cycle.

In contrast to their previous findings, Eckstein and Lansberg (1963) manometrically determined a decrease in SDH activity following PMS administration to immature rats.

Eckstein disagreed with the homogenate dilution interpretation of this phenomenon given by Meyer et al., (1945) because an increase in isocitric dehydrogenase and malic dehydrogenase was observed. This effect has also been observed by Ringler and Kliman (1958).

The effects of FSH and LH on SDH activity determined by manometric techniques has recently been investigated on the cellular level. Oxygen consumption was significantly increased in a medium containing sodium succinate after in vitro addition of LH (Ahren et al., 1965). Isolated theca and interstitial cells of prepubertal rats have been shown to increase SDH activity following in vivo and in vitro addition of FSH (Hamberger and Ahren, 1967; Hamberger, 1968). LH had no stimulating effect on these cell types and FSH was found to have no effect on granulosa cells. Thus, a two cell theory concerning LH and FSH stimulation was advanced by Hamberger (1968) similar to that proposed for estrogen biosynthesis (Fevold, 1941; Ryan and Short, 1965, 1966).

A review of the literature indicates that a conspicuous dearth of research has revolved around ovarian mitochondrial activities. In fact, a private literary search conducted by Reproduction Research Information Service Limited on the topic ovarian metabolic activity in mammals produced only twenty four articles related to this area. The number of references searched was 76,476 included in 7,000 journals

from September, 1962 to September, 1969. No article dealt with ovarian mitochondria.

In the present study, activities of isolated ovarian and liver mitochondria, reflected by the rate of oxygen consumption, was investigated. This method has the advantage of avoiding the homogenate dilution effect mentioned previously and extramitochondrial factors that may be operating in a homogenate. Oxygen consumption, contrary to histochemical techniques, is not subject to arbitrary judgements as are staining intensities that cannot be quantitated.

From the above discussion, it is apparent that contradictory or inconclusive evidence of SDH activity throughout the estrous cycle has been observed. Accordingly, the mitochondrial activity of the estrus, diestrus and proestrus stages was determined employing sodium succinate and sodium glutamate as substrates. The increase in oxygen consumption following the addition of ADP was also determined in each case.

MATERIALS AND METHODS

Animals

Forty-eight nulliparous albino female rats of the Holtzman strain between the ages of 144-264 days were utilized in the present investigation. Thirty-two additional rats were used in preliminary work. The animals were housed in an air-conditioned room in which cycles of 14 hours light (5:00 A.M. - 7:00 P.M.) and 10 hours darkness (7:00 P.M. - 5:00 A.M.) were repeated. Purina Laboratory Chow and water were available *ad libitum*.

Estrous Phase Determination

Cytological examination of vaginal smears of at least 25 female rats was performed daily between 3:00 P.M. - 5:00 P.M. On days in which testing was conducted, examinations were performed between 1:00 P.M. - 3:00 P.M. The vaginal smears were classified as follows:

1. Diestrus - predominate leucocytes with occasional nucleated epithelial cells.
2. Proestrus - many nucleated epithelial cells with cornified epithelial cells rarely present.
3. Estrus - cornified epithelial cells only.

4. Metestrus - leucocytes and occasional cornified epithelial cells.

5. Transitional - leucocytes, nucleated and cornified epithelial cells (Barnea et al., 1968).

Only animals exhibiting normal 4-5 day cycles for two consecutive cycles before testing were used, and data were confined to diestrus, proestrus and estrus.

Isolation of Mitochondria

The ovaries of three female rats in the same phase of the estrous cycle were used in each determination. A rat was killed by cervical dislocation. Each ovary was removed through a dorso-lateral incision immediately posterior to the most caudal rib and placed in a beaker containing ice cold preparation medium (0.25M sucrose, 1mM EDTA, pH 7.5). The two ovaries were washed twice with clean medium and transferred to a Kontes hand homogenizer containing 1 ml of cold preparation medium. The ovaries were homogenized and the homogenate poured into an iced centrifuge tube. The above procedure was repeated with each of the two remaining test animals. The homogenates from three rats were combined.

