FACTORS INFLUENCING THE OXYGEN CONSUMPTION OF THE ISOLATED UTERUS OF RATS AND MICE.

by

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PART II : The Estimation of the Oxygen Consumption of the Isolated Uterus in Animals to which the Various Sex Hormones have been administered.

INTRODUCTION

Regular cyclic changes in the functional activity, histology and weight of the secondary female sexual organs are known to be dependent on the sex hormones.

Mammary cycles in the unmated female mammal have been described in the rat by Meyers (52), and Sutter (67), in the guinea pig by Loeb and Hisselberg (48), in the cow by Hammond (33), and in the human by Dieckmann (23).

Vaginal cyclic changes are known to occur in certain species of animals. Marshall (51) and Hammond and Marshall (34) described such changes in the ferret. Loeb (47), Stockard and Papanicolow (66) and Voss (68) studied these phenomena in the guinea-pig. Lataste (45), Long and Smith (50), Morau (54), Long and Evans (49), and Allen and Doisy (2) described them in the mouse and rat.

Uterine cyclic changes, however, occur in all classes of mammals. These changes are of a very profound character, and this fact suggested that the estimation of the oxygen consumption of the uterus would offer an indication of the mode of action of the endocrine/

endocrine secretions which brought about the changes. The investigations described below were carried out by the writer in order to study the effect of the sex hormones on the oxygen consumption of the isolated uterus. Mice and rats were chosen as experimental animals, because the vaginal smear technique of Long and Evans (49) and Allen and Doisy (2) offered a simple method of diagnosing the various stages of the oestrous cycle in these animals.

These investigations have been carried out in two parts: <u>Part I</u> - The Estimation of the Oxygen Consumption of the Isolated Uterus during the various Stages of Sexual Development, and also during the different Stages of the Oestrous Cycle, and <u>Part II</u> - The Estimation of the Oxygen Consumption of the Isolated Uterus in Animals to which the various Sex Hormones had been Administered.

Part I - The Estimation of the Oxygen Consumption of the Isolated Uterus during the various Stages of Sexual Development, and also during the Different Stages of the Oestrous Cycle.

(a) Review of the Literature.

Sexual Periodicity.

Parkes (55) divided the sexual periodicity in the female mammal into three stages:

1. attainment of puberty.
2. stage of sexual maturity.
3. stage of sexual decline.

During the first or immature stage the uterus differentiates and grows gradually and continuously. In the early part of this stage, it appears that growth is independent of the sex hormones. Though Courrier (18) claimed to find growth effects on the foetal uteri after injection of large doses of the female hormone in pregnant guinea pigs, yet subsequent work by Allen, Francis and Craig (4), and Parkes and Bellerby (61) did not confirm his results. In fact the last authors found that the uterus of the new born mouse did not react to oestrin, Recently Wiesner (71) found that differentiation of the uterus of the immature rat, which occurs during the first 14 days of life, is not accelerated by administration of/

of the oestrifying hormone. He asserted that the reactivity to this hormone developed gradually.

In the later part of the immature stage, the uterus reacts to the administration of the female sex hormones by a marked increase in growth and activity of the muscular as well as of the glandular layers. Such changes are similar to those occurring at the first oestrus, or ~~in~~ the attainment of puberty.

Allen and Doisy (3) described the induction of premature puberty in mice and rats following the injection of active female hormone extracts, and if the injections were stopped, the cycles ceased until the normal time for puberty arrived. Laqueur (44) published a detailed study on the increase in weight and size of the sex organs in rats in which premature puberty had been induced. The increase in weight sometimes reached 300 per cent. He got similar results in guinea pigs, mice and bitches.

Smith (65) and Zondek and Ascheim (76, 77) found that implantation of fresh anterior lobe of the pituitary gland or injection of active extracts of that gland were rapidly followed by oestrous phenomena in the immature animal.

The duration of the immature state in the mouse and rat is of importance for the purpose of these investigations.

In the rat it was described by Long and Evans (49) to be about 10 to 11 weeks. In the mouse

Kirkham /

Kirkham(41) stated that males and females are both sexually mature when about six weeks old. Parkes (59) recorded that the establishment of the vaginal orifice, a sign of sexual maturity occurred at the end of the seventh week. Mirskaia and Crew (53) stated that the earliest first oestrus appeared on the 28th day of life, and the latest on the 75th. The diagnosis of immaturity is based on the age of the animal and the absence of the vaginal orifice. This stage ends abruptly by the appearance of the first oestrus in animals and menstruation in the human female.

Stage of Sexual Maturity.

During this stage cyclical changes occur in the uterus. These changes are now known to be brought about by ovarian hormones which in their turn are dependent on gonadotropic principles secreted by the anterior pituitary gland.

Long and Evans (49) described the uterine changes during the oestrous cycle in the rat as follows: In pro-oestrus the uterus becomes distended with fluid, and increases in diameter from 2.3 to 3.7 mm. In the early part of oestrus it reaches its greatest dimensions (5 mm.) and its epithelium becomes thin. In/

In the late part of oestrus the epithelium undergoes vacuolar degeneration, and the diameter of the uterus diminishes to 2 mm. In metoestrus some vacuolar degeneration of the uterus occurs, but also regeneration. The diameter of the uterus becomes 2.2 mm. In dioestrus the epithelium regenerates and the diameter of the uterus reaches 1.7 mm.

Allen (1) described similar changes in the uterus of the mouse.

Besides the above mentioned changes, periodic variations have been observed to occur in uterine activity by various workers. Considerable conflict of opinion, however, exists in the observations of different workers.

