CXCIII. THE EFFECT OF THE OESTROUS CYCLE ON THE METABOLISM OF ISOLATED RAT UTERUS

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THE respiration of isolated rat [Khayyal & Scott, 1934-5] and mouse [David, 1931] uterus has been shown to vary with the phase of the oestrous cycle. Raab [1929], working with human uterine mucosa, found variations in metabolism with the phase in the menstrual cycle, but Adler [1930] was unable to confirm these results. Victor *et al.* [1936] have shown that the respiration rate of rat-liver slices is increased during oestrus, whereas kidney slices from the same animals showed no cyclic changes. Victor & Andersen [1936], working with anterior pituitary gland (from rats), found respiration and anaerobic glycolysis to be higher during the pro-oestrous and oestrous phases of the cycle, but they were not able to show significant changes in aerobic glycolysis.

The experiments presented here show that the anaerobic glycolysis of isolated rat uterus also varies similarly to the respiration, and that aerobic glycolysis varies, but in the opposite sense to respiration and anaerobic glycolysis.

METHODS

Animals. Black and white rats (10-14 weeks old) from the Lister Institute breeding stock were used, except for a few experiments made on albino rats from the Glaxo colony (marked with letters in the tables). The phase of the oestrous cycle was determined from daily vaginal smears, the majority of the rats had 5-day cycles, but those in the group used for the aerobic experiments had longer cycles, 6-7 days. The following stages were distinguished:

1. Pro-oestrus: epithelial cells only.
2. Oestrus: (a) epithelial and cornified cells; (b) cornified cells only.
3. Metoestrus: cornified cells and leucocytes, or a mass of leucocytes with no epithelial cells.
4. Dioestrus: (a) first day, epithelial cells and leucocytes; (b) second day, epithelial cells and leucocytes.

Stages 1 and 3 are of shorter duration than the others; stage 1 was not observed in all cycles and stage 3 only occasionally. Bilateral ovariectomy was performed on adult rats, which were used for experiments 17-37 days afterwards.

Experimental technique. The rats were anaesthetized by an intraperitoneal injection of sodium amytal, the uterus dissected out and each horn slit longitudinally. No attempt was made to separate muscle from mucosa. For anaerobic experiments a single horn was used; for aerobic experiments one horn was used to determine the initial bicarbonate content, the other for measurement of O₂ consumption and CO₂ production. The tissue was weighed rapidly before immersion in Ringer's solution; at the end of the experiment it was washed and dried to constant weight at 105° in air. All calculations are based on dry weight figures; considerable variation of dry weight was found (mean 17 % wet weight), but no cyclic variation (confirming David [1931]).

The symbols used are those recommended by M. Dixon [1934]. Anaerobic glycolysis was measured in Warburg flasks, filled with a mixture of N_2 and 5% CO_2 (freed from O_2 by passing over heated copper), respiration and aerobic glycolysis in Dixon-Keilin [1933] flasks, filled with a mixture of O_2 and 5% CO_2 . The flasks were shaken in a thermostat at 37.5° . In order to reduce the constants of the Dixon-Keilin flasks they were kept on the small side, 32 ml., the manometers were made longer than is usual, 250 and 300 mm. and were filled with saturated lead perchlorate solution [Krebs, 1930]. It was only possible to use 2 ml. Ringer-bicarbonate solution, but this was sufficient for a single horn of rat uterus. The constants were much smaller than those of the apparatus as originally described (5.05–5.54) and the accuracy rather greater, $\pm 3 \mu$ l. With the small pressure changes recorded the average error, due to the apparatus, is high, $\pm 6\%$ for O_2 consumption and larger for the smaller changes due to CO_2 production. This is reflected in the results, especially in the figures for R.Q.

The experiments lasted 1–2 hr., all times being reckoned from the beginning of the manometric measurements, about 20 min. after the dissection of the uterus.

Chemical analyses. The tissue was ground in the Ringer solution, to which mercuric chloride had been added, the volume adjusted, and lactic acid [Friedmann *et al.* 1927] and glucose (Hagedorn & Jensen [1923], before and after yeast fermentation) determined on aliquots of the mercury-free filtrate.