The isolation of liver mitochondria was initiated by removal of the apex of the left lateral lobe of the liver of the third rat through an abdominal incision. The

approximate wet weight of liver equivalent to that of six ovaries (210-230 mg) was removed by rapidly cutting small slices of liver into ice cold preparation medium contained in a previously tared beaker. The slices were washed twice with cold medium and transferred to a Kontes hand homogenizer containing 1 ml of cold preparation medium and homogenized. The homogenate was poured into an iced centrifuge tube, and the homogenizer was washed twice with 1 ml of cold preparation medium to remove residual liver homogenate from the homogenizer. The two 1 ml washes were poured into the centrifuge tube containing the original 1 ml of liver homogenate.

The two centrifuge tubes containing 3 ml of either ovarian or liver homogenate were removed from the ice bath and centrifuged at 600 G and 2-4^{o}C in a Sorvall refrigerated centrifuge to remove unbroken cells and cell debris. The centrifuge tubes were removed and the supernatant fluids poured into clean, cold centrifuge tubes. The supernatant fluids were then centrifuged at 8,700 G. The resulting supernatant fluids were poured off and each mitochondrial pellet was suspended in 3 ml of preparation medium and washed twice (Jackanicz and Armstrong, 1968). The final ovarian and liver mitochondrial pellet, following removal of the supernatant fluids, were suspended in 0.4 ml of preparation medium by agitation with a glass rod. The mitochondrial suspensions were then uniformly dispersed in glass

teflon homogenizers and were transferred to separate, iced 50 ml flasks in an ice bath.

Oxygen Consumption Determination

The oxygen consumption was measured by a Clark oxygen electrode (Yellow Springs Instrument Co.). The reagents used were as follows:

Phosphorylation medium: 0.1M sucrose, 0.04M KCl, 0.01M potassium phosphate buffer, pH 7.4, 5mM $MgCl_2$, 0.1mM EDTA

Sodium succinate: 0.5M, pH 7.4

Sodium glutamate: 0.5M, pH 7.4

ADP: 40mM, pH 6.84

Phosphorylation medium at room temperature was well shaken and 2.7 ml added into a reaction vessel where the solution was constantly agitated by a small magnetic stirring bar. The diffusion of oxygen from the medium across the membrane of the oxygen electrode and the electro-reduction of this oxygen to water at the platinum cathode surface, which was maintained at 0.80 volts, effected a flow of current proportional to the oxygen contacting the cathode surface. The current was then amplified and recorded on the chart paper of a Servo Riter II recorder (Texas Instruments Inc.).

The chart drive was previously adjusted to one-half inch per minute. The recorder pen was adjusted to 100%, and a sufficient number of sodium hydrosulfite crystals added to the phosphorylation medium to remove all oxygen. The pen was then adjusted to 0%. The recorder being calibrated, the medium was aspirated from the reaction vessel, and the vessel was washed twice with deionized water. After the above calibration, 2.7 ml of fresh, well shaken phosphorylation medium was added, and the pen adjusted to 90% to record any possible upward deflection of the pen due to subsequent addition of other solutions.

To the fresh 2.7 ml of phosphorylation medium, 0.2 ml of ovarian mitochondria was added. Approximately two minutes later, 50 ul of well shaken sodium succinate at room temperature was added to the reaction vessel, and oxygen consumption was recorded automatically on the recorder chart paper. After three minutes, 10 ul of 40mM ADP was added to the mitochondria-succinate mixture and the resulting increase in oxygen consumption was recorded. The entire contents of the reaction vessel was then transferred into a clean, cold appropriately marked test tube and immediately placed in a freezer compartment (-20°C) until mitochondrial protein was determined. The reaction vessel was then washed twice with deionized water. The procedure given above, with the exception of instrument calibration, was conducted with the remaining 0.2 ml of ovarian mitochondria with glutamate as

substrate. Since the mitochondria were uniformly dispersed, the protein value obtained in the succinate test was used for calculations involving data concerned with glutamate. The entire procedure was repeated for liver mitochondria.

The rates of oxygen consumption per mg mitochondrial protein were calculated from the records obtained for both the active and resting state. The active state refers to the steady state within the mitochondria created by the presence of a large amount of an oxidizable substrate, ADP and oxygen. At this state, mitochondria carry out active oxidative phosphorylation. The resting state refers to another steady state at which only an oxidizable substrate and oxygen are available to the mitochondria. No active oxidative phosphorylation occurs at this state (Chance and Williams, 1956).

