97. THE EFFECT OF OESTRONE ON THE METABOLISM OF RAT UTERUS¹

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It has been established that injection of oestrogenic hormone causes an increase in the rate of metabolism of certain tissues. This has been shown for uterus by several workers (for literature see Tesauro [1936]), for ovary by Kiyohara & Izawa [1936], for anterior pituitary and for liver by Victor & Andersen [1937], Andersen & Victor [1938]; the latter workers, as well as Kiyohara & Izawa, showed further that kidney tissue is unaffected by injection of hormone. Another effect of injecting such hormones is to produce rapid growth of the uterus. This growth can be measured by observing either the increase in dry weight [Astwood, 1938; Varangot, 1939] or the number of cell divisions after injection of colchicine [Allen *et al.* 1937]. Both methods show that growth can first be observed 6-9 hr. after injection, and that it is maximal after 36 hr. The results to be reported here show that the changes in metabolic rate closely follow these growth changes, the first increase occurring from 6 to 12 hr. after injection, whilst the maximal rate is reached after 24-36 hr. During the subsequent regression period metabolic rate is depressed, the original rate being regained 96 hr. after injection, when the weight has returned to the pre-injection value [Varangot, 1939].

Methods

Rats aged about 4 months were used for experiment from 17 to 43 days after bilateral oöphorectomy. No influence of the time after operation on the results was observed. For two series of experiments younger rats were used, in one oöphorectomy was performed at 6 weeks, hypophysectomy 1 week later and the rats were used after the lapse of a further 5 days; the second series, which served as controls for the hypophysectomized rats, were oöphorectomized only at the age of 6 weeks.

Isolated uteri, in a glucose-Ringer medium, were used in all cases, and the aerobic and anaerobic metabolic rates measured by the methods previously described [Kerly, 1937].

Crystalline oestrone was used for all injections; both it and the oestriol used in some of the *in vitro* experiments were prepared by Prof. Marrian. A stock solution of oestrone in 10% alcohol was prepared and diluted with 10 vol. of water immediately before use; 5 subcutaneous injections of 0.5 ml. (= 0.5 μ g. oestrone) were given at 0, 6, 22, 26 and 30 hr. [see Voss, 1936]. The activity of all preparations was checked by testing the vaginal smears; cornified cells were observed in some cases 48 hr. after, and in all cases 72 hr. after, the first injection.

Anaerobiosis

The rates of anaerobic glycolysis of uteri from uninjected rats showed great variation (Fig. 1), ranging from the high values typical of uteri from intact rats in pro-oestrus to the low values of di-oestrus. The few values previously

¹ Some of the material contained in this paper was reported at a meeting of the Biochemical Society in March 1938.

reported for oöphorectomized rats [Kerly, 1937] chanced to come from animals showing low values. Zuckerman [1938] has suggested that the accessory reproductive organs of spayed female animals undergo cyclic changes due to the influence of some extra-ovarian factor. This would explain the large variation in metabolic rates of uteri from oöphorectomized rats, where there is no longer any change in the vaginal smear and therefore no means of judging the stage of the uterine cycle.

Fig. 1. The effect of injection of oestrone on the anaerobic glycolysis of isolated uterus. • Oöphorectomized rats, aged approximately 4 months. ○ Oöphorectomized rats, aged approximately 2 months. x Hypophysectomized and oöphorectomized rats, aged approximately 2 months. Values plotted at 0 hr. are those of uteri from uninjected rats.

After injection of oestrone some rise in the rate of anaerobic glycolysis was observed 6 and 12 hr. after the first injection (Fig. 1), but as the rate before injection varied over such a wide range it was difficult to be certain of small increases. After 24 hr. there was a large increase and after 36 hr., when the maximal rate had been reached, the rate was almost double that of uteri from uninjected rats. At this time there was no change in the vaginal smear. After 48 hr., when both cornified and epithelial cells were usually present, the glycolytic rate had fallen; this fall continued until, after 72 hr., the rate was somewhat lower than for uninjected rats. 24 hr. later (96 hr. after the first injection) it had risen again to the original value. Injection of a large single dose of oestrone did not produce an earlier increase in rate; 6 hr. after injection of 5 and 25 μ g. oestrone the values found for Q_G^N were 9.6 and 9.1 respectively. The high rate of anaerobic glycolysis induced by injection of oestrone was much greater than the pro-oestrus values of the normal cycle (mean Q_G^N 9.7 [Kerly, 1937]), but there was a similar time interval (36 hr.) between the highest and lowest rates observed after injection and the high pro-oestrus and the low late oestrus values (mean Q_G^N 3.5) found in the intact rat.