RESULTS

(1) *Effect of substrate and of composition of the Ringer's solution on anaerobic glycolysis*

The anaerobic glycolyses of each horn of a single uterus, measured separately, agreed reasonably (Table I, mean difference 4%), so that one horn may be used

Table I. *Comparison of anaerobic glycolysis, $Q_G^{N_2}$, of one horn (a) of rat uterus with the other (b); and the effect of varying the composition of the Ringer's solution*

No.	(a) Dixon's Ringer	(b) Dixon's Ringer			
		Alone	+ KH_2PO_4	+ $MgSO_4$	+ KH_2PO_4 + $MgSO_4$ + KCl } Krebs & Henseleit's Ringer
		No added carbohydrate			
14	1.11	1.10	—	—	—
18	1.11	1.15	—	—	—
7	1.17	1.08	—	—	—
48	2.67	—	—	—	2.16
17	1.38	—	—	—	1.36
27	1.43	—	—	—	2.24
22	1.29	—	—	—	1.24
31	1.20	—	—	1.18	—
28	1.42	—	1.65	—	—
		+ Glucose			
21	8.73	9.32	—	—	—
24	3.56	3.59	—	—	—
44	6.16	5.96	—	—	—
40	4.87	4.53	—	—	—
13	9.12	9.11	—	—	—
16	4.94	—	—	—	5.01
30	5.20	—	—	—	4.53
		+ Fructose			
35	1.73	—	—	—	1.63
25	1.66	—	—	—	1.43

as a control on the other in comparative experiments. The composition of the Ringer's solution employed by different workers varies somewhat. In these experiments M. Dixon's solution [1934, p. 75], both with and without glucose, was used. In preliminary experiments the effect of adding KH_2PO_4 and MgSO_4 in the proportions used by Krebs & Henseleit [1932], was investigated. Their solution also differs from that used by Dixon in containing twice as much KCl, otherwise its composition is the same. Neither addition of KH_2PO_4 or MgSO_4 , nor of both together with additional KCl (=Krebs & Henseleit's solution) caused any significant difference in the rate of glycolysis. This was the case whether or not the medium contained carbohydrate (Table I).

When the Ringer's solution contained no carbohydrate, glycolysis was low and fell off rapidly at first, reaching a constant rate after 30–60 min. (Fig. 1). Addition of fructose or glycogen (to give a concentration of 0.2% in the Ringer) increased the rate very slightly and did not alter the type of curve obtained (Fig. 1, Table II). In the presence of glucose (0.2%) the rate of glycolysis was much higher and remained constant throughout the experiment (Fig. 1 and Table II).

Fig. 1. Effect of substrate on $Q_G^{N^s}$. Typical curves from dioestrous rats.
 — 36, A no addition, B + glycogen. --- 5, A no addition, B + fructose.
 — 19, A no addition, B + glucose.

In some experiments the sugar disappearing was compared with the lactic acid formed, estimated both chemically and manometrically. Agreement was reasonable and it may be concluded that the tissue converts glucose quantitatively into lactic acid (Table III).

(2) *Effect of oestrous cycle on respiration, aerobic and anaerobic glycolysis*

Anaerobic glycolysis was found to be highest in the pro-oestrous phase, to fall to low values during oestrus and to rise again during dioestrus (Fig. 2, Table II). Some high values were recorded on the second day of dioestrus,

Table II. *Effect of added carbohydrate and of oestrous cycle on anaerobic glycolysis, $Q_G^{N_2}$, of rat uterus*

