OVARIAN AND ADRENAL METABOLIC ACTIVITY IN RABBIT AND GUINEA-PIG PREGNANCY

J. F. ROUX, E. BLOCH AND S. L. ROMNEY

*Departments of Obstetrics and Gynecology and Biochemistry,
Albert Einstein College of Medicine and the Bronx Municipal
Hospital Center, New York 61, N.Y., U.S.A.*

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Summary. (1) Oxygen consumption, conversion of glucose or pyruvate to carbon dioxide and/or lipid are higher in the pregnant rabbit ovary, compared to the non-pregnant ovary when determined *in vitro*. Ovarian succinic oxidase and cytochrome oxidase activity increases 50 % during pregnancy. These findings correlate well with a rise in oxidative ovarian metabolism.

(2) Corpora lutea are more active than the rabbit ovarian interstitial tissue, both in metabolizing glucose and in its cytochrome and succinic oxidase activity.

(3) Ovarian metabolic activity during pregnancy is suggestively related to the production of progesterone in the rabbit.

(4) Guinea-pig ovarian metabolic activity decreases during pregnancy.

(5) Adrenal activity does not change, *in vitro*, in the pregnant rabbit but appears to increase in the pregnant guinea-pig.

(6) The findings are discussed in relation to the known features of placental and/or ovarian hormonal support essential to the maintenance of normal gestation.

INTRODUCTION

Numerous studies have demonstrated in a variety of species that ovarian morphology and/or hormone production is altered during the course of normal pregnancy (Amoroso, 1960). Indeed, successful maintenance of pregnancy is dependent in part upon the existence of a functioning ovary and/or placenta (Amoroso, 1960). On the other hand, the state of ovarian metabolic activity during gestation has received less attention. Cleasson & Hillarp (1953) demonstrated that in the pseudopregnant rabbit, ovarian cholesterol and phospholipids turnover increased. In the pregnant rat, Meyer, McShan & Erway (1945), Copenhauer, Meyer & McShan (1949), Biddulph, Meyer & McShan (1946) and McShan, Erway & Meyer (1948) correlated elevated ATPase, succinic dehydrogenase, cytochrome oxidase and malic dehydrogenase with an increase in progesterone production.

Adrenal involvement in pregnancy has been indicated by the observation of adrenal hypertrophy in guinea-pig, rabbit and rat (Amoroso, 1960) and possible changing activity in man (Amoroso, 1960; Wallace & Carter, 1960; Migeon, Bertrand & Wall, 1957; Gemzell, 1953).

In view of the morphological and hormonal changes of ovarian and possibly adrenal function during pregnancy and the relative paucity of data on metabolic activity, aspects of carbohydrate metabolism of these two endocrine glands during different gestational periods were studied.

MATERIAL AND METHODS

White New Zealand rabbits and English short-hair guinea-pigs were obtained (Rockland Farms, New York) at different periods of their reproductive cycle. These consisted of mature non-pregnant rabbits (at least 6 months old), pregnant rabbits (18 to 30 days *post coitum*), lactating rabbits (1 to 2 weeks *post partum*), mature non-pregnant guinea-pigs (400 to 500 g) and pregnant guinea-pigs (50 to 60 days *post coitum*), the state of gestation and/or lactation being known ± 1 day. They were maintained on a Rockland diet.

The animals were sacrificed by a blow on the head and the abdominal wall opened. The ovaries and adrenals were carefully removed, placed in cold 0.9% saline solution and slices prepared by free-hand cutting with a razor blade. The tissue slices were weighed and placed in incubation vessels. The entire preparation was completed within 15 min after sacrifice. In several experiments, corpora lutea were separated from the interstitial tissue by careful dissection.