As regards the rat's uterus Blair (7), ignoring the small contractions, stated that contractions were fewest at oestrus and most at dioestrus. In a later paper (8) he found that the total number of contractions in young virgins was the same in oestrus and dioestrus, but that major contractions were greater in dioestrus. Frank, Bonham and Gustavson (31) on the other hand found that in dioestrus contractions were greater and more frequent than they were in oestrus, and that in the castrated rat they were similar to those in dioestrus/

diooestrus. They also found that administration of potent female sex hormone extract to a castrated animal caused slow major contractions similar to those characteristic of oestrus. They pointed out that this phenomenon was dependent on the female sex hormone and suggested the use of this reaction in the castrate as a method for testing or assaying the hormone. Later Durrant and Rosenfield (25) confirmed the conclusions of Frank and co-workers, that major contractions were more frequent in dioestrus. Clark, Knaus and Parkes (13) counted all contractions and found no difference in the number of contractions throughout the cycle, but that conduction was much better in oestrus.
Blair (8) found that contractions were more powerful in dioestrus whilst Durrant and Rosenfield (25) noted no significant cyclic difference in the amplitude of contraction.

Corner (17), Wislocki and Guttemacher (75) and Keye (38) observed cyclic variations in the mechanical activity of the uterus of the sow. The significance of these observations is doubtful because Koch and Seel (43) found that the activity of the Fallopian tube was greatest during dioestrus whilst that of the uterus was greatest during oestrus.

Knaus /

Knaus (42) found that the mechanical activity of the rabbit's uterus was decreased by the development of the corpus luteum. Sigmund (63) showed however that this effect did not occur in the rat.

The facts mentioned above indicate that the nature of the cyclic activity of the rat's uterus is distinctly uncertain. It will be shown later that extensive variations in metabolic activity of the uterus occur within the dioestrous period. Hence it is unprofitable to attempt at present to correlate cyclic changes in the mechanical activity of the uterus with the changes observed in the metabolic activity that will be described below.

Castration in the adult causes cessation of all these cyclic changes and produces retrogression of the uterus. Administration to the castrated animal of potent sex hormones brings about changes indistinguishable from those occurring during the normal cycle. A detailed account of these phenomena will be given in Part II.

The gaseous metabolism of the uterus without reference to the oestrous cycle has been investigated by some workers. E. Kehrer (39) studied the CO_2 production of the uterus of the cat in the non-pregnant and pregnant states. He found that in the former/

former state, the uterus produced 0.1 to 0.15 mg. or 0.05 to 0.075 c.c. CO_2 per gram of uterine tissue per hour, while in the latter state it produced 0.2 to 0.3 mg. or 0.1 to 0.15 c.c. CO_2 per gram per hour.

Lovatt Evans (26) estimated the oxygen consumption of the rabbit's uterus and found it to vary from 0.22 to 0.31 c.c. per gram per hour, but pointed out that the length of the muscle fibre was an important factor in determining its oxygen utilisation, because increased tonus produced by drugs such as histamine, produced a decrease in the oxygen consumption. In a later paper, the same author (27) estimated the oxygen consumption of the isolated guinea pig's uterus to be 0.46 c.c. per gram per hour in the relaxed state, and 0.39 c.c. per gram per hour in the tonic state. He therefore concluded that there was distinctly less oxygen used during the tonic than the relaxed state, but argued that the thickening of the tissue during tonus decreased the available surface for the interchange of gases. David (20) recorded a slight variation in the oxygen consumption of the isolated uterus of the mouse according to the stage of the oestrous cycle. He chose the mouse's uterus for his investigations because/

because he concluded from the consideration of Warburg's (70) and Hill's (36) formulae for oxygen diffusion in flattened and cylindrical tissues respectively, that the isolated uterus of the rabbit, guinea pig and rat were unsuitable for investigating problems related to gaseous metabolism of these tissues. He gave the following figures for the average rates of oxygen consumption of the isolated uterus of the mouse at the various stages of the oestrous cycle.

(1) Adult dioestrous uterus consumed 1.7 c.c. O_2 per gram per hour.
(2) Adult oestrous uterus " 1.8 do.
(3) Uterus of the immature mouse " 1.8 do.
(4) Uterus of the castrated mouse " 1.5 do.

The chief point of importance in this connection is that the figures for the oxygen usage of the uterus obtained by David are much higher than those which were obtained by the earlier workers.

(b) /

(b) Methods of Estimation of Oxygen Consumed by Isolated Tissues.

Cohnheim and Pletnow (16) in investigating the respiratory quotient of isolated pieces of the small intestine of the cat used an apparatus devised by the former author (15). This consisted of a closed circuit apparatus through which circulated air that had been previously freed from CO_2 by passing it through soda lime. The CO_2 which was produced during the experiment was absorbed by a known quantity of soda lime included in the circuit. The diminution in volume of the circulating air, due to the oxygen consumed, was indicated by a manometer. At the end of the experiment oxygen was admitted from an oxygen flask so as to bring the manometer to its original level. The difference in weight of the oxygen flask was the weight of the oxygen consumed by the tissue, while the difference in the weight of the soda lime receptacle gave the weight of the CO_2 produced.

Harden and Maclean (35) described a modification of that method for estimation of oxygen consumed by isolated tissues. In this case a continuous stream of oxygen was circulated through a closed circuit apparatus. At one point of the circuit, a vessel containing strong standard potash was introduced, and/

and at another point was placed a flask containing the minced up organ or tissue in Ringer's solution. Oxygen was passed first through the minced tissue and afterwards through the potash so that any CO_2 given off by the tissue was immediately absorbed by the alkali and could be subsequently estimated. The oxygen that was consumed by the tissue was indicated by a scale on a burette.