No.	No added carbohydrate		Glucose 0-60 min.	Fructose		Glycogen	
	0-15 min.	45-75 min.		0-15 min.	45-75 min.	0-15 min.	45-75 min.
Phase 1. Pro-oestrus							
11	3.66	0.90	9.36	—	—	—	—
41	2.68	0.72	9.95	—	—	—	—
Mean	3.17	0.81	9.66				
Phase 2 <i>a</i> . Oestrus							
10	4.80	0.83	9.13	—	—	—	—
21	—	—	9.00	—	—	—	—
26	2.70	0.80	9.30	—	—	—	—
16	—	—	4.96	—	—	—	—
31	2.46	0.75	—	—	—	—	—
48	4.00	1.18	—	—	—	—	—
32	—	—	3.91	—	—	3.89	1.48
35	—	—	—	3.12	1.11	—	—
Mean	3.49	0.89	7.26				
Phase 2 <i>b</i> . Oestrus							
17	2.48	0.87	—	—	—	—	—
15	2.33	—	2.88	—	—	—	—
23	2.41	0.84	4.18	—	—	—	—
18	1.96	0.75	—	—	—	—	—
24	—	—	3.58	—	—	—	—
3	—	—	3.26	1.97	0.93	—	—
Mean	2.30	0.82	3.48				
Phase 3. Metoestrus							
2	1.55	0.56	—	2.15	0.88	—	—
9	5.06	0.74	4.15	—	—	—	—
30	—	—	5.26	—	—	—	—
Mean	3.30	0.65	4.71				
Phase 4 <i>a</i> . Dioestrus							
36	1.60	0.83	—	—	—	2.06	0.98
44	—	—	6.16	—	—	—	—
40	—	—	4.87	—	—	—	—
8	2.98	0.98	4.44	—	—	—	—
22	1.70	1.08	—	—	—	—	—
27	3.10	0.83	—	—	—	—	—
4	1.86	1.27	—	—	—	—	—
7	1.80	0.80	—	—	—	—	—
5	1.41	0.90	—	2.29	1.26	—	—
6	—	—	6.63	1.91	0.72	—	—
Mean	2.06	0.96	5.53				
Phase 4 <i>b</i> . Dioestrus							
19	1.40	0.78	6.53	—	—	—	—
12	—	—	6.18	—	—	—	—
28	2.48	0.92	—	—	—	—	—
47	1.99	1.15	10.10	—	—	—	—
13	—	—	9.11	—	—	—	—
45	2.10	0.76	9.29	—	—	—	—
39	—	—	7.83	—	—	—	—
25	3.60	1.75	—	—	—	3.18	1.82
29	—	—	—	2.50	1.20	—	—
Mean	2.31	1.07	8.17				
Ovariectomized							
A 1	5.38	1.98	5.82	—	—	—	—
A 2	1.18	0.81	5.58	—	—	—	—
4 B	2.34	0.73	5.20	—	—	—	—
111	—	—	6.20	—	—	—	—
1 A	2.23	0.98	—	—	—	—	—
Mean	2.78	1.13	5.70				

probably because the metabolic changes taking place in the tissue precede the changes in the vaginal smear, so that the uterus of a rat that is at the beginning of the pro-oestrous phase will have a high metabolism while leucocytes are still present in the smear. A similar explanation will account for the occurrence during the first phase of oestrus (2a) of high values typical of pro-oestrus and low values typical of the second oestrous phase (2b). In ovariectomized animals the rate was of the same order as that found in normal animals on the first day of dioestrus.

Table III. *Comparison between sugar utilized and lactic acid formed, measured both manometrically and chemically, during anaerobiosis*

No.	Sugar used mg.	Lactic acid formed, measured	
		Manometrically mg.	Chemically mg.
22	2.62	2.40	2.36
8	1.54	1.14	1.19
9	1.19	0.91	1.30
5	0.74	0.77	0.82
17	1.59	1.83	—
3	2.30	2.21	2.16

When the Ringer's solution contained no glucose, in spite of considerable variations, the initial rate followed the same general course, glycolysis was highest during pro-oestrus and in the first stage of oestrus. In these experiments the pressure changes measured are small and the results less accurate; small differences in the time after immersion in anaerobic Ringer's solution at which measurement is begun will have a marked effect on the initial rate recorded.

Fig. 2. Effect of oestrous cycle on:
 Anaerobic glycolysis, ⊙. Respiration, ×. Aerobic glycolysis, □.
 Blocks represent mean values.

The mean value of the final constant rate was rather higher during dioestrus, but the differences were hardly significant and the phase in the cycle seems to have little or no effect on this constant rate of glycolysis in the absence of glucose.

Table IV. *Effect of oestrous cycle on O_2 consumption, Q_{O_2} , aerobic glycolysis, $Q_G^{O_2}$, and R.Q. of rat uterus*

