THE OXYGEN CONSUMPTION OF THE UTERUS, AND THE ACTION OF OESTRIN THEREON.

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THE OXYGEN CONSUMPTION OF THE UTERUS, AND THE ACTION OF OESTRIN THEREON.

Part I.

1. Literature.

The oxygen usage of plain muscle has been studied by various workers. Lovatt Evans (1) estimated the oxygen consumption of the rabbit's and guinea pig's uterus and found that the oxygen use varied from 0.22 c.c. to 0.31 c.c. per gram per hour for rabbit's uterus. An important factor in determining the oxygen usage, he thought, was the actual length of the plain muscle fibres, the consumption decreasing with increased tonus of the muscle caused by histamine and other stimulants. In a later paper (2) he gives the figures for the oxygen usage of the uterus in the relaxed state to be 0.46 c.c. per gram per hour, while for tonic state it was 0.39 c.c. There is therefore distinctly less oxygen used in the tonic state than during the state of relaxation. He recognised, however, that this was probably because, owing to the thickening of the tissue on contraction it was able to get oxygen less readily than when it was in the relaxed state. His experiments proved that there can be no definite increase of metabolism during the upkeep of tonus. The guinea pig's uterus which used 0.11 c.c. per gram per hour under conditions of normal oxygen supply, used 0.21 c.c. per gram per hour in the early recovery from a two hour period of anoxybiosis.

As regards other plain muscle, Brodie and Vogt (3) calculated the oxygen consumption of small intestine, muscle and mucosa, as varying from 0.336 c.c. to 1.878 c.c. per gram per hour with a mean of 1.074 c.c. Fenn (4) observed the oxygen use of small intestine of the frog to be from 0.24 to 0.3 c.c. per gram per hour and that of its stomach as from 0.156 c.c. to 0.223 c.c. per gram per hour. Tsang-gi-Ni and Lim (5) vivi-perfused the stomach of a dog and calculated that the quiescent vivi-perfused stomach consumes oxygen at the rate of about/

about 0.42 c.c. per gram per hour. Lovatt Evans (1) found that different portions of the cat's bowels consumed oxygen at different rates, the figure for duodenum being 0.45 c.c. per gram per hour, the middle of the jejunum 0.31 c.c. and the lower ileum 0.288 c.c. per gram per hour, thus confirming the observation of Alvarez and Starkweather (6) of the existence of a 'metabolic gradient' from the duodenum to the colon.

It may be interesting to note here the oxygen consumption of another type of involuntary muscle, that of the heart. Clark and White (7) found that the resting metabolism of cardiac tissue is much higher than that of skeletal muscle, their figures being in c.c. of oxygen per gram per hour:-

Frog: auricle - 0.4-0.8.
ventricle- 0.5-1.0.
Tortoise: auricle - 0.1-0.2.
ventricle 0.3-0.6.

They also estimated that the oxygen consumption of the auricle increases with increase of diastolic volume and that the maximum oxygen consumption of the extended auricle during activity may be five times that of the empty auricle.

The/

The experimental procedures adopted by these investigators were various. Evans (1) used a modification of Winterstein's micro-respirometer as well as a Ringer bath containing oxygenated Ringer's solution in which the uterus was suspended; the fluid was periodically drawn off and its oxygen content calculated analytically. Fenn (4) estimated the oxygen intake and carbon dioxide output by the conductivity method. Clark and White (7) used a specially designed bulb attached to a Barcroft's manometer and suspended the frog's auricle inside the bulb at the end of a suitable cannula in an atmosphere of oxygen. The carbon dioxide given off was absorbed by a roll of filter paper soaked in a 5 per cent. solution of NaOH kept in a receptacle communicating with the bulb. Howard and Sollmann (8) in their work on the metabolic gradient of non-rhythmic strips from different levels of the turtle's heart used a modified Osterhout micro-calorimeter in a closed system in which the contained gases are made to circulate continuously and in such manner that the CO_2 given off by the respiring tissue is led through a M/10,000 $NaHCO_3$ solution appropriately colored with/

with an indicator. The rate at which CO_2 is produced was found by the time required to change the pH of $NaHCO_3$ buffer solution from pH 7.78 to pH 7.60. Dixon and Elliot (9), working on the action of cyanide on the oxygen usage of animal tissues, used differential Barcroft micro-respirators, into the right-hand flasks of which the tissue was placed in cyanide solution with 2 c.c. of phosphate buffer. They found no difference in action in using Ringer's solution for phosphate buffer. CO_2 was absorbed by filter paper soaked in 6 per cent. KOH rolled round the inside of the leading tubes. Raab (10) who estimated the metabolism of scrapings of human endometrium, however, found that a piece of tissue left in Ringer's solution for about six hours showed far reaching destruction of cell structures, whereas in serum, no such degeneration occurred.

2. Method.

For my study of the oxygen consumption of the uterus, I adopted the method of Clark and White(7), also used by me in determining the action of drugs on the oxygen consumption of the frog's isolated auricle/

auricle (11). The oxygen usage is determined by means of a Barcroft's manometer with a three-way stopcock on either side. Glass bulbs similar to the diagram in Fig. 1 were fixed to the manometer on either side, by means of a small piece of pressure tubing. The tissue was tied to loops at either end of a thin glass rod about 4-5 cm. long and stretched between them as shown in the figure (Fig. 1, a and b) and suspended inside the bulb on one side, usually on the right. A roll of filter paper soaked in a 5 per cent. solution of NaOH (f) was placed in the side tube. The bulb on the left side of the manometer acted as control. The bulbs were filled with oxygen at atmospheric pressure and the rubber corks, with a small pledget of cotton wool soaked in Ringer's solution sitting on the top of each of them, were then inserted into the bottom opening of the bulbs, which were then immersed into a water-bath kept at a constant temperature of 37°C. by means of a suitable thermostatic arrangement. In order to obtain a uniform temperature throughout the bath, compressed air was rapidly bubbled through the water all the time. The stopcocks were kept open to the

atmosphere/

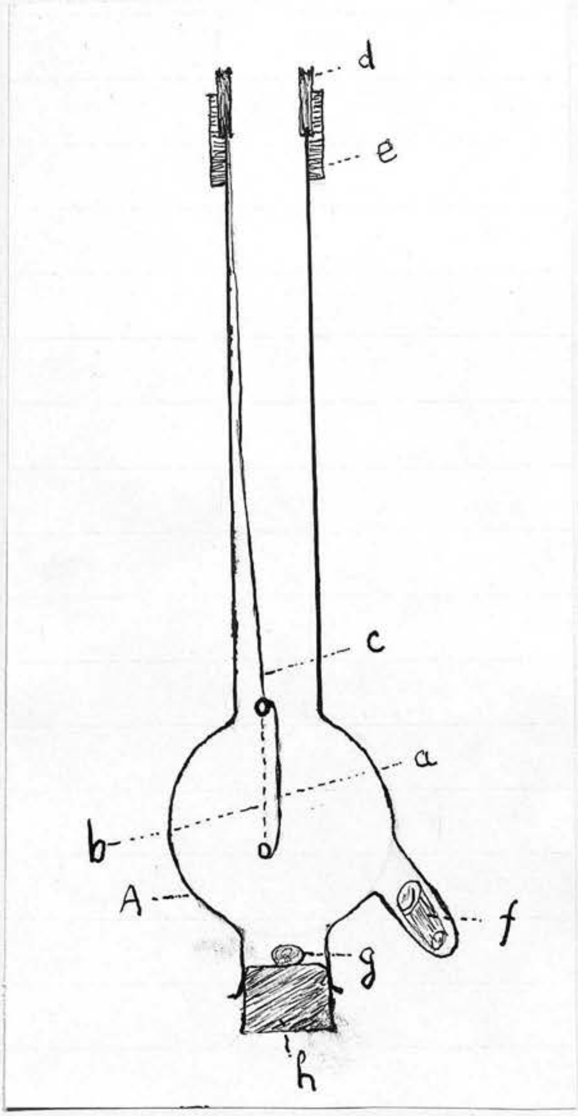

Fig. 1.