If injections were continued beyond the standard 30 hr. period (2 injections daily of 0.5 μ g. oestrone) the very high 36 hr. values were not maintained (Table 1). 72 hr. after the initial injection the glycolytic rate had fallen below

Table 1. *Anaerobic glycolysis of isolated uterus after prolonged injection of oestrone*

0.5 μ g. oestrone injected at the following times: 0, 6, 22, 26, 30, 46, 54, 70, 78 and 96 hr. Times for $Q_G^{N_2}$ reckoned from the first injection.

$Q_G^{N_2}$ at		
36	72	100 hr.
15.1	9.3	10.4
16.2	8.7	11.0
13.7	—	10.7
14.7	—	—
14.6	—	—

the 36 hr. value, but not to so great an extent as in those rats in which injections ceased at 30 hr.; after 100 hr. the rate had risen again to an intermediate value. The cyclic character of the response to the initial injections appears to be maintained in spite of further injection of hormone. As the time after injection increased there was less variation of rate between uteri from different rats, which would be expected if an artificial cycle had been superimposed on animals initially at different phases of their natural cycles.

Aerobiosis

The rates of aerobic metabolism reported here are considerably higher than those recorded previously both for intact and oöphorectomized rats [Kerly, 1937]. This is due to the fact that an error was made in calculating the earlier values,

Table 2. *Aerobic metabolism of isolated uterus*

	$-Q_{O_2}$	$Q_G^{N_2}$
(a) Normal rats:*		
1. Pro-oestrus	9.0	1.2
2a. Early oestrus	9.0	2.1
2b. Late oestrus	6.4	2.8
3. Met-oestrus	7.8	3.8
4a. Early di-oestrus	6.2	4.9
4b. Late di-oestrus	7.9	3.5
(b) Oöphorectomized rats	3.7*	3.3*
	1.2	1.4
	4.5	1.7
	8.5	3.2
	3.3	2.8
(c) Oöphorectomized rats, injected with oestrone:		
24 hr. after injection	3.3	3.3
	5.2	1.5
36 hr. after injection	7.6	4.7
	8.0	2.8
	9.0	2.6
	10.0	5.8
48 hr. after injection	9.2	3.1
72 hr. after injection	6.2	4.0
	6.9	3.6
	7.4	3.9
	6.1	2.4
	5.2	3.5
96 hr. after injection	5.4	3.5
	6.2	3.5
	6.8	5.2

* Mean values, corrected, of results previously reported [Kerly, 1937].

which require to be multiplied by 2. The mean values given in Table 4 of the former paper have been recalculated and the corrected values are given here in Table 2. The conclusions previously reached are not affected and the corrected results for rate of O_2 consumption for uteri from intact rats at different phases in the reproductive cycle are now in good agreement with those of Khayyal & Scott [1934-5].

As in the anaerobic experiments, large variations in rate of both O_2 consumption and aerobic glycolysis were found in uteri from uninjected rats. Increases in O_2 consumption after injection of oestrone (Table 2) were not so striking as the increases in anaerobic glycolysis, but a significant increase was found 36 hr. after the first injection; after 72 hr. the rate had returned to, but had not fallen below, the pre-injection level. The rate of aerobic glycolysis varied so much, and with the small amount of tissue available the individual values were not sufficiently accurate, to be certain of any changes of rate consequent upon injection of oestrone.