Brodie and Vogt (9) in their investigations on the oxygen consumption of the small intestine, isolated a loop of the latter with the blood vessels intact and determined the oxygen consumed by the difference in the value of the oxygen tension in the arterial and venous blood.

Osterhout (56) estimated the oxygen consumption of marine animals by a colorimetric method using limulus blood as indicator. The blood is blue when fully oxygenated and nearly colourless when deoxygenated. He measured the CO_2 produced by Haas's (32) method.

Alvarez and Starkweather (5) in their work on the metabolic gradient along the small intestine, estimated the rate of oxidation at different levels in terms of the catalase content of the tissues. The

CO_2/

CO_2 produced was measured by Haas's method, which is essentially a colorimetric method.

Warburg (70) while working on the oxygen consumed by tumour tissue slices used a manometric method, based on the original Barcroft differential manometer (6) for blood gas analysis. He described a constant volume manometer in which the level of the manometric fluid was adjustable by means of a reservoir. In this case the tissues were immersed in Ringer or serum, and the apparatus was fitted to a mechanical shaker, which revolved at the rate of 120 revolutions per minute.

Evans (26) used two different methods for estimating the oxygen consumed by the isolated uterus. One was a modification of Winterstein's micro-respirometer, and the other consisted of a Ringer bath through which oxygen was bubbled, and in which the uterus was suspended. The fluid was drawn off periodically and its oxygen content calculated analytically by a micro modification of Winkler's (73, 74) method.

Fenn (28) in calculating the oxygen consumed by the small intestine of the frog, used a differential volumeter. The CO_2 produced was absorbed by $Ba(OH)_2$ solution 0.0066 M. and was estimated/

estimated by conductivity, while the oxygen used was determined volumetrically.

Dickens and Simer (21) described a modification of Fenn's method for the determination of the R.Q. of tumour tissues. In a later paper the same authors (22) described a modification of this method. Clark and White (14) used a modified Barcroft manometer for calculating the oxygen consumption of cardiac tissues. The same method was adopted by David (20) in his work on the oxygen consumption of the isolated uterus.

(c) Method and Technique.

In my experiments a modified Barcroft method was used. This was previously described by Scott and myself (40). The apparatus consisted of a Barcroft manometer attached to two chambers. One chamber (Fig. 1) was a dummy and contained about 2 c.c. of water so that the atmosphere in it was always saturated with water vapour. It was attached to the limb of the manometer and served to compensate for alterations in temperature and pressure./