TISSUE SLICE INCUBATION

Incubations were performed in Warburg vessels which contained 3 ml of a medium consisting of 40 μ moles K^+ , 80 μ moles Na^+ , 10 μ moles Mg^{2+} , 100 μ moles Cl^- and 40 μ mole PO_4^{3-} /ml, adjusted to an initial pH of 6.9 ± 0.1 . After flushing the flasks with 100% oxygen for 9 min, 30 μ moles glucose-1- ^{14}C , glucose-6- ^{14}C or 60 μ moles pyruvate-2- ^{14}C were tipped into the main reaction chamber from the sidearm of the vessel and incubation carried out for 1 hr at $37.8^\circ C$. At the end of the incubation period, the slices were removed, blotted in filter paper, weighed and homogenized in chloroform:methanol for extraction of lipids by the method of Folch, Lees & Sloane-Stanley (1957). Aliquots of the incubation medium were analysed for glucose (glucose-oxidase, Eli Lilly*), lactate (Barker & Summerson, 1941), pyruvate (Friedemann & Haugen, 1943), and $^{14}CO_2$ (Roux & Villee, 1960). Respiratory quotient was determined by dividing the barium carbonate (precipitated from the alkali in the centre well) by the tissue oxygen consumption. The lipids and $^{14}CO_2$ were transferred to stainless steel planchets for radioactivity determinations. Radioactivity was measured in a thin-window flow counter (Nuclear-

* We are indebted to Eli Lilly & Sons, Inc. for having provided us with glucose-oxidase.

Chicago, Model D47) and was corrected for self-absorption. Each sample was counted for a period of time sufficient to insure a counting precision of $\pm 3\%$.

TISSUE HOMOGENATE INCUBATION

For the determination of succinic oxidase and cytochrome oxidase activity, rabbit and guinea-pig ovaries were disrupted in a Potter Elvehjem homogenizer in cold distilled water to yield a 10% homogenate which was centrifuged at 0° C for 10 min at 650 g. Aliquots of the resulting supernatant were incubated for 1 hr at 37.8° C by a modified Potter's manometric technique (Schneider & Potter, 1943). The modification consisted of the addition of 5×10^{-4} moles of cytochrome C and omission of aluminium chloride and calcium chloride in the cytochrome oxidase determination. Aliquots of the homogenate were analysed for nitrogen by a micro-Kjeldahl method. All values were corrected for the appropriate blanks and endogenous activity. The data were treated statistically by analysis of variance or by Student's Test.

TABLE 1
CYTOCHROME AND SUCCINIC OXIDASE
ACTIVITIES OF RABBIT AND GUINEA-PIG OVARIES

Reproductive stage	Tissue	Cytochrome oxidase	Succinic oxidase
		$\mu\text{mole O}_2/\text{mg N/hr} \pm \text{s.e.}$	
		Rabbit	
Mature, non-pregnant Pregnant	Whole ovary	85 $\pm$ 7 (20)	10 $\pm$ 0.7 (20)
	Whole ovary	128 $\pm$ 7 (20)	*16 $\pm$ 0.8 (9)
	Corpora lutea	*206 $\pm$ 36 (4)	*14 $\pm$ 0.7 (4)
	Int. tissue	113 $\pm$ 32 (4)	6.7 $\pm$ 0.7 (4)
Lactating	Whole ovary	105 $\pm$ 10 (10)	14 $\pm$ 1.4 (9)
		Guinea-pig	
Mature, non-pregnant Pregnant	Whole ovary	*12 $\pm$ 2.4 (9)	1.8 $\pm$ 0.8 (6)
	Whole ovary	3.0 $\pm$ 0.8 (9)	3.2 $\pm$ 0.7 (6)

* $P \leq 0.05$ when compared to the mature group.
(), number of determinations.

RESULTS

OVARIAN SUCCINIC OXIDASE AND CYTOCHROME OXIDASE ACTIVITY

As seen in Table 1, cytochrome and succinic oxidase activity of the rabbit ovaries increased 50 to 60% during pregnancy and were still somewhat elevated during lactation. In gestation, cytochrome oxidase activity of guinea-pig ovaries decreased without changes in succinic oxidase activity. The corpora lutea and interstitial cell tissue of two rabbits (23 days pregnant) were incubated separately. The cytochrome and succinic oxidase activities of corpora lutea were found to be higher than that of the interstitial tissue, succinic oxidase being twice as active in corpora lutea.