A. Bulb for measuring oxygen consumption.

a. Thin glass rod looped at either end; b. Uterine tissue; c. Thread supporting glass rod; d. Vertical tube of Barcroft's manometer; e. Rubber tubing; f. Roll of filter paper soaked in 5% NaOH; g. Pledget of moist cotton wool; h. Indiarubber cork.

atmosphere until a uniform temperature was secured inside the bulbs. This usually took about 10-20 minutes. They were then closed simultaneously, thus communicating the manometer with the bulbs only. This procedure involved the loss by diffusion of a certain proportion of the oxygen, and hence the term "filled with oxygen" does not imply more than a relatively rich oxygen mixture.

The first reading was taken when the level of the fluid in the manometer (kerosine) became steady, and thereafter readings were taken at regular intervals, say, every 15 minutes. It may be mentioned here that such readings have been found to be remarkably regular in the case of the uterus. Several such apparatuses could be placed in the bath together. Readings were taken for a period of 2-4 hours. The exact duration depended on the regularity of the readings. The uterine tissue was weighed at the end of the experiment.

Each apparatus was calibrated by means of a pipette containing mercury. This constant varied, in the case of the bulbs used from 2.5 to 3. That is to say, one millimetre reading on the manometer scale indicated 2.5 to 3 cubic millimetres of oxygen consumed.

The figures obtained for oxygen consumption of the uterus were, in every case, corrected to normal/

normal temperature and pressure using the following formula:-

$$Vo = V \frac{b_o - C}{760(1 + 0.003670.t)}$$, where Vo is the corrected volume, V the uncorrected figure, C the millimetres of mercury of water vapour pressure at 37°C, which is 47, t the temperature and b_o the observed barometric pressure in millimetres. To simplify calculations, the value of the formula

$$\frac{b_o - C}{760(1 + 0.003670.t)}$$ when t was 37°C.,

was worked out for each barometric reading from 730 to 760 mm. This figure varies between 0.7914 and 0.8262. To obtain the corrected result, the uncorrected volume was multiplied by the factor for the barometric pressure observed at the time.

Pieces of uterus from rabbits, guinea pigs, rats and mice were used in my experiments. The animal was killed by a blow on the neck and the uterus was dissected out and put into a dish containing Ringer's solution of the following composition:-

NaCl, 18 per cent.	50 c.c.
KCl , 4.2 " "	10 c.c.
$CaCl_2$,2.4 " "	10 c.c.
Na_2HPO_4 , 2.0 "	25 c.c.
Water to	1000 c.c.

An/

An adequate portion of the tissue devoid of adherent connective tissue was cut out and suspended in the Barcroft's bulb as already described. The adherent Ringer's fluid as well as the moist pledget of cotton wool placed on the top of the cork sufficed to prevent the tissue from drying during the course of the experiment.

3. Experimental Results.

I measured the oxygen consumption of the uteri of various animals in order to find a tissue suitable for investigation. The chief difficulty was that the oxygen supply to the tissue depended on diffusion and hence it was necessary to find a tissue sufficiently thin for it to obtain an adequate oxygen supply by diffusion. Warburg (12) has given a formula relating the thickness of tissue with the oxygen that can be supplied to it by diffusion. He used tissues immersed in serum or Ringer's solution. Clark and White (7) found that this formula was approximately correct for the frog's auricle suspended in oxygen or air. They also found that strips of cardiac tissue, more than 0.02 cm. thick, do not receive an adequate oxygen supply when suspended in air and that the oxygen/

oxygen consumption of the filled auricle is nearly equal in air and in oxygen. Warburg's formula is as follows:-

$d = \sqrt{8.c.D/A}$ where d is the thickness in cm. of a section exposed on both sides that will receive an adequate oxygen supply by diffusion, A is the observed oxygen consumption of the tissue in c.c. per gram per minute, c is the concentration of oxygen in atmospheres, and D is Krogh's diffusion coefficient for oxygen. This last figure is 1.4×10^{-5} for muscle at 20°C., and since it rises 1 per cent. per 1°C. rise of temperature, it is about 1.64×10^{-5} for 37°C. It is, therefore, necessary to apply the above formula in order to find out if the observed consumption of oxygen is adequate to the needs of the tissue or whether any part of the tissue is being starved for want of proper diffusion. This would, incidentally, be also a check on the adequacy of the proposed method.

(a) Oxygen consumption of rabbit's uterus.

Twelve experiments were done with portions of rabbit's uterus. The average oxygen consumption per gram per hour at N.T.P. was found to be 0.564 c.c. in oxygen and 0.341 c.c. in air (vide Table I).

A few experiments done on strips of guinea pig's uterus gave the average figure of 0.533 c.c. per gram wet weight per hour in oxygen.

Table I.

The oxygen usage of rabbit's uterus.

No.	Moist weight in grams	Oxygen use per gram per hour at N.T.P.	
		In oxygen c.c.	In air c.c.
1	0.06	0.666	
2	0.321	0.644	
3	0.67	0.299	
4	0.33	0.631	
5	0.363	0.573	
6	0.283	0.495	
7	0.256	0.640	
8	0.246	0.368	0.324
9	0.352	0.453	
10	0.223	0.609	0.358
11	0.15	0.746	0.341
12	0.258	0.619	
	Average	0.564	

Table/

Table I shows that the intake of oxygen when only air is supplied is on the average about three-fifths of that observed when the tissue is suspended in oxygen; it may also fall as low as one half. This indicates that the oxygen supplied by diffusion is inadequate when the uterus is suspended in air, and that it may be inadequate even when the uterus is suspended in oxygen. The thickness of tissue which can be supplied with adequate oxygen by diffusion can be calculated from Warburg's formula. The actual thickness of the tissue can be calculated from the formula $l \times b \times d = V$, where $\underline{l}$ is the length, $\underline{b}$ the breadth, $\underline{d}$ the depth and V is the volume. The length and breadth can easily be measured and the figure for the weight of the tissue gives the volume if we take its specific gravity as unity, which is sufficiently accurate for practical purposes. The following table shows the values obtained for the thickness of the tissue, from typical experiments.

Table II /

Table II.

Rabbit's Uterus.

No.	Moist wt. of uterine strip in grams	Length x breadth of strip in c.c. m	O_2 use per gram per minute in		Calculated thickness in cm.	Maximum thickness of tissue in cm. which, according to Warburg's formula, receives an adequate oxygen supply when suspended in	
			oxygen c.c.	air c.c.		air	oxygen
6	0.283	1.3 x 1.3	0.008		0.168		0.126
7	0.256	1.1 x 1.3	0.011		0.197		0.111
10	0.223	2.5 x 0.7	0.010	0.007	0.128	0.061	0.114
11	0.150	2.0 x 0.5	0.012	0.006	0.150	0.068	0.103
12	0.258	1.4 x 1.0	0.0103		0.184		0.113

These results indicate that a strip of rabbit's uterus, even when suspended in oxygen can receive adequate oxygenation only through about seven-tenths of its thickness. When suspended in air, the tissue cannot receive sufficient oxygen even through half its thickness. Evans (2) found that the rabbit's uterus consumed 0.22 to 0.31 c.c. oxygen per hour. These values are considerably lower/

lower than those that I obtained, and it appears that he also failed to ensure proper diffusion of oxygen through the whole depth of the tissue. These results indicated that the rabbit's uterus was an unsuitable material on which to estimate the oxygen consumption.

(b) The oxygen usage of rat's uterus.

A few experiments were conducted with rats' uteri, but could not be continued owing to the difficulty of getting an adequate supply of virgin rats at the time. The following is a record of the experiments:-

Oxygen use of rat's uterus. Table III.

No.	Moist weight in grams	Oxygen use per gram per hour at N.T.P. when suspended in oxygen.
1	0.153	0.758 c.c.
2	0.133	1.056 c.c.
3	0.038	1.536 c.c.
4	0.096	0.60 c.c.
5	0.058	1.08 c.c.

The mean figure is about 1.006 c.c. per gram per hour.

With reference to the calculation of the limits/

limits of oxygen diffusion by Warburg's formula, in the case of the rats' uteri, it may be pointed out that the cylinder is a more favourable shape than a sheet of muscle.

For example, supposing that in a sheet of muscle the calculated limit of thickness for oxygen consumption is 30 per cent. less than the observed thickness, this will mean that 30 per cent. of the muscle mass is getting an inadequate oxygen supply and the consequent reduction in oxygen use may be anything up to 30 per cent. ~~of~~ less than the true value.