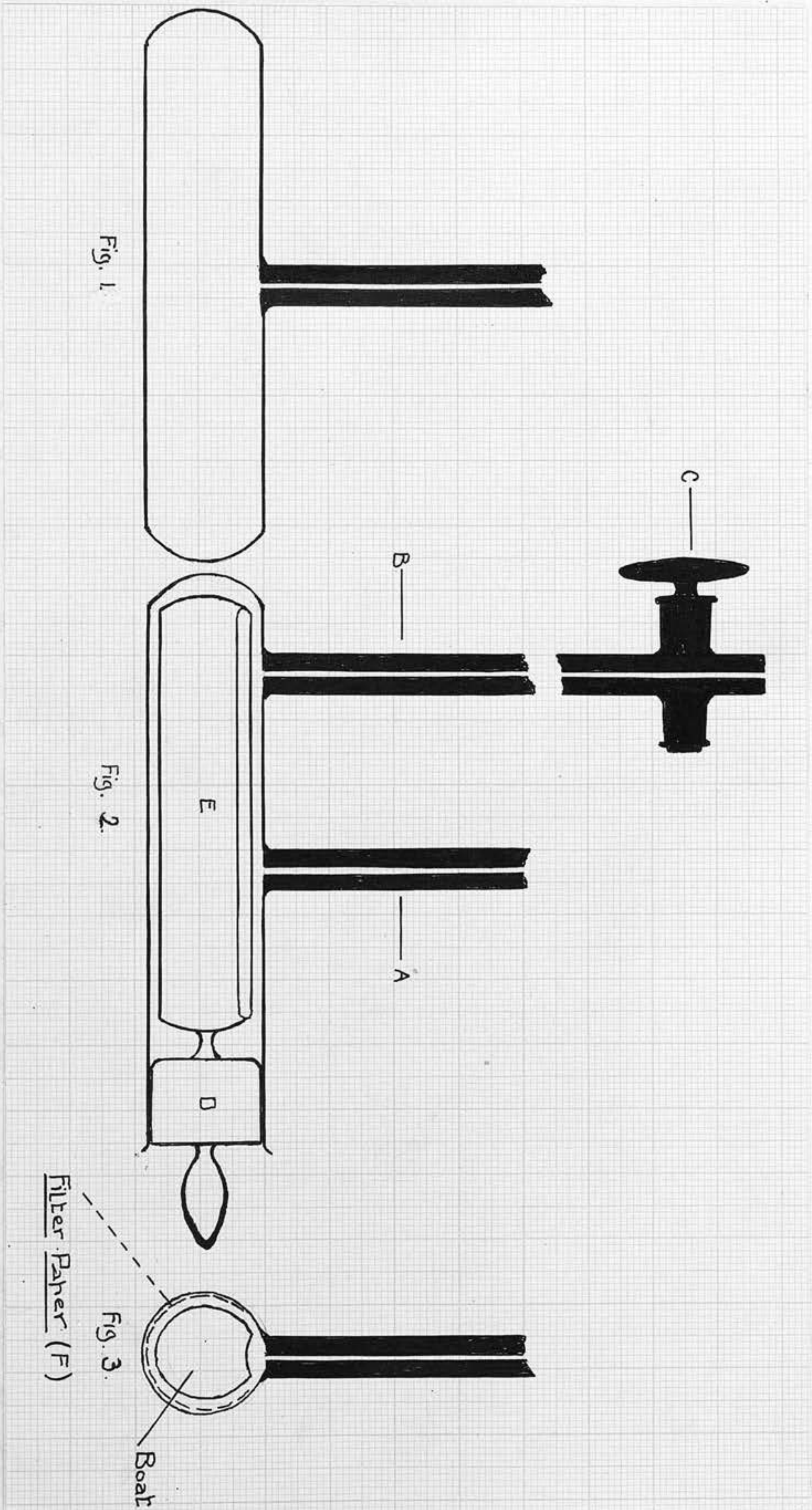


Fig. 1.

Fig. 2.

Fig. 3.

pressure. The other or experimental chamber (Fig. 2) contained a glass boat (E)(Fig. 2) fixed to a stopper. The stopper fitted the chamber by means of a ground glass joint (D). The chamber was connected with the manometer by a fine glass tube (A). The experimental tissue, of which the oxygen consumption was to be determined, was contained in the boat (E), immersed in a known volume of Ringer-Locke's solution or serum. A second limb (B), provided with a two-way stopcock (C), allowed connection of the inside of the chamber with the atmosphere and was so arranged that a fine pipette could be introduced through it, for addition of test fluid to that contained in the boat. A piece of filter paper (F) size 3 x 1.5 inches, soaked in 6 per cent. KOH solution was used for absorption of CO_2. The filter paper was placed adjacent to the wall of the chamber underneath and around the boat (Fig. 3).

Both chambers were contained in a thermostatically controlled bath, which was kept constantly at 37°C. with a maximum variation of 0.05° C. The water in the bath was thoroughly mixed by a rapid stream of air bubbles to ensure a uniform temperature throughout the bath. A circulation was kept up in the/

the atmosphere of the experimental chamber by fixing the whole Barcroft apparatus to a moving platform which was shaken in a horizontal plane to the extent of 3 inches in either direction. The number of movements of the platform were 140 per minute.

The apparatus was standardised before using it by withdrawing a known volume of gas from the experimental chamber, and comparing that volume with the alteration in height of the column of kerosine in the Barcroft manometer. A micro-pipette was used for this purpose. 1 cm. variation in the height of the kerosine meniscus on the right limb of the manometer represented from 0.041 to 0.049 c.c. of gas removed from the different experimental chambers.

Technique.

The animal was killed by a blow on the head, and was bled. The abdominal wall was opened and both horns of the uterus, including the ovaries, were dissected out quickly. The uterus was severed from the vagina, and transferred to a small porcelain dish containing a little Ringer-Locke's solution. Then the ovarian ends were cut at the junction of the uterine horn with the Fallopian tube, and any remains/

remains of the broad ligament were dissected off the uterus.

The uterus was immersed in Ringer-Locke's solution in the experimental boat,(diagram 2), which was fitted to the experimental chamber. The apparatus was then fixed to the moving platform of the thermostatic bath and the experimental chamber oxygenated. Oxygenation was carried out in the following manner. The chamber was shut off from the manometer, and connected to an oxygen cylinder by means of the three-way stopcock on that side. Oxygen circulated freely in the chamber and was allowed to escape to the atmosphere through stopcock. (c).

The three stopcocks were kept open to the atmosphere for 15 minutes, after which the experimental chamber was reoxygenated. Five minutes later the stopcocks were so arranged that the two chambers were only connected to the manometer, which always showed constant readings by that time. The barometric pressure at the moment was taken. The reading of the manometer was then taken, and thereafter every 20minutes for 2-3 hours or longer.

At the end of the experiment, the tissue was weighed/

weighed and its oxygen consumption calculated in the number of c.c. of oxygen which were consumed per gram of uterine tissue per hour. All figures were corrected to N.T.P. from the formula:

$$Vo = V \frac{bo - c}{760(1 + .00367\ t)}$$

where Vo = corrected volume
V = uncorrected volume
c = water vapour pressure in mm. Hg at t°C.
t = temperature
bo = observed barometric pressure.

The composition of the Ringer's fluid in these experiments was as follows: 0.09% NaCl, 0.042% KCl, 0.024% $CaCl_2$ and 0.02% NaH_2PO_4. The pH of this solution was adjusted to pH 7.4.

Efficiency of the Method.

Clark and White (14) in their investigations on the oxygen consumption of the isolated auricle of the frog's and tortoise's hearts, suspended these tissues in an atmosphere of oxygen in a stationary apparatus. David (20) used the same technique as the previous authors in dealing with the isolated uterus.

Warburg (70) suspended the experimental tissue slices in Ringer or serum and fitted his apparatus to/

to a mechanical shaker that revolved at the rate of 120 revolutions per minute.

Evans (26) stated that in a deficiency of oxygen there is an accumulation within the tissue of metabolic products that are removed to some extent by immersing the tissue in Ringer. He therefore thought it advisable to immerse experimental tissues in Ringer.