No.	$-Q_{O_2}$	$Q_G^{O_2}$	R.Q.
Phase 1. Pro-oestrus			
A 4	3.03	0.61	0.90
126	6.07	0.68	1.14
136	4.78	0.37	0.98
AA	4.09	0.77	0.87
Mean	4.50	0.60	0.97
Phase 2a. Oestrus			
A 3	4.63	1.20	0.92
118	3.56	0.51	1.02
127	4.44	0.13	1.24
129	4.36	1.48	0.72
135	4.76	1.84	0.86
A 6	3.36	0.71	0.85
108	6.32	1.64	0.75
Mean	4.49	1.07	0.91
Phase 2b. Oestrus			
207	2.09	1.95	0.89
210	3.46	1.48	0.92
B 3	3.64	1.35	0.68
1 B	3.31	0.78	0.78
106	3.54	1.49	0.85
Mean	3.21	1.41	0.82
Phase 3. Metoestrus			
112	3.89	1.89	0.94
Phase 4a. Dioestrus			
212	3.48	3.03	0.69
C 3	3.40	2.96	0.67
101	3.12	1.78	0.80
100	3.18	2.24	0.62
203	2.47	2.31	0.73
Mean	3.13	2.46	0.70
Phase 4b. Dioestrus			
B 4	3.63	1.33	0.80
121	3.70	2.10	0.70
132	3.30	1.80	0.69
133	3.87	1.36	0.88
117	3.55	2.10	0.88
213	4.54	1.95	0.80
202	5.00	1.60	1.04
Mean	3.94	1.75	0.83
Ovariectomized			
102	1.6	1.9	—
103	1.7	1.1	0.94
110	2.2	0.7	0.81
109	1.9	1.1	0.50
Mean	1.85	1.2	0.75

No attempt was made to study the effect of alterations in the composition of the Ringer's solution on the rate of O_2 consumption and aerobic glycolysis; all determinations were made on M. Dixon's solution, containing glucose. The rate of O_2 consumption varied with the phase in the oestrous cycle in a similar manner to the rate of aerobic glycolysis (Fig. 2, Table IV). As the differences due to the phase in the cycle were not so pronounced and individual variations were

large, partly on account of the greater errors involved in the experimental technique, considerable overlapping of values was found.

Aerobic glycolysis, on the other hand, was found to be low in pro-oestrus and oestrus, when O_2 consumption was high, and to rise to its highest rate on the first day of dioestrus, when O_2 consumption was at its lowest level.

In ovariectomized animals O_2 consumption and aerobic glycolysis were rather lower than in uteri from normal animals on the first day of dioestrus. Since the uteri were very small, the pressure changes were smaller and the accuracy of the results less than in experiments with normal animals.

DISCUSSION

The results are in general agreement with those of Khayyal & Scott [1934-35] for O_2 consumption of rat uterus and of Victor *et al.* [1936] for rat liver and of Victor & Andersen [1936] for rat anterior pituitary. Khayyal & Scott did not distinguish the pro-oestrous phase, neither did they divide the oestrous phase into two groups and their dioestrous phase was divided on a weight basis. They state that their dioestrous group II, large uteri, probably represents early dioestrus, and group I, small uteri, late dioestrus. The values given in Table IV for oestrus and early dioestrus are of the same order, though rather lower, than those they found, but the highest values found in these experiments, in pro-oestrus, do not approach those they found in late dioestrus. The same spreading of pro-oestrus values over the second day of dioestrus and the first day of oestrus was found as in the anaerobic experiments. Fewer high values were found on the second day of dioestrus, probably because the majority of the rats in this group had longer cycles (6-7 days) than those used for the anaerobic experiments. Khayyal & Scott's higher values may be accounted for by the difference in the composition of the Ringer's solution which they used (NaCl and KCl 0.9 and 0.042 g. per 100 ml. instead of 0.69 and 0.018 g. respectively, Na_2HPO_4 instead of $NaHCO_3$ and no glucose), or by a difference in the strain of rats.

The type of curve obtained for anaerobic glycolysis in glucose-free Ringer's solution may be explained by supposing that the tissue converts glucose into lactic acid at a much higher rate than glycogen. Kerr & Ghantus [1937] have found that in brain in anaerobic conditions glucose disappears much more rapidly than glycogen. If this is the case for uterus the high initial rate will be due to glycolysis of glucose and will decrease as the glucose contained in the tissue is exhausted and be succeeded by a slow rate which will remain constant while an excess of glycogen is available. Glucose and glycogen were estimated in a few uteri and were found to be of the order of 70 and 200 mg. per 100 g. wet weight respectively. If the dry weight of the tissue is taken as 17% and $Q_{C_6}^{N_2}$ as 5.5 (mean value for first day of dioestrus in glucose-Ringer), then the glucose present in the tissue would all be converted into lactic acid in 10 min. For the steady rate the mean value of $Q_{C_6}^{N_2}$ found was 0.9, at this rate it would take $2\frac{1}{2}$ hr. to convert all the tissue glycogen into lactic acid. As the experiments lasted 1-2 hr. no falling off in rate is to be expected, neither would added glycogen cause any increase in rate. The small increase found experimentally is hardly significant, and the results suggest that in uterus as in brain glucose is converted into lactic acid more rapidly than glycogen.