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TABLE 2
RABBIT OVARIAN AND ADRENAL METABOLISM OF ^{14}C -LABELLED GLUCOSE *in vitro*

	Mature		Pregnant		Lactating	
	Ovary	Adrenal	Ovary	Adrenal	Ovary	Adrenal
Oxygen consumption	15.8 $\pm$ 0.6	24.8 $\pm$ 0.7	*23.3 $\pm$ 0.6	27.0 $\pm$ 0.7	*21.0 $\pm$ 1.3	*31.0 $\pm$ 0.9
Glucose utilization	11.6 $\pm$ 0.8	10.4 $\pm$ 1.5	12.5 $\pm$ 1.0	17.6 $\pm$ 3.3	12.0 $\pm$ 2.2	10.2 $\pm$ 1.7
Respiratory quotient	0.9 $\pm$ 0.06	0.9 $\pm$ 0.2	*1.4 $\pm$ 0.06	1.2 $\pm$ 0.2	1.0 $\pm$ 0.06	0.9 $\pm$ 0.01
Conversion of Carbon 1 of glucose to carbon dioxide	0.03 $\pm$ 0.01	0.2 $\pm$ 0.07	*0.18 $\pm$ 0.01	0.2 $\pm$ 0.07	*0.08 $\pm$ 0.01	0.2 $\pm$ 0.06
Conversion of Carbon 6 of glucose to carbon dioxide	0.05 $\pm$ 0.006	0.1 $\pm$ 0.03	*0.10 $\pm$ 0.006	0.1 $\pm$ 0.03	0.08 $\pm$ 0.03	0.1 $\pm$ 0.04
Conversion of Carbon 1 of glucose to lipids	0.18 $\pm$ 0.2	0.30 $\pm$ 0.05	*0.91 $\pm$ 0.2	0.38 $\pm$ 0.1	*0.82 $\pm$ 0.1	0.39 $\pm$ 0.2
Conversion of Carbon 6 of glucose to lipids	0.16 $\pm$ 0.2	0.30 $\pm$ 0.01	1.0 $\pm$ 0.2	0.36 $\pm$ 0.07	0.44 $\pm$ 0.004	*0.40 $\pm$ 0.01
Lactate production	9.2 $\pm$ 0.3	0.5 $\pm$ 0.6	7.9 $\pm$ 0.6	9.5 $\pm$ 0.6	9.5 $\pm$ 0.5	10.3 $\pm$ 2.0

The results are expressed in $\mu\text{moles per g of wet weight per hr of incubation at } 37.8^\circ \text{C}$. The results are average $\pm$ s.e. of the mean of twenty-four determinations.

* $P < 0.05$.

METABOLISM OF LABELLED GLUCOSE BY OVARIAN AND ADRENAL TISSUE

Oxygen consumption by ovarian tissue from pregnant rabbits increased 50 %, and the conversion of glucose-1-¹⁴C and glucose-2-¹⁴C to ¹⁴CO₂ rose two- to sixfold compared to mature non-pregnant rabbits (Table 2). Lactate production tended to decrease somewhat during gestation. Conversion of glucose-1-¹⁴C or glucose-6-¹⁴C to lipids increased fivefold in the pregnant rabbit ovary. The respiratory quotient rose 40 % during pregnancy. In the ovaries

TABLE 3

GUINEA-PIG OVARIAN AND ADRENAL METABOLISM OF GLUCOSE *in vitro*

	Mature		Pregnant	
	Ovary	Adrenal	Ovary	Adrenal
Oxygen consumption	20.0 ± 1.8	8.0 ± 5.0	*13.5 ± 0.6	*15.5 ± 2.0
Glucose utilization	97.0 ± 2.2	21.0 ± 1.5	*17.0 ± 1.1	27.0 ± 4
Respiratory quotient	0.6 ± 0.1	0.9 ± 0.0	*1.5 ± 0.1	*1.9 ± 0.5
Lactate production	10.4 ± 2.0	9.6 ± 0.4	*34.0 ± 1.0	*17.8 ± 1.8

The results, which are from eight determinations, are expressed as in Table 2.