Dixon and Elliot (24) showed that the efficiency of a Barcroft apparatus used for the determination of oxygen consumption by tissues depended on the following factors:-

1. Surface area of the filter paper used to absorb the CO_2 produced during respiration, as this determines the time necessary for the apparatus to be in equilibrium. They used a filter paper 4 x 2.5 cm. and found that the time thus necessary was about 20 mins.

2. The concentration of the caustic used for absorbing CO_2. They found if the caustic used was of a higher concentration than 6 per cent. oxidation of the filter paper took place. Even with 6 per cent. KOH they advised the insertion of a similar piece of filter paper with caustic in the compensation chamber for/

for accuracy.

3. The surface area of the suspension fluid of the tissue as this affects the relative rate of diffusion of oxygen between the atmosphere of the chamber and the fluid bathing the tissue.

4. The rate of shaking of the apparatus. This they stated had to be proportional to the velocity of the gaseous metabolism of the tissue. With reactions of average velocity the rate of shaking of 120 per minute was found to be quite efficient.

Raab (62) who estimated the metabolism of scrapings of human endometrium, found that a piece of tissue left in Ringer's solution for about 6 hours showed far reaching destruction of cell structure, whereas in serum no such degeneration occurred.

The Barcroft apparatus which I used had the following advantages:

(1) The tissue could be contained in a fluid so that a known concentration of a drug might be applied directly to it.

(2) A large surface for the absorption of CO_2 was provided by the use of the filter paper (f), Fig. 3.

(3) Limb (b) (Fig. 2) provided an easy method of adding drugs to the fluid bathing the tissue during the course of the experiment.

(4) /

(4) The same limb afforded an easy way for circulation of oxygen during the oxygenation of the experimental chamber.

Six control experiments were performed to determine the amount of oxygen consumed by the filter paper. The consumption was never greater than 2 cmm. per hour, a figure which falls within the limits of the experimental error of the investigations.

Four experiments were carried out in which the oxygen consumption of the uterus of the same rat was measured over a period of 3 hours in Ringer and in serum. For this purpose a single uterine horn split longitudinally into two halves was used. One half was immersed in Ringer while the other was immersed in serum. The results are recorded in Protocol 1. These results show that there was no alteration in the rate of oxygen consumption when Ringer's solution was substituted for serum.

In my experiments, therefore, the oxygen consumption of the uterus immersed in Ringer's solution was measured.

Warburg (70) and Hill (36) have shown that when the oxygen consumption of a tissue is measured, it/

it is necessary that every cell should receive an adequate supply of oxygen. The thickness of the tissue is therefore of importance because the cells lying deeply must receive their oxygen supply by diffusion. Warburg and Hill calculated the critical thickness of a tissue which would allow sufficient oxygen diffusion to provide all the cells of the tissue with an adequate supply of oxygen. They assumed that the tissue was using oxygen at a constant rate and that diffusion of oxygen took place from a gaseous or liquid phase in which its concentration was constant.

Hill's formula deals with cylindrical tissues. David (19) applied this formula in his discussion on the oxygen consumption of the isolated uterus of the rat and mouse. He stated that rats' uteri and those of adult mice in oestrus fall beyond the limits of Hill's formula, whereas the uteri of immature, adult dioustrous and adult castrated mice are within the limits of the formula, i.e. receive an adequate supply of oxygen if suspended in an atmosphere of this gas.

I confirmed the measurements of the last author and agree with his conclusions. Since my experiments were/

were conducted on rat's and mice's uteri, which are cylindrical tissues, and although a cylindrical tissue may theoretically be considerably too thick for full oxygen diffusion, yet the experimental error may be small. For example if full oxygenation can occur to a depth of 0.5 mm. then in the case of a cylinder with a radius 50 per cent. greater (i.e. 0.75 mm.) the percentage of tissue that will not be oxygenated fully is only

$$\text{vol. of cylinder} = l \times \pi r^2 \quad \text{when } l = \text{length, } r = \text{radius.}$$

$$\text{vol. of cylinder} (r = 0.75) = l \times \pi \times (0.75)^2$$

$$\text{vol. imperfectly oxygenated} = l \times \pi \times (0.25)^2$$

$$\text{Per cent. imperfectly oxygenated} = \frac{100 \times l \times \pi \times (0.25)^2}{l \times \pi \times (0.75)^2}$$

$$= 11 \text{ per cent.}$$

But although the rate of oxygen consumption of the rat's uterus and that of adult mice in oestrus cannot therefore be measured in absolute figures, yet the results which have been obtained for these tissues were found useful for comparative purposes. For instance the ovariectomised rat's uterus consumes less oxygen per gram of tissue than the uterus of the/

the uncastrated rat at any part of the oestrous cycle in spite of the fact that the diameter of the uterus of the ovariectomised animal is always less than that of the uncastrated animal.

A few experiments were carried out in order to separate the mucous membrane of the uterus from the muscular layer, so as to study the oxygen consumption of each part separately. Two different methods were attempted:

1. Separation of the fresh tissues by means of fine forceps under a dissecting microscope. This procedure could only be attempted in the case of oestrous uteri, and it was found difficult even in such cases to strip the mucous membrane completely in one piece.

2. It was found that freezing the uterus does not alter appreciably its oxygen consumption. An attempt was therefore made to separate the two layers of the uterus by means of the freezing microtome.

For this purpose the oxygen consumption of one horn of the uterus was measured as soon as the uterus was removed from the body. This served as a control. The other horn was then split longitudinally/

longitudinally along the line of attachment of the broad ligament into two halves. Both halves were immersed in 6 per cent. gum saline and then both were frozen on the freezing microtome. One half was then removed; thawed, and its oxygen consumption measured. The other half was split by means of the microtome into its two layers. The oxygen consumption of each of these was then measured.