On the other hand, in the case of a cylinder with a radius of one unit, if the radius of a cylinder that gets a true oxygen supply is 0.7, then the mass of tissue that is below the depth at which an adequate oxygen supply can be obtained is about $1 \times \pi(1.0 - 0.7)^2 = 1 \times \pi \times 0.09$, which is $\frac{100 \times 1 \times \pi \times 0.09}{1 \times \pi \times 1} = 9$ per cent. Hence the experimental error produced by a cylinder of tissue being too thick to allow adequate diffusion to its centre is not very great.

Hill (13) has provided a formula for calculating the "critical" radius or diameter of a cylindrical solid using up oxygen by metabolic process at/

at a constant rate, the oxygen being diffused from a gaseous or liquid phase in which its concentration is maintained constant. This formula is as follows:- $r_o = \sqrt{\frac{4 KYo}{a}}$, where r_o is the radius of the cylinder, K is Krogh's constant, Yo the concentration of oxygen in atmosphere~~s~~, and a is the oxygen usage observed, per gram per minute.

Table IV.

Rat's Uterus.

State of uterus	Length in cm.	Weight in grams	Oxygen use per Gm. per min. in c.c.	Calculated radius of uterus in cm.	Maximum radius of cylinder that will receive adequate oxygen supply according to Hill's formula in cm.
Weighted	4.0	0.133	0.017	0.100	0.06
No wt.	2.0	0.133	0.010	0.141	0.08
Weighted	3.0	0.038	0.256	0.063	0.05
No wt.	2.0	0.038	0.189	0.077	0.06

Table IV shows the relation between the actual radius of the "uterine cylinder" and the "critical radius" which would according to Hill's formula/

formula receive an adequate diffusion of oxygen. The actual radius was calculated from the formula $\pi r^2 \times l$ = volume and where the length and volume are known the radius can be easily deduced.

The figures given in Table IV show that when the rat's uterus is stretched its radius is slightly greater than the radius of a cylinder that would receive an adequate oxygen supply. When the rat's uterus is allowed to contract the oxygen consumption falls and the ratio between the observed radius and the critical radius is nearly the same as that in the stretched uterus.

That this result is to be expected will be seen from the following considerations. In the case of the stretched uterus, probably about 90 per cent. receives an adequate oxygen supply and the figure for the oxygen consumption is about 10 per cent. too low and in consequence the critical radius calculated is somewhat too high. When the uterus contracts, the thickness is increased from 20 to 40 per cent.; hence the amount of unoxygenated tissue in the centre is increased, and the observed oxygen consumption is much below the true value and in consequence the critical radius calculated is considerably greater than its true value.

For/

For these reasons the effect of contraction on the oxygen consumption of the uterus cannot be calculated unless it is quite certain that the oxygen diffusion is adequate both in the relaxed and in the contracted state. This obviously is not the case with the rat's uterus, and my results support the conclusion of Evans (2) that by far the most probable reason for the diminution of oxygen use in the contracted uterus is deficient diffusion of oxygen through the thickened tissue.

The results indicate that the rat's uterus is too thick to permit accurate estimation of its oxygen consumption.

(c) Experiments with Mouse's Uterus.

The oxygen consumption of the uteri of immature mice or adult mice in dioestrus varies between 1 and 2 c.c. per gram per hour with a mean of about 1.70 c.c.

Table V shows the oxygen consumption of uteri suspended in air and in oxygen.

Table/

Table V.

Mouse's uterus (dioestrus).

No.	Moist wt. in grams	Length in cm.	Oxygen use per gram per min. in		Calculated radius.	Radius which according to Hill's formula is adequately supplied with oxygen when suspended in	
			air	oxygen		air cm.	oxygen cm.
1	0.022	2.0	0.009	0.019	0.054	0.024	0.058
2	0.030	2.0	0.009	0.012	0.069	0.025	0.074
3	0.020	2.0	0.014	0.019	0.056	0.032	0.058
4	0.012	2.0	0.025	0.036	0.043	0.022	0.042
Average			0.014	0.021	0.055	0.026	0.058

The calculations in Table V show that the uterus when suspended in oxygen, should receive an adequate supply of oxygen because the critical radius is slightly less than the actual radius of the tissue. The calculations also show that the uterus is considerably too thick to receive an adequate supply of oxygen when suspended in air. This conclusion is confirmed by the fact that the oxygen consumption in air is less than that in oxygen. The average figures for the two conditions are 0.014 and 0.021, a ratio of 1 to 1.5. The diffusion in air and/

and in oxygen respectively would be as $\sqrt{1}$ to $\sqrt{5}$, i.e. as 1 to 2.2. The fact that the difference between the observed figures is considerably less than this supports the view that the diffusion in oxygen is more than adequate for the oxygen needs of the uterus.

Test of Accuracy of Method. In order to test the accuracy of my method a few experiments were done to ascertain if the two horns of the same uterus consumed equal volumes of oxygen, weight for weight. If this were so, tests done on the two horns would also serve as an excellent check on experimental errors. Table VI is typical of the results.

Table VI.

Oxygen use of two horns of the same uterus of the mouse.

Serial No.	Moist weight in grams	Oxygen use per gram per hour at N.T.P. in c.c.
9	0.023 0.021	(a) 2.084 (b) 2.256
13	0.023 0.023	(a) 2.182 (b) 2.028
14	0.023 0.025	(a) 2.400 (b) 2.056
33	0.032 0.032	(a) 1.424 (b) 1.796

Excepting in one case the differences are well within experimental errors. This observation is interesting as a great deal of individual variation/

variation is found to occur in the oxygen use of the mouse's uterus.

(d) The influence of temperature on the oxygen consumption of the uterus.

Lovatt Evans (1) found that oxygen usage increased first, slowly, then more rapidly, then again slowly, until a maximum was reached at about 43°C., beyond which the value quickly fell off. The phenomenon of heat paralysis occurs at a temperature of 49°C. Fig. 2 illustrates an experiment done to elucidate this.

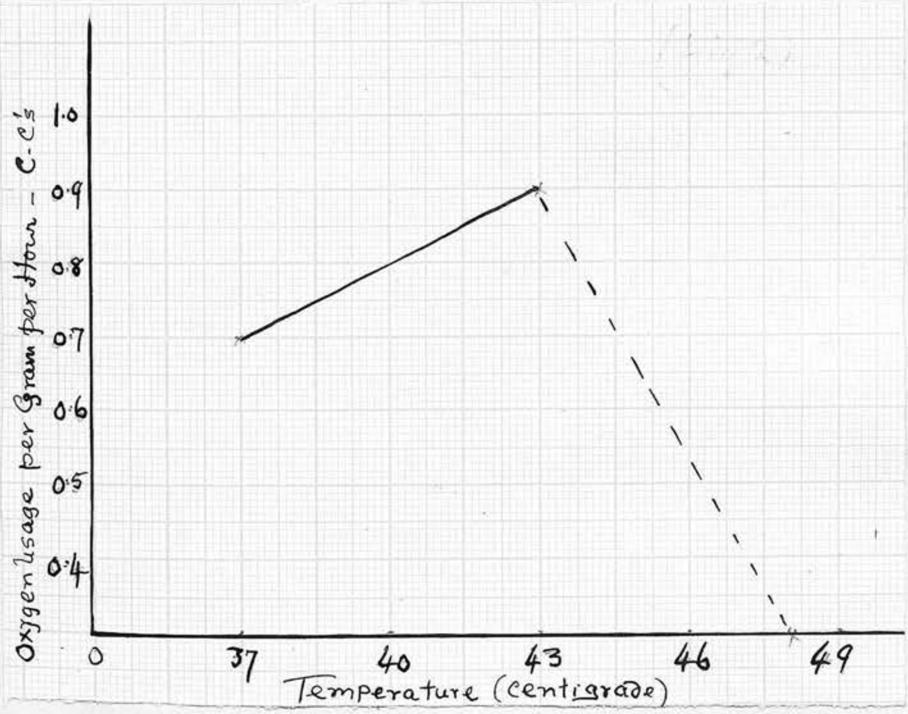

Fig. 2.

There is a rise in metabolism from 37°C. up to about 43°C, beyond which there is a falling off, heat paralysis setting in at about 48°C., the respiration completely stopping at this level.