Oxygen content of the reaction medium was assumed to be equal to that in an air-equilibrated pure water medium. The molar content was calculated from the data in a chemistry handbook. It was approximately 240 uM at 25°C.

Protein Determination

The method of Folin-Ciocalteu as described by Cowgill and Pardee (1957) was employed in protein determinations. Unknown samples of liver and ovarian mitochondria were removed from the freezer compartment and thawed under cool, running tap water. The liver mitochondria samples were

tested following a 1:1 dilution with deionized water and ovarian mitochondria samples were tested without dilution.

To five test tubes, each unknown sample was added in 0.1 ml increments (0.1, 0.2, 0.3, 0.4, 0.5). All unknown samples were then brought to 0.5 ml by the addition of an appropriate volume of deionized water which was followed by 0.5 ml of 1N NaOH to give a 0.5N NaOH suspension. A tube containing both 0.5 ml deionized water and 0.5 ml 1N NaOH was used as a reference blank.

To all tubes, 5 ml of reagent A (1ml 2.7% sodium-potassium tartrate·4 H_2O and 1 ml 1% $CuSO_4$·5 H_2O added to 100 ml 2% Na_2CO_3) was added, and the samples were allowed to stand for 10 minutes. This was followed by the addition of 0.5 ml of reagent B (1N commercial phenol reagent) which was mixed immediately. After 30 minutes, the absorption of all tubes was determined by a Klett-Summerson colorimeter with a red filter.

The same procedure was followed with a known standard (crystalline bovine serum albumin). The maximum amount of the standard protein in a tube was 200 ug. The protein concentration of the unknown samples was calculated by the comparison of the unknown samples with the standards.

RESULTS AND DISCUSSION

An analysis of covariance applied to a factorial design was calculated to determine statistically significant differences between activity of ovarian and liver mitochondria before and after the addition of ADP. A similar analysis of activity was determined for both types of mitochondria resulting from variations in the estrous cycle and the possible interaction of ovarian and liver mitochondria in respect to changes in activity. An analysis was made for each of the substrates used.

It was shown that the increase in activity of liver mitochondria following the addition of ADP was significantly greater than that of ovarian mitochondria when glutamate was employed as a substrate ($p = .01$). All other calculations were not significant. It is, however, interesting to speculate on trends manifested in the data.

From Appendix A, it is obvious that no significant difference was found in mitochondrial protein concentrations throughout the cycle stages tested. Although the average value during diestrus appears lower than that of proestrus and estrus, this stage also had the greatest range.

The average resting state activity of ovarian mitochondria in the presence of succinate was found to be slightly greater during diestrus (Appendix B). This supports the contentions of Meyer *et al.*, (1945) and Okano *et al.*, (1966)

but does not agree with earlier studies involving oxygen consumption of whole ovaries (Fujita, 1928; Lipschutz and Vesnjakov, 1928, 1929; Brouha-Dubbison, 1938). The absence of a significant difference may be due to the fact that SDH activity is greater in lutein tissue than in interstitial tissue (Meyer et al., 1945). This difference may contribute negligibly to the mitochondrial activity of the entire ovary. Similarly, according to Pupkin et al. (1966), the SDH activity of the newly formed corpora lutea may be masked by the more active corpora lutea formed during previous cycles. This effect would also be subject to the number of previous ovulations.

It should be pointed out that, in addition to the variation of the average mitochondrial activity between cyclic phases being slight, wide ranges were observed between individual determinations within each group; therefore, caution should be given in this interpretation. Since the liver also displayed the same phenomenon, the possibility of no change in SDH activity should not be discounted. Surprisingly, ovarian mitochondria demonstrated resting state SDH activities twice that of liver mitochondria in all cycle phases tested.

Contrary to observations in the resting state, ovarian and liver mitochondrial SDH activity during the active state was found to be greatest during estrus. Thus, the amount of ADP in various tissue preparations may partially account for conflicting reports found in the literature.