The results showed that the oxygen consumed per unit of weight by the two layers of the uterus was nearly equal to that of the control, but that the oxygen consumed by each layer separately showed very wide variations. This was mainly due to inclusion in the muscular layer of a variable part of the glandular layer of the endometrium and this fact was confirmed histologically.

As no definite conclusions could be drawn from these experiments, it was decided to use the uterus (muscle and mucosa) for investigating its oxygen consumption.

Experimental Animals.

The isolated uterus from the following classes of animals was used for investigation:

A. Adult female rats, (1) in dioestrus
(2) in oestrus
(3) castrated

B. /

B. Adult female mice, (1) in dioestrus
(2) in oestrus
(3) castrated

C. Immature mice.

The sexual cycle was diagnosed by the vaginal smear technique of Long and Evans (47), and Allen and Doisy (2).

Coward and Burn (19), and Burn (11) used a spatula or a blunt probe for obtaining the smear in mice, and examined the unstained smear under a low power microscope. Frank (30) advocated the use of a capillary pipette and stained the films with a simple stain. David (20) used a platinum loop, and stained the films in cases of suspected oestrus.

I used a modification of Frank's method for rats. A pipette with a fine drawn end containing a drop of Ringer's solution was introduced in the vagina, the drop was then mixed with the vaginal contents, and a little was drawn off to be smeared on a slide. It was found convenient to put the rat, head first in a long narrow cylinder, so that it would not be able to turn round. The animal was held in this position and the smear obtained. For mice a platinum/

platinum loop holding a drop of Ringer was used. All smears were examined immediately, and in any case of suspected oestrus the smear was stained with methylene blue.

The criterion for oestrus was the complete absence of polymorphnuclear leucocytes from the vaginal smear. The presence of any polymorph nuclear leucocytes was considered to indicate the dioestrous phase. Allen and Doisy (2) and Long and Evans (49) described the appearances of the smear in detail.

Castration in both mice and rats was conducted according to the method described by Burn (12), but as the skin in these animals is freely mobile a single dorsal incision was usually made and both ovaries removed. Animals were not experimented upon for at least 14 days after the operation.

Dr Wiesner of the Animal Genetics Department of the University of Edinburgh kindly supplied mice between 3 and 4 weeks old. The vaginal orifice in these animals was not yet established and their weights varied from 8-12 grams with an average of 9.5 grams. They were therefore definitely immature animals.

The average length of the oestrous cycle in the rat was given by Long and Evans (49) as 5.4 days for/

for 1,999 cycles. Of these 82 per cent. had an average of 4.6 days. In the mouse Allen (1) gave the average length of 4-5 days; whereas Parkes (60) gave a mean of 6.2 days for 1,000 cycles. I found that the average length of the oestrous cycle in rats was 5 days and in mice 5.5 days. Only animals in which the cycle was regular, were used for investigations.

(d) Factors influencing the Water Content of the Uterus.

The oxygen consumption of the isolated uterus is expressed in terms of the number of c.c. of oxygen which are consumed in one hour by one gram of uterine tissue.

Previous workers on the oxygen consumption of the isolated uterus used the wet weight, i.e. the weight of the tissue when taken freshly out of the body as the basis for their calculations.

Okey, Bloor and Corner (55) however, working on the lipoid content and oestrus in the uterine mucosa of the pig, found a great variation in the water/

water content of the tissue at different stages of the oestrous cycle, and that the highest water content was at the stage of congestion.

Frank (30) on the other hand, using rabbits' uteri for his investigations stated that the increase in weight and size of the uterus during oestrus was not due to a great increase in the water content of the uterus.

David (20) using isolated mice's uteri at the various stages of the oestrous cycle, found that regardless of the stage of the cycle, uteri weighing from 10-60 mgm. had a dry weight which was 21 per cent. of the wet weight, and that in the case of uteri of less than 10 mgm. wet weight, the dry weight rose to 30 per cent. He suggested that this rise in the case of the small uteri was largely due to evaporation of water during weighing the fresh tissue.

Scott and myself (40) on the other hand, reported marked variation in the water content of the isolated mouse's uterus during the different phases of the oestrous cycle.

It was therefore necessary to calculate the wet as well as the dry weight in every case in these investigations. After measuring its oxygen consumption/

consumption the uterus was dried with filter paper, and the wet weight was obtained by weighing on a torsion balance. It was then transferred to a clean watch glass and desiccated in vacuo over phosphorus pentoxide for at least 48 hours and the dry weight measured on a micro balance.

In six control experiments the wet weight of the uterus was determined immediately before and after the measurement of its oxygen consumption. No difference in the wet weight was found, and therefore immersion in Ringer did not alter appreciably the water content of the uterus.

Hill (37) has shown that all the water in the muscle can be considered as being free, and that there was no part of it combined. No attempt was made here to repeat this work in the case of the uterus as that consideration did not affect these calculations.

The water content of the isolated uterus of the rat in the various stages of the oestrous cycle is shown in Table I.

Table I /

Table I.

The water content of the isolated uterus of the rat during the various stages of the oestrous cycle.

State of animal	No. of animals	Av. wet weight	Av. dry weight	% water	% solids	Standard deviation
Normal in oestrus	16	196.1	37.34	80.9	19.1	0.92
Normal in di-oestrus	12	95.2	18.09	81.0	19.0	2.11
Adult castrates	17	50.1	9.83	80.4	19.60	1.32
Artificial oestrus by Female Hormone Ciba	2	132.5	22.55	82.9	17.10	

These results show that there is no increase in the water content of the rat's uterus at the various stages of the cycle.