Part II.

Influence of Oestrous Cycle on Oxygen Use of the Mouse's Uterus.

1. Literature.

Previous workers have attempted to find out the influence of the oestrous cycle and of oestrin itself on the basal metabolism, but there is no mention in literature of any attempt to study the influence, if any, of the oestrous cycle on the metabolism of the uterus itself. Lacqueur, Hart and De Jongh (14) made accurate gasometric measurements of the oxygen and carbon dioxide consumption of six mice simultaneously. It was found that an increase of basal metabolism resulted from injection of female sex hormone into female castrates. On the other hand, Geist, Goldberger, Reiss and Lande (1926) (15), made a study of the basal/

basal metabolism, body weight and blood chemistry following bilateral oöphorectomy and found no variation of the basal metabolism and body weight, nor did they find any change in the blood chemistry or blood picture. Lee (1927) (16) found an increase of about 12 per cent. in heat production in the rat during the last ten hours of the dioestrum and during the beginning of the pro-oestrum. During the period of oestrus the average heat production was below the general mean. Lee interpreted this as indicating that the oestrus-producing hormone of the ovary was not primarily a metabolic stimulant. Fraser and Wiesner (1929) (17) found an apparent slight rise in metabolism following injections of follicular fluid and extracts, but the behaviour of the experimental animals was not consistent, nor were the increases in carbon dioxide output very great. In the normal rat, they found a definite increase in CO_2 output after the stage of full cornification, there being a rise in metabolism anywhere from 12 to 48 hours after the time when the smear showed full cornification, with a mode of about 12 hours. Loewy and Richter (18) found diminution of 10 to 20 per cent. in the oxygen consumption and evolution of CO_2 /

CO_2 of a castrated dog and bitch while at rest. They fed the animals upon ovarian substance. Normal animals did not react at all; a castrated bitch, on the other hand, showed a greatly increased metabolism, 30 to 50 per cent. above the normal values observed before the operation. When the glandular tissue was withheld the consumption of oxygen sank slowly to normal. Lüthje(19) on the other hand was unable to confirm these results.

From the above cited literature, it is obvious that while no information is available as to the effect of oestrin on the uterine metabolism, its effect on the body metabolism is obscured by a cloud of contradictory experimental results.

2. Methods.

I measured the oxygen consumption of uteri taken from the following classes of mice:

(a) Adult intact mice in dioestrus.
(b) Adult intact mice in oestrus.
(c) Immature intact mice.
(d) Adult castrated mice.

I also determined the effect produced by injections of oestrin on the oxygen consumption of uteri from classes (c) and (d).

The/

The sexual cycle was diagnosed by the vaginal smear technique of Long and Evans (20). I used a small platinum loop for obtaining the vaginal secretion. Coward and Burn (21) used a spatula and examined the unstained smear under the low power objective. Frank (22) advocates the use of a capillary pipette and stains the film with a simple stain. While I found the former method quite a convenient and rapid way of diagnosing dioestrus, I also found it advisable to confirm the diagnosis of oestrus by staining the smear with methylene blue in order to make sure that it contained only cornified cells.

Firstly, I measured the oxygen consumption of the dioestrous uterus. For this purpose I employed immature and adult mice. Owing to the reconstruction of buildings, it was found impossible to maintain my own stock of animals; and hence the mice were all obtained from one dealer.

The immature mice were between three and four weeks old and weighed between 9 and 13 grams, the average weight being about 10 grams. There is a certainty of such mice being immature, for Kirkham (23) states that "males and females are both sexually mature when about six weeks old". And Parkes/

Parkes (24) records that the vaginal orifice is established at the end of the seventh week. Also Engle and Rosasco (25) found that the vaginal introitus could be recognised between the 28th and 49th day of life. Lastly, Mirskaia and Crew (26) state that the earliest first oestrus was on the 28th day of life and the latest on the 75th. No attempt was made to take a vaginal smear in the case of immature mice as the vagina was unopened.

3. Oxygen use of adult dioestrous uterus.

The uterus of the adult mouse in dioestrus consumes oxygen at the average rate of about 1.700 c.c. per gram moist weight per hour, the standard error of the mean being as small as 0.03. The results of sixteen experiments are plotted in Fig.3. It may be noted that 69 per cent. of the total lie between 1.5 and 2.0 c.c. per gram per hour and 31 per cent. between 1.0 and 1.5 c.c. per gram per hour.

Fig. /

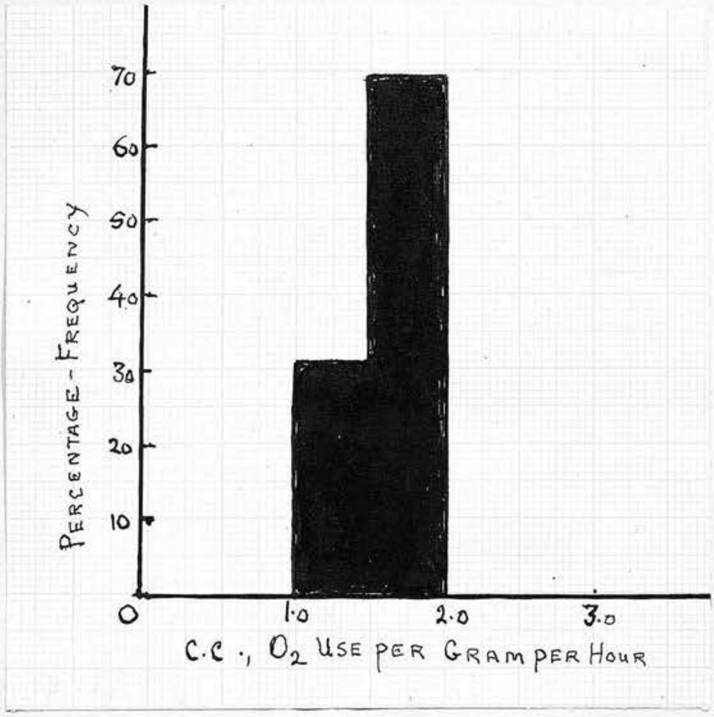

Fig. 3. Oxygen usage of adult uterus of mouse in dioestrus.

Table/

Table V shows that the adult mouse's uterus in dioestrus is sufficiently thin to permit of an efficient diffusion of oxygen through its whole thickness.

4. Oxygen use of immature uterus of mouse.

The uterus of the immature mouse is a thin filamentous affair and the tissue used in the experiments was usually not more than 2 cm. long.

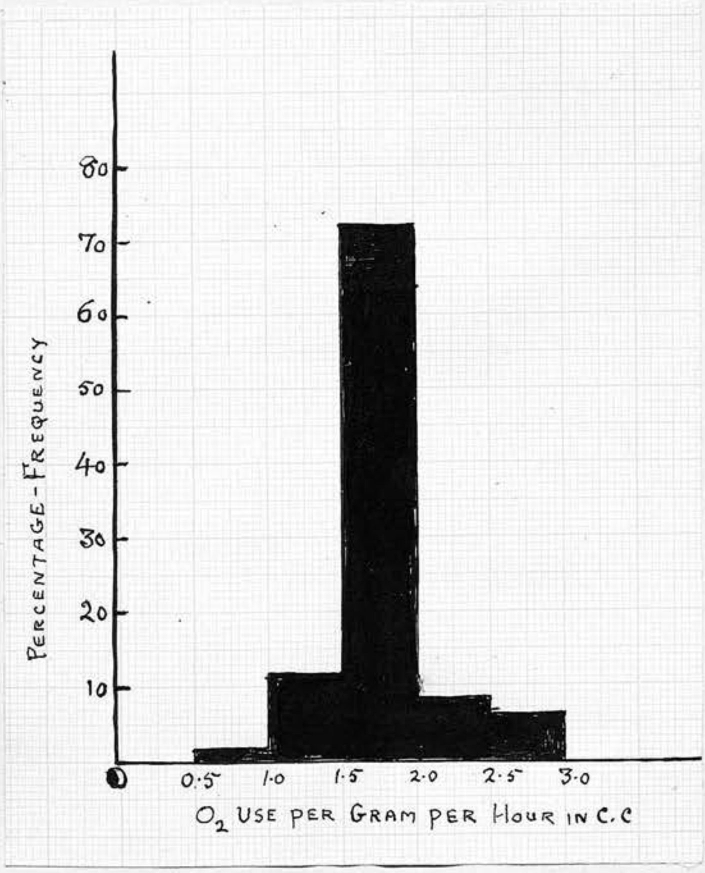

Fig. 4. Oxygen use of immature uterus of mouse.