When glutamate was used as substrate, an increase in the resting state activity was observed during diestrus (Appendix C). This was observed in both ovarian and liver mitochondria.

Since glutamate dehydrogenase is responsible for the production of the Krebs cycle intermediate alpha-ketoglutarate by the oxidative deamination of glutamate (Lehninger, 1965), an increased production of alpha-ketoglutarate by this enzyme is compatible with the increase in activity of Krebs cycle postulated to be requisite for the energy necessary for progesterone synthesis as proposed by Meyer et al., (1945). The implication of this phenomenon occuring in the liver is not certain. As noted previously, the increase in activity of liver mitochondria over ovarian mitochondria during the active state was statistically significant in all cycle stages tested when glutamate was utilized as substrate.

The active state of liver mitochondria utilizing glutamate as substrate, as in the case of both ovarian and liver mitochondria using succinate, was found to be highest in estrus. The ovarian mitochondrial active state GDH activity, however, was greatest during proestrus (Appendix C).

A possible explanation of the high ovarian active state GDH activity in proestrus may be found in a recent study in which progesterone concentrations were determined from the ovarian vein effluent of the rat during the estrous cycle (McDonald et al., 1969). In this investigation, a peak in progesterone secretion was observed during late proestrus as

well as diestrus. Lowest concentrations were observed in early proestrus. All synthesis of the hormone during diestrus was attributed to the corpora lutea as determined by Golgi apparatus disintegration, but it's origin in proestrus was not observed by this technique. In reference to the unknown origin of the proestrus progesterone, it is perhaps germane that Jackanicz and Armstrong (1968) have demonstrated progesterone biosynthesis in the rabbit ovarian interstitial tissue mitochondria. Regardless of the source, the proestrus progesterone synthesis was found to function only briefly, but the quantity produced may have influenced the results obtained with ovarian mitochondria.

It is interesting that the addition of ADP (active state) did not increase the rate of oxygen consumption proportional to that exhibited by mitochondria in the presence of either substrate alone (resting state). This is evident when the respiratory control ratios (RCR) are compared. The diestrus stage of both liver and ovary mitochondria displayed the lowest RCR in the presence of both succinate and glutamate (Appendix D). The estrus stage mitochondria exhibited the highest RCR in all cases except in ovarian mitochondria utilizing glutamate. In this instance, the highest RCR was found in proestrus. Although the low RCR seen in diestrus may be a reflection of the high activity during the resting state, it does not contribute to the fact that the highest activities of mitochondrial oxygen consumption in the active state is

found during estrus in all cases except during proestrus when ovarian mitochondria utilized glutamate (Appendix D).

The uncoupling of oxidation from phosphorylation by progesterone (Kupperman, 1965) may have contributed to the results obtained. In the presence of an uncoupling agent, the mitochondria often increase the rate of substrate oxidation because of the loss of the slower, rate limiting phosphorylation steps (Clark, 1964). The presence of progesterone during diestrus possibly exhibited this effect on ovarian and liver mitochondria resting state GDH activity (Appendix C). In respect to this, progesterone has also been shown to increase the activity of ATP-ase in liver mitochondria (Kupperman, 1965). If uncoupling does not occur *in vivo*, perhaps this effect may partially account for the greater resting state activity of liver and ovarian mitochondria during diestrus.

Because uncoupling is postulated to occur between DPNH and cytochrome c along the respiratory chain in the presence of progesterone (Kupperman, 1965), the SDH activity should not be affected by the presence (or absence) of progesterone. This was the case (Appendix B).

It was possible to isolate ovarian mitochondria showing respiratory control. This indicated that ovarian mitochondria were not generally different from liver mitochondria which had been studied extensively by many investigators. Since RCR can be considered as a criterion of structural integrity of mitochondria, high RCR of liver mitochondria during estrus

may be because of the low concentration of progesterone-type uncouplers, or because of the mitochondria being more structurally stable during this phase of the estrous cycle. The low RCR of ovarian mitochondria may possibly have reflected the relative difficulty experienced in isolation. It should be mentioned, however, that Krebs cycle intermediates are found in greatest concentrations in the human ovary previous to ovulation (Behrman and Duboff, 1960). In the present investigation, rats used for estrus determinations were selected on the afternoon previous to ovulation. The RCR found was greatest in this stage except in the ovarian mitochondria utilizing glutamate as substrate.