The water content of the isolated uterus of the mouse at the various stages of the oestrous cycle and under the influence of certain substances is shown in Table II.

Table II.

The water content of the uterus of the mouse during the various stages of the oestrous cycle and under the influence of certain hormones.

State of animal	No. of animals	Av. wet weight	Av. dry weight	% water	% solids	Standard deviation
Normal in dioestrus	22	36.1	6.68	81.5	18.5	2.94
do. in oestrus	17	101.3	16.22	84.2	15.8	1.42
Normal immatures	36	6.36	1.43	77.5	22.5	2.53
Artificial oestrus in immature mice by						
1. Female Hormone 'Ciba'	4	29.0	4.13	85.2	14.2	
2. Sistomensin	2	34.5	4.77	86.17	13.83	
3. Dihydroxyoestrin	6	36.8	5.99	83.7	16.3	
4. Trihydroxyoestrin	2	20.0	4.69	76.57	23.43	
Castrates	74	15.99	3.25	79.7	20.3	1.62
Artificial oestrus in castrated mice by -						
1. Female hormone 'Ciba'	8	47.62	6.16	87.02	12.92	0.88
2 /						

Table II contd.

	State of animal	No. of animals	Av. wet weight	Av. dry weight	% water	% solids	Standard deviation
	Artificial oestrus in castrated mice by						
2.	Di-oestrin	4	61.0	7.92	87.0	13.0	
3.	Tri "	2	37.0	5.78	84.4	15.6	
4.	Equal parts of 2 and 3	4	49.3	6.81	86.2	13.8	
5.	Pregnant human female serum	2	141.0	16.5	88.3	11.7	
	Castrates treated by corpus luteum extract	6	36.6	5.60	84.5	15.5	

Table II shows the following changes in the water content of the mouse's uterus:

This is lowest in immature mice (77.5 per cent) and in castrates (79.7 per cent),but rises in adult mice and is higher in oestrus(84.2 per cent)than in dioestrus (81=5 per cent).

Production/

Production of artificial oestrus by various means caused a well marked rise in the water content in all cases, except where pure trihydroxyoestrin was given. The general averages for the figures excluding the experiments with trihydroxyoestrin were 84.6 per cent. in the case of immature mice and 86.9 per cent. in the case of castrates. These figures represent a respective increase of 7.1 and 7.2 per cent. of water content. The few figures obtained with trihydroxyoestrin suggested that this substance produced oestrus without causing as great a change in the water content. The injection of corpus luteum extract also caused a rise in the water content of the uterus.

These changes in the water content are significant in relation to the oxygen consumption of the uterus, for it must be assumed that the oxygen consumption is dependent on the solids present and the increase in water content in artificial oestrus represents a reduction by one third of the percentage of solids. For this reason all figures for oxygen consumption are given in terms of dry weight of the uterus at N.T.P.

(e) The Influence of the Oestrous Cycle on the Oxygen Consumption of the Isolated Uterus of the Rat and Mouse.

Slonaker (64), and Wang, Richter and Guttemacher (69) found that the spontaneous activity of rats underwent cyclical variation in correlation with the oestrous cycle, and that the maximum activity occurred during oestrus. Bugbee and Simond (10) stated that this increased activity stopped after ovariectomy, but could be brought about in the ovariectomised animal by the injection of oestrin. Further they showed that continuous injection led to continuous activity. These experiments would suggest that the metabolism was raised during oestrus.

Lee (46) found, however, that during oestrus the average heat production in the rat was below the general mean, and reported an increase of about 12 per cent. in heat production during the last 10 hours of dioestrus, and the beginning of pro-oestrus. According to that author oestrin is not primarily a metabolic stimulant.

Wiesner and Fraser (72) on the other hand, found a definite increase in the CO_2 output in rats

12-24 hours after full cornification, and that ovariectomy diminished the output of CO_2 greatly and also the variability of output from day to day. They stated that in dioestrus the basal metabolism reaches that of the ovarectomised animal. They suggested that the rise in metabolism may be due to

(1) oestrin
(2) other substance or substances.

Oestrin in this respect would have a delayed effect i.e. cornification, enlargement and secretory activity of the uterus occurred much earlier than the rise in metabolism. It seems probable that the rise in metabolism culminates shortly after rupture of the follicle and during the tubal journal of the ovum, thus it might be that any substance or substances other than oestrin, and causing the rise in metabolism, are liberated from the follicles.

David (20) investigated the effect of the oestrous cycle on the oxygen consumption of the isolated uterus of the mouse. He found slight variation in the mean figures for the various phases of the oestrous cycle, but a better marked variation in the distribution graphs for the rates of oxygen consumption in each group of animals. He gave the following/

following figures.

Mouse's uterus in	dioestrus	consumed	1.7 c.c. O_2	per gram per hour
" "	oestrus	"	1.8 c.c.	"
Immature mouse's uterus		"	1.8 c.c.	"
Castrated " "		"	1.58 c.c.	"

He therefore found a greater oxygen consumption rate in adult mice in oestrus and in immature mice than in adult mice in dioestrus. He also found that the castrated mouse's uterus had a lower oxygen consumption rate than any type of non-castrated mice.

I repeated David's experiments using the apparatus which has already been described.

Experimental Results in Rats.