Hypophysectomized rats

Victor & Andersen [1937] found that theelin injected into oöphorectomized rats causes a transitory rise in O_2 consumption of the anterior pituitary, lasting less than 12 hr., whilst its effect in increasing the metabolism of the liver is apparent much later, 72 hr. after injection [Andersen & Victor, 1938]. They were able to show [Victor & Andersen, 1938] that the effect on the liver is indirect, due to the action of the thyroid, itself stimulated by the increased activity of the anterior pituitary. In order to determine whether the effect of oestrone on the uterus was also indirect and due to stimulation of the anterior pituitary, in one series of animals the hypophysis was removed as well as the ovaries. Anaerobic glycolysis was measured rather than O_2 consumption, since the effect of injection was so much more pronounced on anaerobic metabolism. Increases in glycolytic rate occurred at the same time intervals after injection as in those rats which had been only oöphorectomized (Fig. 1). The increases observed were rather greater than in the main series of experiments, probably because younger rats were used; a control series of rats of the same age, which had had only their ovaries removed, gave similar values (Fig. 1).

In vitro action of oestrogenic hormones

Victor & Andersen [1937] were able to show that theelin has a direct *in vitro* action on the O_2 consumption of anterior pituitary tissue from oöphorectomized rats, but they found that it had no effect on liver slices, results in accord with their demonstration of the secondary effect of theelin in raising the metabolic rate of the liver. No increase in anaerobic glycolysis was observed during a 2-3 hr. experimental period when oestrone or oestriol was added to isolated uteri from either normal or oöphorectomized rats (Table 3). One horn of the uterus was used as a control, and hormone dissolved in various solvents was added to the Ringer solution surrounding the other horn, an equal amount of solvent being added to the control horn. The very high values found in several experiments must be attributed to the action of the solvent and not to that of the hormone. It is possible that uterine tissue is less permeable than anterior pituitary and that in these experiments no hormone penetrated to the active tissue. On the other hand the failure to show an increase in glycolytic rate may be due to the fact that, although oestrone would appear to act directly on the uterus, and not indirectly through its action on the hypophysis, the increased metabolic rate following its injection is correlated with the uterine growth which it induces and which may occur only in the intact animal.

Table 3. *Effect of adding oestrone or oestriol in vitro on anaerobic glycolysis of isolated uterus*

	$Q_G^{N_2}$	
	Control horn	Experimental horn
1. 0.1 μ g. oestrone (dissolved in 0.1 ml. 1 % alcohol) added to 3 ml. Ringer	5.5	5.2
2. 2.0 μ g. oestrone (dissolved in 0.2 ml. 10 % alcohol) added to 3 ml. Ringer	14.8	18.3
3. Uterus soaked 6 hr. in oxygenated Ringer containing 2.0 μ g. oestrone (dissolved in 0.2 ml. 10 % alcohol) transferred to fresh Ringer for measurement of glycolysis	14.4	13.1
4. As no. 3, but oestrone dissolved in sesame oil	16.3	16.1
5. 25 μ g. oestrone dissolved in 2N NaOH, neutralized with HCl and made to 3 ml. with Ringer (Victor & Andersen's method)	11.0	9.5
6. 25 μ g. oestrone dissolved in 2N NaOH, neutralized with HCl, diluted with Ringer and placed in the side arm of the flask; total vol. 3 ml. Control = $Q_G^{N_2}$ before addition of oestrone, experimental = $Q_G^{N_2}$ after tipping in hormone, 1 hr. after beginning of anaerobiosis	11.0	8.3
7. As no. 5, but 25 μ g. oestriol used instead of oestrone	5.4	3.2

SUMMARY

1. Anaerobic glycolysis of uteri from oöphorectomized rats showed great variation in rate, ranging from the high values typical of pro-oestrus to the low values found in late di-oestrus in intact rats.

2. Following injection of oestrone an increase in the rate of anaerobic glycolysis was observed after 6–12 hr., but the maximal rate was not reached until 24–36 hr. after injection, at the same time as maximal growth is known to occur. After 72 hr. the rate had fallen below, but after 96 hr. it had returned to, the pre-injection level; at this time the weight is known to return to the original value.

3. If injections were continued for 5 days the glycolytic rate was not maintained at the maximal level.

4. The effect of injection on the O_2 consumption was not so pronounced as on the rate of anaerobic glycolysis, but 36 hr. after injection the rate of O_2 consumption had increased.

5. Hypophysectomy did not affect the action of oestrone in raising the rate of anaerobic glycolysis.

6. Neither oestrone nor oestriol had any *in vitro* action on the rate of anaerobic glycolysis of isolated uterus.

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