If the sugar usage in O_2 and in N_2 in glucose-Ringer solution, $Q_{C_6}^{O_2}$ and $Q_{C_6}^{N_2}$, is calculated as suggested by K. C. Dixon [1936] from the rates of O_2 consumption and of aerobic and anaerobic glycolyses, Table V, it is apparent that more sugar is used anaerobically than aerobically at all stages of the cycle, so that the

Table V. *Glucose utilization of rat uterus in glucose-bicarbonate Ringer's solution in O₂ and in N₂, expressed as μ l. gas per mg. dry weight per hr., $Q_{C_6}^{O_2} = (Q_{O_2}/6 - Q_{G_2}^{O_2}/2)$ and $Q_{C_6}^{N_2} = (-Q_{G_2}^{N_2}/2)$, respectively (calculated from mean values in Tables II and IV)*

Phase in cycle	$-Q_{C_6}^{O_2}$	$-Q_{C_6}^{N_2}$	R.Q.
1	1.1	4.8	0.97
2a	1.3	3.7	0.91
2b	1.3	1.8	0.82
4a	1.7	2.8	0.70
4b	1.5	4.1	0.79

Pasteur effect is always in operation. Whilst the cyclic changes in anaerobic glycolysis are reflected in similar cyclic changes in anaerobic sugar utilization, the differences in aerobic sugar usage are much smaller, and the maximum is found during dioestrus and not during pro-oestrus or oestrus. It has been pointed out that under the conditions of these experiments the determination of the R.Q. involves a considerable error, but in spite of the large variations recorded in Table IV it is seen that the high values all occur during oestrus and the low during dioestrus, from which it may be inferred that glucose is the main metabolite during oestrus, but that other substances are metabolized during dioestrus (under the conditions of these experiments). Therefore the assumption made in calculating $Q_{C_6}^{O_2}$, that all the O₂ consumed is utilized for oxidation of sugar, is only correct during oestrus, so that the figures for glucose usage during dioestrus will be too high. It seems probable, therefore, that glucose usage, in O₂, does not vary much throughout the oestrous cycle. It is interesting to speculate that the limiting factor in glucose usage may be the amount of glucose which can reach the cell, which K. C. Dixon & Holmes [1935] have suggested is itself limited in O₂ by the effect of O₂ in decreasing the permeability of the cell wall. If this is the case, then some other factor, varying with the oestrous cycle, controls the amount of glucose oxidized, the rest of that reaching the cell being converted into lactic acid. This second factor, controlling oxidation, is probably the same as that controlling anaerobic glycolysis, which varies with the phase in the cycle in the same manner as O₂ consumption.

It should be emphasized that the figures in Table V represent glucose usage per mg. dry weight of tissue, and that glucose usage of the whole uterus increases during oestrus owing to the large increase in dry weight at this stage of the cycle. The values in Table V have been calculated from mean values of experiments showing wide variations, and while the differences are large enough to show conclusively that the Pasteur effect is operative at all stages of the cycle, they can only suggest the constancy of sugar usage in O₂. It has been shown that anaerobically glucose consumption accounts quantitatively for lactic acid production, Table III, but more rigorous proof is required before it is proved that aerobically glucose utilization is constant throughout the oestrous cycle.

SUMMARY

1. Anaerobic glycolysis and O₂ consumption of isolated rat uterus are highest during pro-oestrus and lowest at the end of oestrus. The changes in respiration are not so pronounced as those in anaerobic glycolysis.

2. Aerobic glycolysis varies in the opposite sense and is lowest during pro-oestrus and highest immediately after oestrus.

3. Pro-oestrus values are found in some rats at the end of dioestrus and at the beginning of oestrus (as judged from vaginal smears).

4. When glucose is present in the Ringer's solution anaerobic glycolysis is constant, when no glucose is present it is lower and falls rapidly to a constant, very low, value.

5. Addition of glycogen or fructose to the Ringer's solution increases the rate only slightly and does not alter the type of curves obtained.

6. Addition of KH_2PO_4 , MgSO_4 and extra KCl to the Ringer's solution does not alter the rate of anaerobic glycolysis.

7. During anaerobic glycolysis, in the presence of glucose, glucose consumption is equal to lactic acid production, measured both manometrically and chemically.

8. The Pasteur effect is operative throughout the cycle, i.e. anaerobic sugar usage is always greater than aerobic.

9. Anaerobic sugar usage varies throughout the cycle, but aerobic sugar usage is approximately constant (both calculated from manometric results).

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