SUMMARY

Rat ovarian and liver mitochondrial succinic dehydrogenase (SDH) and glutamate dehydrogenase (GDH) activities (n moles of O_2 consumed per mg of mitochondrial protein per minute) were determined in the diestrus, proestrus and estrus stages of the reproductive cycle. The resting state activity (activity of mitochondria in the presence of sodium succinate or sodium glutamate and oxygen) and active state activity (activity of mitochondria in the presence of sodium succinate or sodium glutamate, oxygen and ADP) were determined for both types of mitochondria in each trial. The respiratory control ratio's (RCR) were calculated from the average active and resting state activities in each cycle phase tested.

The average resting state SDH and GDH activity of both ovarian and liver mitochondria was slightly higher in diestrus. Wide ranges in SDH resting state activities occured in both mitochondrial types in all stages of the estrous cycle. Ovarian mitochondrial resting state SDH activity was approximately twice that of liver throughout the estrous cycle.

The GDH resting state activity increase was more apparent in both mitochondrial types. The uncoupling of oxidation from phosphorylation by progesterone between DPNH and cytochrome c along the respiratory chain may account for the increased resting state GDH activity since this steroid is known to be produced during diestrus. It is also possible

that an increase in production of the Krebs cycle intermediate alpha-ketoglutarate by GDH may be a mechanism involved in the energy production postulated to be required for progesterone synthesis.

The active state SDH activity of both ovarian and liver mitochondria was greatest in estrus. Liver mitochondria active state GDH activity was also highest in this stage but, to the contrary, ovarian GDH activity was increased during proestrus. The recent observation of a brief period of ovarian progesterone synthesis during proestrus and the possibility of "uncoupling" occuring in its presence may account for this observation. The increase in active state activity of ovarian and liver SDH and liver GDH supports a previous report which showed a rapid increase in Krebs cycle intermediates in human ovaries at ovulation.

The lowest RCR values were in diestrus in both ovarian and liver mitochondria in all cases. The highest RCR values were during estrus except in ovarian mitochondria utilizing glutamate. In this case, the highest RCR was in proestrus. All liver mitochondrial RCR values were greater than the highest ovarian RCR value.

Since RCR is considered a criteria of mitochondrial structural integrity, the high RCR of liver mitochondria during estrus may be due to the low concentration of progesterone-type uncouplers, or because of the mitochondria being more structurally stable during this phase of the estrous

cycle. The low RCR of ovarian mitochondria may possibly have reflected the relative difficulty experienced in isolation.

APPENDIX A

Each value represents the total mitochondrial protein in 0.2 ml of the 0.4 ml of ovarian mitochondrial suspension prepared from six pooled ovaries from three rats. The values in parenthesis are averages of all determinations in each cycle.

APPENDIX A (continued)

Total Ovarian Mitochondrial Protein

(ug protein/0.2 ml mitochondrial preparation)

Stage of Cycle	Total Protein
Diestrus	98.0 165.9 168.5 180.5 191.7 278.7 (180.55)
Proestrus	120.1 153.0 206.5 217.9 245.4 (188.58)
Estrus	168.5 174.8 180.6 201.7 224.5 (190.02)

APPENDIX B

The succinic dehydrogenase activity values in the following table were calculated from the oxygen consumption per minute values (see oxygen consumption determination in methods and materials) of ovarian and liver mitochondria and the appropriate protein concentrations from Appendix A in the case of ovarian mitochondria. The resting state activities of both types of mitochondria were based on the oxygen consumption in the presence of succinate and oxygen. The active state activities were based on the increased oxygen consumption exhibited by the mitochondria following the addition of ADP.

The active and resting states of both ovarian and liver mitochondria from one determination (3 rats) may be found by following any row horizontally. The amount of ovarian protein present in the reaction medium of any determination is the respective value from the column in Appendix A. The values in parenthesis are averages.