Fig. 4 graphically gives the results of 55 experiments. It will be noticed that 72 per cent. of the total show an oxygen consumption of between 1.5 and 2.0 c.c. per gram per hour, the mean figure lying about 1.80 c.c. per gram moist weight per hour, whilst the standard deviation of the mean is 0.016.

Table VII.

Immature Uterus of Mouse

Weight in grams	Approx. length cm.	Absolute oxygen use c.c. per min.	Oxygen use c.c. per gram per minute	Radius of tissue in cm.	Critical radius in cm. calculated from Hill's formula.
0.0074	2.0	0.000207	0.030	0.034	0.046
0.004	2.0	0.00015	0.030	0.025	0.046
0.006	2.0	0.00035	0.046	0.034	0.037
0.0028	2.0	0.00013	0.037	0.021	0.041
		Average	0.036	0.028	0.042

Calculations similar to those already given in Part I for the oxygen diffusion into the uterus of the adult mouse, show that the uterus of the immature mouse receives an adequate supply of oxygen by diffusion when it is suspended in oxygen.

The figures given in Table VII show that a cylinder/

cylinder with a radius one and a half times that of the uterus would receive an adequate oxygen supply. The application of Hill's formula shows that a uterus of the size of those under discussion would receive by diffusion oxygen sufficient to maintain a consumption of 0.084 c.c. per gram per minute, an amount which is 2.3 times that actually consumed.

5. (a)-Oxygen use of adult uterus in oestrus.

Eight experiments were done to ascertain the oxygen consumption of the adult uterus of the mouse in oestrus. The average per gram moist weight per hour is about 1.802 c.c.; and within the limits of experimental error and individual variation, this figure does not differ perceptibly from the average obtained for the dioestrous uterus. 75 per cent. of the total lie between 1 and 2 c.c. and about 25 per cent. fall above this figure but within 3 c.c. Figure 5 illustrates this point.

Unfortunately Table VIII shows that the actual thickness of the tissue is greater than that which can receive a supply of oxygen adequate to its needs, as calculated from Hill's formula.

Fig. /

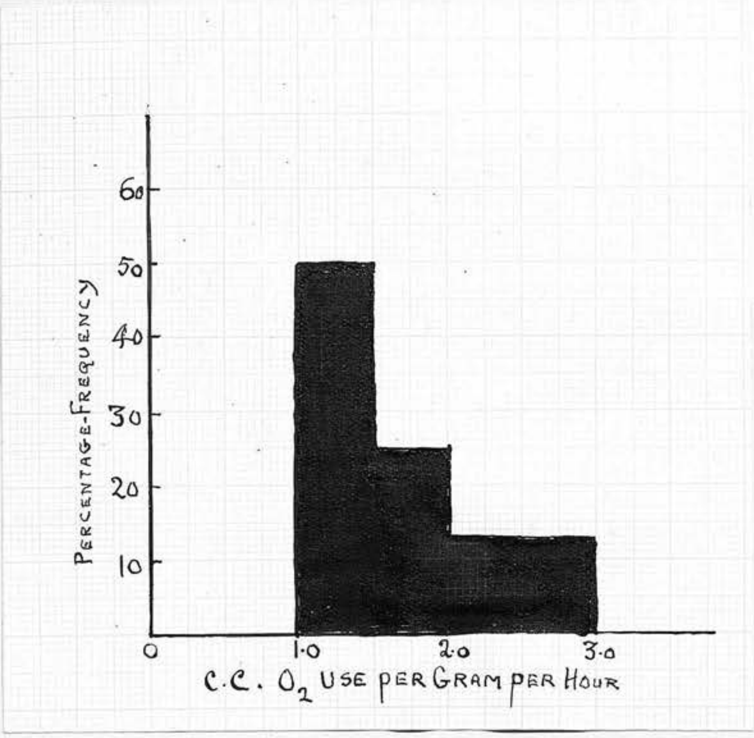

Fig. 5. Oxygen use of adult uterus of mouse in oestrus.

Table /

Table VIII.

Adult Uterus of Mouse - Oestrus.

Length of tissue cm.	State of uterus	Weight in grams	Absolute O_2 use per min. in c.c.	O_2 use per gram per min. in c.c.	Observed radius of tissue in cm.	Radius in cm. from Hill's formula.
2.0	Pro-oestrus	0.014	0.0009	0.051	0.048	0.036
2.0	meta-oestrus	0.022	0.0012	0.042	0.057	0.039
3.0	oestrus	0.057	0.0015	0.021	0.076	0.058
2.0	pro-oestrus	0.026	0.0010	0.032	0.064	0.045
2.0	prooestrus	0.024	0.0006	0.021	0.068	0.055
2.5	meta-oestrus	0.032	0.0011	0.029	0.064	0.047
2.0	oestrus	0.025	0.0006	0.019	0.063	0.058
3.0	oestrus	0.050	0.0015	0.024	0.073	0.052
			Average	0.029	0.064	0.049

The observed oxygen consumption may therefore be considerably less than that which would occur with an adequate oxygen supply. These figures are chiefly/

chiefly of interest for comparison with the results obtained after injection of oestrin.

5.(b) Possible error due to variation in water content.

Okey, Bloor and Corner (27),working on the lipoid content and oestrus in uterine mucosa of the pig,found a great variation in water content of the tissue at different stages of the sexual cycle, there being a high water content during the stages of congestion. This deduction is, unfortunately, unsupported by experimental figures in their paper. If the increase in weight during oestrus is due largely to the increase of water in the tissue, it follows that if the oxygen consumption be calculated on the dry weights of the uterus, a rise would be noticed during oestrus. On the other hand, Frank (28) states: "Actual experiments have shown me that the tremendous increase in size of the uterus during oestrus is not due to a great increase in the percentage of water content of the uterus. My attempt to deduce any useful biological information from this fact has proved futile, nor was I able to make use of the weight of the wet compared to the dry/

dry uterus in obtaining constants."