* $P < 0.05$.

TABLE 4

RABBIT CORPORA LUTEA AND INTERSTITIAL
TISSUE METABOLISM OF GLUCOSE *in vitro*

	Corpora lutea	Interstitial tissue
	μmole/g of wet weight/hr at 37.8° C ± s.e.	
Oxygen consumption	*25.0 ± 1.0 (12)	19.0 ± 1 (12)
Glucose utilization	18.0 ± 2.0 (12)	4.0 ± 4.0 (12)
Respiratory quotient	1.5 ± 0.2 (12)	1.3 ± 0.1 (12)
Conversion of Carbon 1 of glucose to carbon dioxide	0.1 ± 0.03 (6)	0.05 ± 0.02 (6)
Conversion of Carbon 6 of glucose to carbon dioxide	0.04 ± 0.004 (6)	0.05 ± 0.006 (6)
Conversion of Carbon 1 of glucose to lipid	1.1 ± 0.40 (3)	0.59 ± 0.26 (3)
Conversion of Carbon 6 of glucose to lipid	0.53 ± 0.14 (3)	0.39 ± 0.10 (3)
Lactate production	*15.5 ± 2.3 (12)	9.5 ± 2.0 (12)

(), number of determinations.

* $P < 0.05$.

from lactating rabbits, the values of oxygen consumption, conversion of glucose-1-¹⁴C or glucose-6-¹⁴C to lipids were close to those of the pregnant ovary. Moreover, glucose utilization, respiratory quotient and carbon dioxide formation was about the same as that of ovarian tissue from non-pregnant animals. Adrenal metabolic activity did not change significantly with change in reproductive function. Glucose-1-¹⁴C or glucose-6-¹⁴C were metabolized to carbon dioxide and lipids to the same extent. In no instance was the carbon dioxide obtained from the glucose-1-¹⁴C higher than the carbon dioxide obtained from glucose-6-¹⁴C.

Ovarian tissue from pregnant guinea-pigs consumed less oxygen and glucose than tissue from the mature non-pregnant animal, but produced more lactic acid (Table 3). In contrast, pregnancy was associated with a higher oxygen consumption, respiratory quotient and lactate production by adrenal slices.

As shown in Table 4, rabbit corpora lutea had a significantly higher oxygen consumption, glucose utilization and lactate production compared to interstitial cell tissue. The conversion of glucose-1-¹⁴C or glucose-6-¹⁴C to lipids had a tendency to be somewhat higher in the corpora lutea. Both corpora lutea and interstitial tissue metabolized glucose-1-¹⁴C or glucose-6-¹⁴C to carbon dioxide to the same extent.

TABLE 5
METABOLISM OF PYRUVATE-2-¹⁴C BY RABBIT OVARIAN SLICES *in vitro*

	Non-pregnant	Pregnant (21 days)
	<i>μmole/g of wet weight/hr at 37.8 C ± s.e. of six determinations</i>	
Oxygen utilization	10.8 ± 2.1	*26.4 ± 1.0
Respiratory quotient	—	1.6 ± 0.1
Pyruvate utilization	32.8 ± 2.3	*78.0 ± 3.7
Conversion of pyruvate to carbon dioxide	1.1 ± 0.5	*4.1 ± 0.1
Conversion of pyruvate to lipid	0.06 ± 0.02	*0.42 ± 0.04
Lactate production	8.0 ± 0.8	12.2 ± 0.9

* $P \leq 0.05$.

METABOLISM OF PYRUVATE-2-¹⁴C BY OVARIAN SLICES

In the pregnant rabbit ovary (Table 5), oxygen and pyruvate utilization doubled while lactate production increased by 50 %. Conversion of pyruvate to carbon dioxide and the lipid increased fourfold and sevenfold respectively.