APPENDIX B (continued)

Succinic Dehydrogenase Activity

(n moles O_2/min/mg mitochondrial protein)

	Ovarian Mitochondria		Liver Mitochondria	
	Resting State	Active State	Resting State	Active State
Diestrus	26.4	52.9	11.5	50.1
	23.9	50.2	16.9	45.0
	13.0	29.6	6.2	9.2
	34.1	60.0	15.7	31.3
	29.5	50.7	10.3	15.6
	24.7	49.5	9.1	40.7
	(25.27)	(48.82)	(11.62)	(31.99)
Proestrus	25.2	53.5	6.8	22.5
	22.4	65.3	9.3	38.4
	17.7	43.2	8.9	35.7
	25.5	32.0	7.5	36.3
	30.6	45.3	18.2	32.2
	(24.28)	(47.86)	(10.14)	(33.02)
Estrus	23.2	44.1	10.3	60.5
	23.4	49.4	6.5	37.9
	19.5	46.9	10.4	46.4
	31.2	62.4	8.4	35.7
	20.0	42.2	11.7	37.5
	(23.46)	(49.00)	(9.46)	(43.60)

APPENDIX C

The glutamate dehydrogenase activity values in the following table were calculated from the oxygen consumption per minute values (see oxygen consumption determination in methods and materials) of ovarian and liver mitochondria and the appropriate protein concentrations from Appendix A in the case of ovarian mitochondria. The resting state activities of both types of mitochondria were based on the oxygen consumption in the presence of glutamate and oxygen. The active state activities were based on the increased oxygen consumption exhibited by the mitochondria following the addition of ADP.

The active and resting states of both ovarian and liver mitochondria from one determination (3 rats) may be found by following any row horizontally. The amount of ovarian protein present in the reaction medium of any determination is the respective value from the column in Appendix A. The values in parenthesis are averages.

APPENDIX C (continued)

Glutamate Dehydrogenase Activity

(n moles O_2/min/mg mitochondrial protein)

	Ovarian Mitochondria		Liver Mitochondria	
	Resting State	Active State	Resting State	Active State
Diestrus	11.0	13.6	2.5	25.3
	4.8	6.4	12.5	32.4
	5.8	7.4	4.6	5.4
	12.1	13.4	19.1	23.9
	5.4	8.0	3.0	5.5
	4.6	7.2	1.1	25.4
	(7.28)	(9.33)	(7.13)	(19.65)
Proestrus	4.9	11.4	5.0	14.0
	4.9	9.9	2.6	14.4
	3.8	6.3	2.3	15.7
	5.6	7.6	3.7	24.5
	8.6	13.6	10.1	11.4
	(5.56)	(9.76)	(4.74)	(16.00)
Estrus	6.6	9.7	2.6	27.3
	6.4	10.9	9.0	20.2
	5.8	7.7	4.0	24.9
	5.1	10.3	2.0	17.0
	4.7	5.7	7.0	21.1
	(5.72)	(8.86)	(4.92)	(22.10)

APPENDIX D

The respiratory control ratios were calculated from the average active and resting state activities from the previous two tables (Appendix B and C). Isolation of ovarian mitochondria demonstrating respiratory control indicated that it was not generally different from liver mitochondria which had been extensively studied.

APPENDIX D (continued)

Respiratory Control Ratios

$$\frac{\text{(average activity of active state)}}{\text{(average activity of resting state)}}$$

Phase	Ovary		Liver	
	Succinate	Glutamate	Succinate	Glutamate
Diestrus	1.93	1.27	2.75	2.77
Proestrus	1.97	1.75	3.27	3.40
Estrus	2.08	1.57	4.59	4.51

APPENDIX E

The abbreviations used throughout the text are listed on the following page.

APPENDIX E (continued)

Abbreviations Used

ADP	=	Adenosine diphosphate
ATP-ase	=	Adenosine triphosphatase
DPNH	=	Diphosphopyridine nucleotide (reduced)
EDTA	=	Ethylenediaminetetraacetic acid
FSH	=	Follicle-stimulating hormone
GDH	=	Glutamate dehydrogenase
HCG	=	Human chorionic gonadotrophin
LH	=	Luteinizing hormone
PMS	=	Serum gonadotrophin
RCR	=	Respiratory control ratio
SDH	=	Succinic dehydrogenase

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