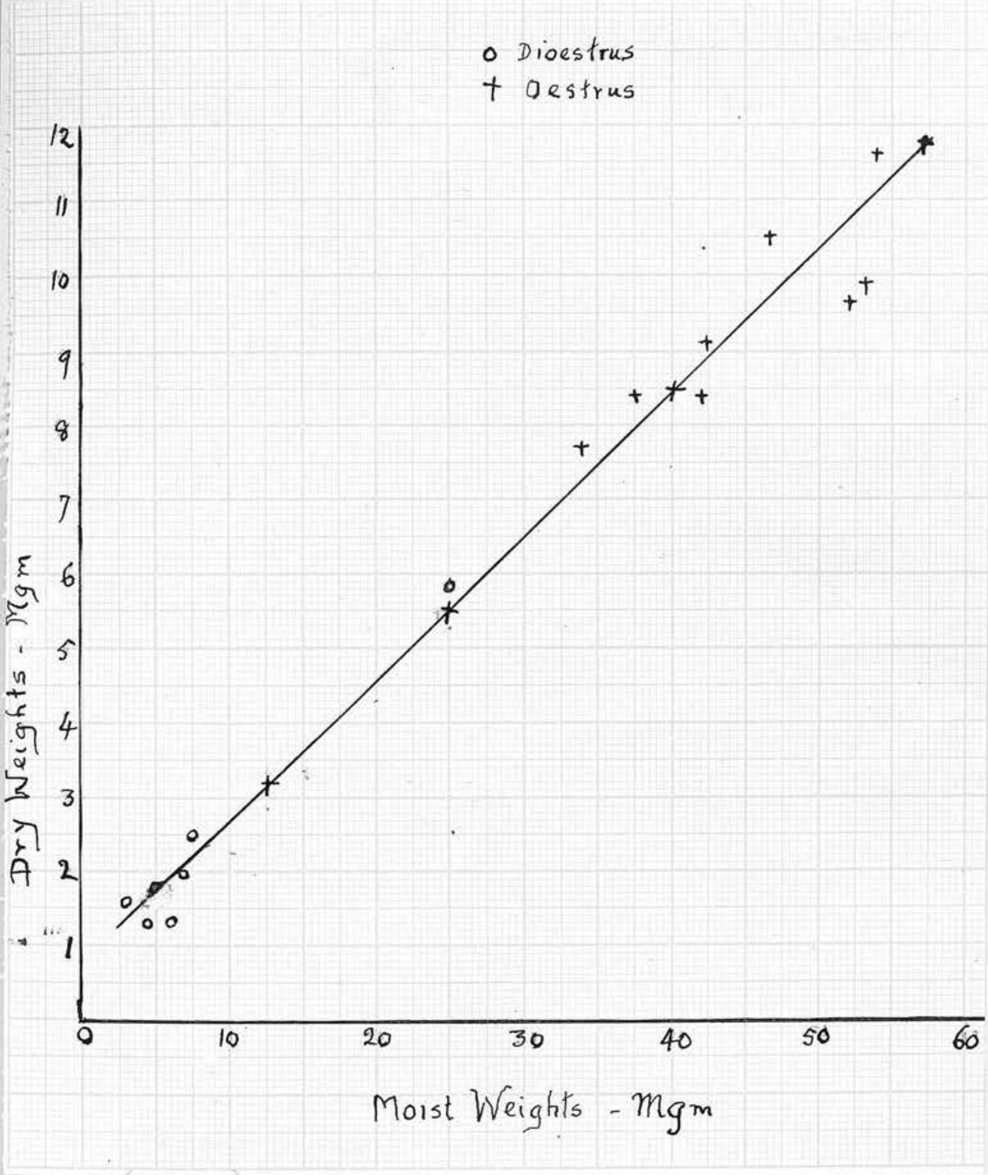

Fig. 6.

In order to clear this point, I noted down the moist and dry weights of the mouse's uterus, immature, adults, and castrates, both in dioestrus and oestrus and attempted to discover any variation in water content. In Fig. 6, the abscissa represents wet weights and the ordinates, the dry weights. The points do not all fall along one straight line; nor does the line passing through or near as many points as possible, cut the zero point. This latter is possibly due to evaporation and consequent loss of weight in the case of the lower figures, those representing immature uteri, before and while the moist weight is taken. The immature uterus is very thin and the smallest moist weight is even less than 2 mgm.,thus leaving a large margin for experimental error.

According to this graph (Fig. 6), no variation in the water content of the uterus during dioestrus and oestrus is apparent.

6. The oxygen consumption of the uterus of the ovariectomised mouse.

Adult mice weighing from 15-20 grams were castrated, the operation being done according to the method described by Burn (29). The oxygen usage/

usage of the castrated uterus was measured after an interval of about a fortnight after the operation. 29 experiments were done.

The oxygen consumption per gram wet weight, per hour, varied between 1 and 2 c.c., rarely as low down as 1 or even as high as 2 c.c., the average being about 1.580 c.c.

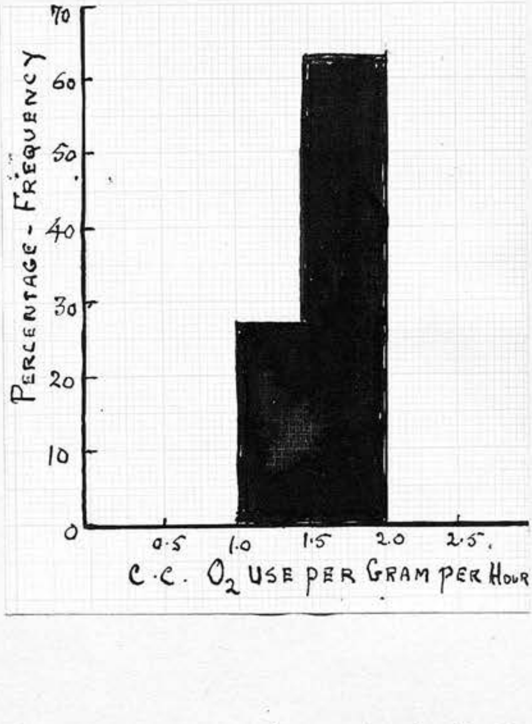

Fig. 7. Oxygen usage of castrated uterus.

Fig. 7 illustrates this graphically. It is obvious from Table IX that there is an adequate diffusion of oxygen right through the depth of the tissue.

Table IX.

Oxygen use of castrated uterus.

Weight gms.	Length cm.	Absolute O_2 use per min. c.c.	O_2 use per Gm. per min. in c.c.	Observed radius of uterus in cm.	Radius from Hill's formula in cm.
0.0054	2.0	0.00015	0.023	0.03	0.052
0.0086	2.0	0.00033	0.037	0.037	0.041
0.0120	2.0	0.00048	0.030	0.043	0.045
0.0163	2.0	0.00045	0.0225	0.059	0.053
			Average	0.042	0.048

The average oxygen consumption per gram per hour in the case of the castrate uterus is 1.58 c.c. which is less than that for the dioestrous adult uterus (1.70 c.c.), and that for the immature uterus (1.80 c.c.) by about 12 per cent.

7. Effect of Injection of Oestrin on:

A. The oxygen use of immature uterus.

An attempt was made to ascertain if, following injections of the oestrus-producing hormone, a rise in oxygen consumption would occur (a) even before/

before vaginal cornification is noticed, and (b) when oestrus is diagnosed by vaginal cornification as well as uterine distension.

Sistomensin (Ciba), an oily product supplied by the Clayton Aniline Company, London, as well as a more concentrated oily solution labelled to contain 40-50 rat units per c.c. were used in my tests.

Standardisation: Firstly, the batch of Sistomensin was biologically assayed according to Coward and Burn's method. Two lots of castrated mice, ten in each, were used, and the hormone was given hypodermically emulsified in 1 per cent. sodium carbonate solution. The first batch of ten had 0.5 c.c. of Sistomensin given to each and 30 per cent. went into oestrus. Double the dose was given to the second batch and again 30 per cent. showed signs of oestrus. Using the graph provided by Coward and Burn, 1 c.c. Sistomensin was found to have in the first batch, a strength of 1.4 units, and in the second, 0.75 units, giving a mean of 1.07 units per c.c. If 1.0 c.c. of this batch of Sistomensin be given by injection to castrated mice, about 54 per cent. would in due course go into oestrus, producing typical vaginal cornification.

(a) Effect of small doses: Doses of Sistomensin from 0.20 c.c. to 1.50 c.c. were given hypodermically and intraperitoneally to immature mice, alone or emulsified with 1 per cent. sodium carbonate solution. The animals were killed after 24, 48, 72 or 96 hours, before cornified cells were detected in the vaginal spread.

In all about 100 mice were thus treated and the oxygen consumption of the uterus measured before the onset of oestrus. Neither was any perceptible rise in oxygen consumption noted in any one of them, nor could the time interval after injection be related to the oxygen use of the uterus in any way.

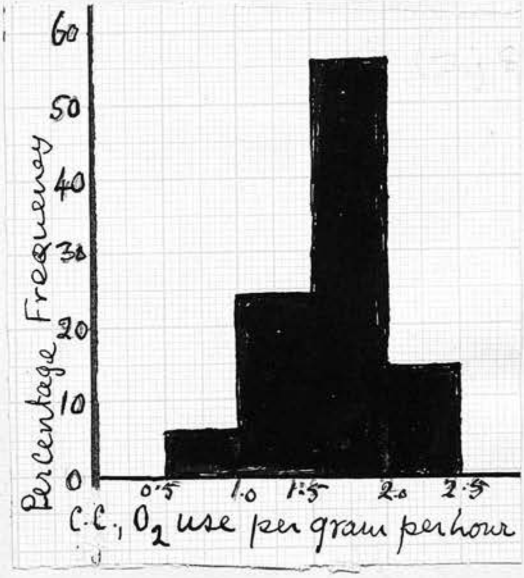

Fig. 8. Effect of small doses of Sistomensin on the oxygen use of the mouse' s immature uterus.

Fig. 8 shows the result. It is seen that about 56 per cent. of the total fall between 1.5 and 2.0 c.c. and about 24 per cent. between 1.0 and 1.5 c.c. The average figure is about 1.70 c.c. per gram per hour, which is about equal to the oxygen consumption of the dioestrous uterus.