DISCUSSION

The metabolic activity of ovarian tissue from pregnant rabbits was markedly elevated over that from non-pregnant animals. Thus, oxygen consumption, the respiratory quotient, pyruvate utilization and the conversion of glucose and pyruvate to carbon dioxide were all increased at least twofold. Conversely, lactate production was decreased somewhat. These results indicate that during pregnancy glucose and pyruvate are metabolized at a higher rate by ovarian tissue. Most of this higher activity would appear to be due to the presence of corpora lutea. Thus, when corpora lutea, separated from interstitial tissue, were incubated with labelled glucose, they utilized more oxygen, more glucose and transformed more labelled hexose to lipids than interstitial tissue.

During pregnancy the finding of increased ovarian pyruvate oxidation,

carbon dioxide production, oxygen consumption would suggest an augmentation of the Krebs' cycle activity. Supporting evidence for this suggestion is to be found in the elevated cytochrome oxidase and succinic oxidase activity which increase 50 % in the pregnant rabbit ovary. Since the conversion of glucose-1- ^{14}C to carbon dioxide and to lipids did not differ from that of glucose-6- ^{14}C , this may mean no increase in hexosemonophosphate shunt activity or that the increase cannot be detected. The use of glucose-1- ^{14}C or glucose-6- ^{14}C does not give the true value of the hexosemonophosphate shunt, if the recycling of fructose-6- PO_4 to glucose-6- PO_4 is elevated and if the Krebs' cycle is very active, as it is in our experiments (Katz & Wood, 1960).

Of interest is the finding that rabbit corpora lutea were both metabolically and enzymatically more active than the interstitial tissue. This latter tissue had a rate of metabolic activity closer to the non-pregnant ovarian stroma.

The data from ovaries from lactating rabbits indicate that the ovarian metabolic activity returns slowly to the non-pregnant level after delivery. Thus some of the activities were similar to those of the non-pregnant state (RQ, carbon dioxide formation), others to those found during pregnancy (oxygen consumption, conversion of glucose-1 or glucose-6 to lipids). In contrast to the rabbit, the metabolic activity of the guinea-pig ovary decreased during pregnancy. Thus, the data reported have demonstrated a decrease, *in vitro*, in glucose metabolism, oxygen consumption and cytochrome oxidase and succinic oxidase activities.

The observed respiratory quotient values support in general the findings of somewhat variably increased lipogenesis by ovarian and adrenal tissue during pregnancy. Although the data suggest a trend toward higher respiratory quotient values, no differences were significant due to the large standard error.

Our results indicated an increase in the oxidative metabolism of the pregnant rabbit ovary, while in the case of the guinea-pig a decrease occurred. These data can be correlated with our knowledge of the endocrine maintenance of pregnancy in the two species. Ovariectomy invariably terminates pregnancy in the rabbit, but not in the guinea-pig (Amoroso, 1960). In the former species, this appears to be due to the lack of progesterone, because progesterone administration to the pregnant spayed animal allows pregnancy to progress to term (Hulming Wu & Allen, 1959). Moreover, ovariectomy decreases blood progesterone (Zarrow, 1956). On the other hand, in the guinea-pig, progestational compounds are produced by the placenta which replaces ovarian function (Amoroso, 1960). Therefore, a correlation between increased metabolic activity and progesterone formation appears to exist. In view of our present knowledge of the requirement for steroidogenesis (Hayano, Saba, Dorfman & Hechter, 1956), it is suggested that part of the increased oxidative metabolic activity is utilized for increased production and availability of cofactors and substrate, i.e. ATP, TPN, DPN, acetate from the Krebs' cycle for steroid synthesis and steroid precursors.

The *in-vitro* metabolism of rabbit adrenal during gestation did not differ from that of the non-pregnant state. In contrast, the guinea-pig adrenal metabolizes more glucose, produces more lactate and utilizes more oxygen during pregnancy ($P \leq 0.05$).

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