(b) Effect of larger doses: As the results with larger doses of Sistomensin were irregular and doses of 1 to 2 c.c. were too voluminous for injection into young mice, I substituted a concentrated oily solution of the ovarian hormone made by the "Ciba" laboratory, Basel, and very kindly supplied by the Clayton Aniline Company of London. This product was supplied in ampoules of 1 c.c. said to contain 40 or 50 rat units respectively. A dose of 5 r.u. invariably produced oestrus in immature mice in about 96 hours, but it was thought advisable to give bigger doses and obtain an unmistakable condition of oestrus. So I gave doses varying from 5 to 20 r.u. to each mouse. Vaginal smears were made daily and the oxygen consumption of the uterus was measured usually when full cornification occurred. Experiments were also done in the pro-oestrous and meta-oestrous/

oestrous stages. Under these conditions oestrous changes could be diagnosed in the injected immature mice, usually on the 3rd day, but also, even as early as 48 hours or sometimes even as late as 96 or 120 hours. The uterus, when exposed, was swollen and turgid. It was carefully dissected away from all connective tissue, the ovaries were removed and one horn or both horns were used, depending on the size of the tissue. The vagina was cut off at its junction with the uterus. The uterus was also split longitudinally along the attachment of the broad ligament so as to get rid of the fluid and debris with which it was distended.

The results of 44 experiments with the uterus of immature mice, in which oestrus was induced, are represented graphically in Fig. 9. In 88 per cent. of the total number, the oxygen consumption is found to be higher than 2.0 c.c. per gram wet weight per hour, 86 per cent. falling between 2 and 3 c.c. Only 12 per cent. lie below 2 c.c. Reference to Fig. 4 shows that in the case of the immature uterus in dioestrous condition, only 14 per cent. gave an oxygen consumption above 2.0 c.c. per gram per hour, whereas 86 per cent. lay below this level.

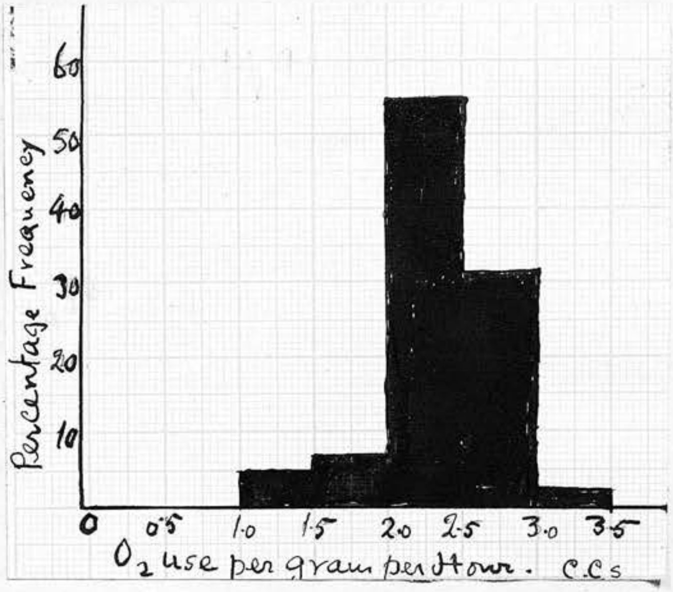

Fig. 9. Effect of large doses of oestrin on the oxygen use of immature uterus.

Oxygen consumption after large doses of oestrin.

The average is 2.300 c.c. per gram moist weight per hour with a standard error of the mean of 0.054, while the average rate for the dioestrous uterus is 1.80 c.c. per gram per hour with a standard error of the mean of 0.016. With tissues such as the mouse's uterus, one invariably comes across such marked individual variation that averages do not necessarily give a proper idea of what really happens. A better and more correct idea is obtained/

obtained by comparing Figs. 4 and 9.

Table X.

Immature mouse's uterus - Induced oestrus.

Serial No.	State of uterus	Weight gms.	Length cm.	Absolute O_2 use per minute.	O_2 use per gram per min. c.c.	Observed radius of tissue cm.	Radius in cm. from Hill's formula
296	Oestrus	0.023	2.0	0.0011	0.040	0.058	0.039
298	Pro-oestrus	0.014	2.0	0.0005	0.031	0.049	0.048
322	Met-oestrus	0.033	3.0	0.0018	0.046	0.059	0.037
335	do.	0.007	2.0	0.0003	0.039	0.032	0.042
342	Oestrus	0.027	3.0	0.0012	0.037	0.053	0.042
344	do.	0.042	2.5	0.0011	0.021	0.073	0.055
301	Pro-oestrus	0.019	2.0	0.0015	0.044	0.055	0.038
344	do.	0.022	2.0	0.0010	0.036	0.059	0.043
				Average	0.037	0.055	0.043

In Table X, with the exception of one case, ~~in all others~~ the observed radius of the uterus is greater than that which would, according to Hill's formula, permit of an adequate diffusion of oxygen right/

right through the tissue. Nevertheless, a marked rise in oxygen consumption is observed. The probable reason for this phenomenon may be that lack of oxygen in a small area in the centre of the uterus does not prevent the peripheral tissue increasing its oxygen use.

It is also noticed that in the case of oestrus induced by oestrin the rise in oxygen consumption starts early during pro-oestrus and continues through full oestrus and meta-oestrus. The oxygen intake falls to the dioestrous level as the meta-oestrus stage merges into dioestrus. I have been unable to find any difference in the oxygen usage during oestrus and pro and meta oestrus.

B. Effect of Oestrin on Uterus of Castrated Mice.

The oxygen consumption of the uterus of the ovariectomised mouse during oestrus induced by injections of large doses of oestrin does not show an increase similar to that observed with the immature ones. The average of eight experiments is 1.420 c.c. per gram per hour with a standard error of the mean of 0.07. This figure is actually lower than/

than that obtained with castrated mice which had received no oestrin. I am unable to suggest any reason for this.

Table XI.

Uterus of Castrated Mouse - Induced Oestrus.

Serial No.	Moist wt. grams	Length cm.	Absolute O_2 use per min.	O_2 use per Gm. per minute c.c.	Observed radius of uterus in cm.	Radius from Hill's formula in cm.
387	0.042	2.0	0.0010	0.020	0.081	0.057
388	0.056	2.5	0.0011	0.016	0.086	0.062
389	0.070	4.0	0.0020	0.022	0.074	0.053
390	0.057	3.7	0.0019	0.027	0.069	0.048
391	0.050	2.5	0.0014	0.022	0.078	0.052
392	0.054	2.0	0.0009	0.013	0.092	0.070
324	0.040	3.0	0.0014	0.029	0.065	0.470
315	0.054	4.0	0.0026	0.038	0.064	0.410
			Average	[illegible]	0.076	0.054

The observed radius of the uterus of the spayed mouse in induced oestrus is greater than the critical radius calculated from Hill's formula, and/

and hence there is obviously a lack of oxygen supply in the centre of the uterus. Therefore the figures for the oxygen consumption in this case, do not mean much, but comparison with the uterus of the immature mouse in artificial oestrus showing a higher oxygen consumption presents an interesting puzzle. It is difficult to explain this difference in the two results.

Fig. /

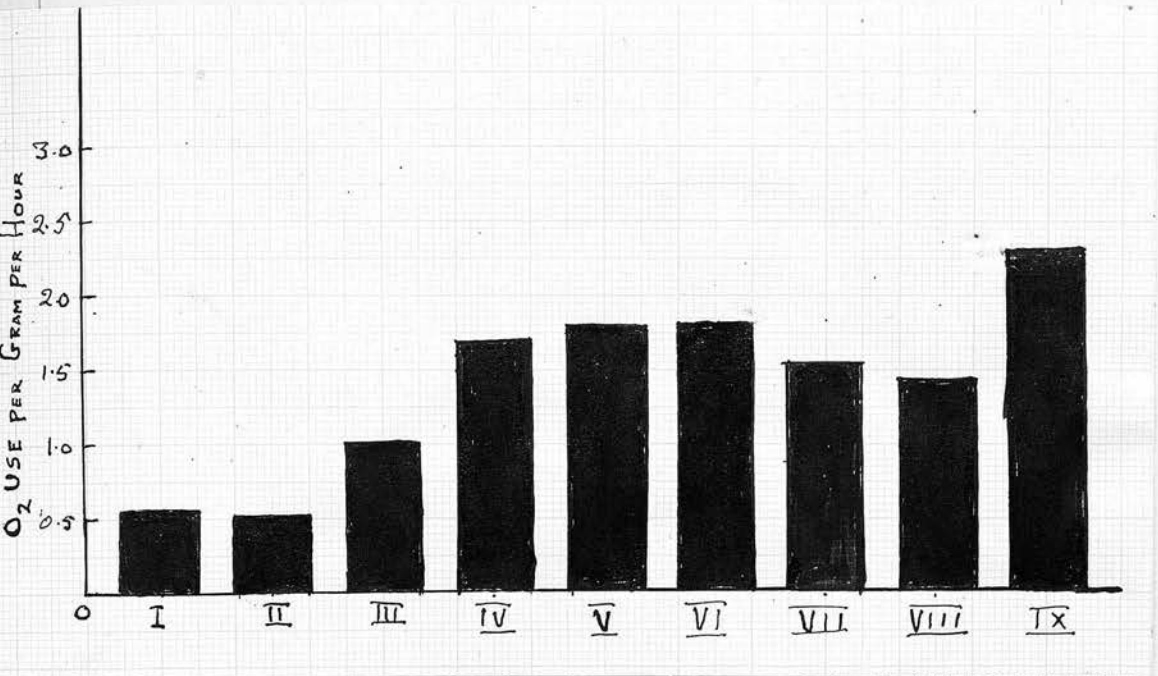

Fig. 10. Comparison Graph of Oxygen Consumption of Uterus.

I. Rabbit's uterus — 0.564 c.c. per gram per hou

II. Guinea pig's uterus — 0.533 do.

III. Rat's uterus — 1.006 do.

IV. Adult mouse - dioestrous uterus 1.700 do.

V. Immature do. do. — 1.800 do.

VI. Adult mouse - oestrous uterus — 1.802 do.

VII. Uterus of castrated mouse — 1.580 do.

VIII. Uterus of castrated mouse (induced oestrus) — 1.420 do.

IX. Uterus of immature mouse (induced oestrus) — 2.300 do.

8. /

8. Discussion.

Fig. 10 shows at a glance the oxygen consumption of the uterus of rabbit, guinea pig, rat and mouse. Under the conditions of the experiments, the mouse's uterus in dioestrus uses a little more than three times the oxygen consumption of the rabbit and the guinea pig, and about one and a half times that of the rat. The uterus of the castrated mouse uses less oxygen than that of the adult or immature uterus. No rise in oxygen consumption is noted during natural oestrus, nor when oestrus is induced in the spayed animal. But, as already pointed out, figures do not mean much as under the conditions of the experiments, the observed thickness of the uterus during oestrus, both natural and induced, happens to be greater than that which would permit an adequate diffusion of oxygen according to Hill's formula for the diffusion of gases into a cylinder. This means that a central area of the tissue is not obtaining sufficient oxygen.

But, surprisingly enough, a definite increase of oxygen consumption by about 23 per cent. occurs when oestrus is induced in the immature mouse, although/

although in this case also the tissue is too thick to allow an adequate diffusion right through its substance. This result is produced by massive doses of oestrin that cause not only cornification of the vaginal smear, but also definite uterine distension. Smaller doses of oestrin that do not produce these changes do not affect the oxygen intake.

The characteristic signs of oestrus and precocious puberty are vaginal cornification, uterine distension and readiness to copulate. The morphological and histological changes are thus described by Parkes (30): "During dioestrus the uterus is constricted and anaemic. At pro-oestrus dilatation of the lumen has begun and at the time of ovulation the uterus has increased to about twice the normal diameter; the stroma and muscular material are then very much attenuated. The increase in size is caused solely by the distension of the lumen. During met-oestrus the uterus gradually returns to its dioestrous size." According to Allen and Doisy (31): "The immature uterus is small and anaemic. A few simple tubular glands have begun to develop. The uterine epithelium is quite undifferentiated and the stroma almost embryonic/

embryonic in structure. In injected animals the cornua are hyperaemic and greatly distended with uterine secretion. Mitoses are abundant in the epithelium which is typically columnar with nuclei ranged along the basement membrane. The stroma cells are spindle shaped. In 48 to 60 hours the differentiation has been induced in the infantile uterus by injection of follicular hormone".

While the increase in size of the uterus during **natural** oestrus is caused solely by the distension of the lumen, it is evident that active anabolic changes are abruptly brought about in order to bring the immature uterus to an oestrous state. This, in itself, would explain the definite increase in the oxygen usage of the immature uterus in induced oestrus. And this occurs in spite of the fact that the uterus in this condition is too thick to permit of an adequate diffusion of oxygen through its whole radius. Perhaps, as has already been observed, it may be that lack of oxygen in a small area in the centre of the uterus does not prevent the peripheral tissue increasing its oxygen use.

My experiments have shown that it is possible to produce not only vaginal cornification but also typical uterine distension by injections of small doses of ovarian hormone from 12 to 20 r.u. to immature/

immature and castrated mice. This is interesting in view of the observation of Marrian and Parkes(32) that "Less than 100 "vaginal cornification" units have no detectable effect on uterine distension, while even at this dosage only one mouse out of four showed this effect."

9. Value of Method in Standardisation.

This research was undertaken in the hope that measurements of the changes in the oxygen consumption of the uterus produced by oestrin could be used as a method to standardise this hormone.

Unfortunately this hope has been disappointed. In the first place, the oxygen consumption of the uterus of the controls varies so much that the effect produced by oestrin can only be estimated by measuring the response of a number of uteri. This makes the method too laborious for practical use. In the second place, the change in the oxygen consumption of the uterus produced by oestrin is of the nature of an all or none phenomenon rather than a graded reaction. There is a certain average effective dose which produces visible changes in the uterus; and doses smaller than this produce no measurable/

measurable effect whilst larger doses produce no greater effect than does the just effective dose.

10. Summary and Conclusions.

(1) A method has been described for measuring the oxygen consumption of plain muscle, such as the uterus, by suspending it in oxygen in specially shaped bulbs connected with a Barcroft manometer.

(2) Measured according to this method, the oxygen consumption of the rabbit's uterus is 0.56 c.c., that of the guinea pig 0.53 c.c. and that of the rat 1.01 c.c. per gram moist weight per hour. (Volumes calculated as dry gas at N.T.P.).

(3) But the observed thickness of these uteri is shown to be greater than that which, according to Warburg's and Hill's formulae, would allow an adequate diffusion of oxygen right through the substance of the muscle.

(4) The uterus of the immature mouse as well as the adult in dioestrus and the castrated animal is sufficiently thin to permit of an adequate diffusion of oxygen when suspended in this gas.

(5) The oxygen consumption of the dioestrous uterus of the mouse is about 1.70-1.80 c.c. per gram per hour; that of the castrated animal is slightly less.

(6) Under the conditions of the experiments no rise in oxygen use has been noticed in natural oestrus and when oestrus is induced in the spayed animal. But the figures are not conclusive as the radius of the uterus in this state has been found to be greater than the "critical radius" of Hill.

(7) A noticeable increase in oxygen consumption, however, occurs in the uterus of the immature mouse during oestrus induced by injection of the oestrus-producing hormone, the average being about 2.300 c.c. or about 23 per cent. above that of the dioestrous uterus. The increase begins at pro-oestrus and is sustained until the declining stage is reached. This increase occurs in spite of the fact that the uterus is too thick to allow proper diffusion of oxygen.

(8) This phenomenon cannot be utilised as a means of standardising oestrin as the response is not graded/

graded and the technique is too laborious.

(8) Doses ranging from 12 to 20 r.u. of oestrin not only produce vaginal cornification but also uterine distension.

(9) The tremendous increase in size of the uterus during oestrus is not due to a great increase in the percentage of water content of the uterus.

I am deeply grateful to Professor A.J. Clark for supervising this work and for his constant help and encouragement during its course. The expenses of this research were met by a grant from the Government Grant Committee of the Royal Society, made jointly to Professor Clark and myself.

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