1. Oxygen consumption of the adult dioestrous rat's uterus.

Rats were used which were known to have a regular oestrous cycle by means of the vaginal smear technique. They were killed when the smear showed the dioestrous characteristics. The uterus was dissected out and immersed in Ringer Locke's solution. The oxygen consumption was measured by means of the technique which has been described. The results of/

of 12 rats are recorded in Protocol 2 and distribution graph (1). The figures given in Protocol 2 show that the metabolic rate of the uterus varies from 4.0 c.c. to 13.30 c.c. per gram per hour, with an average value of 6.3 c.c. and a standard deviation of 2.25. The scatter of the results average can be reduced greatly by dividing the uteri into two groups according to their weight as in Table III.

Table III.

The relationship between the oxygen consumption of the dioestrous rat's uterus and its weight.

	Dry weight in mgm.	No. of animals	Av. dry wt. in mgms.	Av. O_2 consumption per gram per hour at N.T.P.
Group I	>10	8	22.8	4.8 c.c.
Group II	<10	4	6.4	10.1 c.c.

This result shows that the smaller uteri have a metabolic rate more than twice as great as the larger uteri.

The most obvious explanation is that the thickness of the larger uteri is too great to permit adequate/

adequate oxygen diffusion, since the larger uteri have a diameter of more than 2 mm. and hence according to Hill's formula they are too thick for adequate oxygen diffusion.

This possible cause cannot be excluded, but it will be shown later in the case of the uterus of the mouse in dioestrus that a similar variation in oxygen consumption occurs and in this case it cannot be explained by differences in oxygen diffusion.

2. Oxygen consumption of the adult oestrous rat's uterus.

In the same way the oxygen consumption of the adult oestrous rat's uterus was measured. The vaginal smear in these animals showed the presence of cornified epithelial cells in great number and no polymorph nuclear leucocytes.

The results of 6 rats are recorded in Protocol 3 and graph (2).

The oxygen consumption varied from 3.3 c.c. to 6.9 c.c. with an average of 5.2 c.c. per gram per hour, and a standard deviation of 1.20. It has already been stated that these figures for oxygen consumption cannot be considered to be absolute figures/

figures because the diameter of these uteri was on an average 4.7 mm. and would certainly fall beyond the limit's of Hill's formula. They may however be convenient for comparative purposes.

Comparison of distribution graphs (1) and (2) shows that the oxygen consumption rate is greater in dioestrus than in oestrus. It also shows that there is greater variation in the oxygen consumption in dioestrus than in oestrus.

The average rate of oxygen consumption in oestrus which is 5.2 c.c. per gram of dry weight per hour is however slightly greater than the average rate of oxygen consumption of uteri of similar weights in dioestrus (group I, Table III, uteri with dry weights more than 10 mgm.) which is 4.8 c.c. per gram per hour.

(3) Oxygen Consumption of the Adult Castrated Rat's Uterus.

Rats were castrated in the manner which has been described (p. 27). At least 14 days were allowed to elapse between their castration and use in experiments. The oxygen consumption of the uteri of 17 castrated rats is recorded in Protocol 4 and graph (3). The oxygen consumption varied from 2.06 c.c. to 4.78 c.c. per gram per hour with an average of 3.43 c.c., and a standard deviation of 0.72. In this case the oxygen consumption was not affected by the weight of the uterus, for the eight larger uteri had an oxygen consumption of 3.3 c.c. per gram per hour, while this figure in the case of the nine smaller uteri was 3.5 c.c. per gram per hour.

Comparison of the distribution graphs (1), (2) and (3) shows that the oxygen consumption rate of the isolated uterus of the castrated rat is much less than that of the non-castrated animal and shows much less individual variation, although the diameter of the uterus in the castrated animal was on an average 1.5 mm. as compared with 1.7 mm. in dioestrus and 4.5 mm. in oestrus, and the uterus in the first case should therefore receive more edequate amounts of oxygen by diffusion than in the last two cases.

The/

The average figures for the oxygen consumption of the isolated uterus for the various types of rats which were used are summarised in Table IV.

Table IV.

The average oxygen consumption of the rat's uterus at the various stages of the oestrous cycle.

Condition of rats.	No. of animals.	Av. wet wt. in mgm.	Av. dry wt. in mgm.	Average O_2 consumption per gram dry weight per hour at N.T.P.
Adult castrated rats	17	50.1	9.84	3,43 c.c.
Rats in dioestrus with				
(a) small uteri	4	30.88	6.40	10.10 c.c.
(b) large uteri	8	109.46	22.80	4.80 c.c.
Rats in oestrus	6	196.08	37.34	5.20 c.c.

Experimental/

Experimental Results in Mice.

1. The oxygen consumption of the uterus of the adult mouse in dioestrus.

In the case of rats in dioestrus the results have already been shown to fall into two groups. In the first the oxygen consumption was high and the weight of the uterus low, and in the second the reverse was found (Table V). These results suggested that the oxygen consumption might vary according to the stage of dioestrus, whether early or late.

Mice which were known to have a regular oestrous cycle were chosen. They were killed during the period of dioestrus and the oxygen consumption of the uterus was measured. They were divided into two groups:-

(1) Those which had been in oestrus 24 hours previously. The vaginal smear of these animals consisted almost entirely of polymorph nuclear leucocytes.

(2) Those mice which had been in oestrus 66-72 hours previously. The vaginal smear of these animals consisted of leucocytes but also contained a large number of nucleated epithelial cells/

cells. The uterus at this stage appeared on naked eye examination to be more attenuated than at any other stage of the oestrous cycle.

The results of the oxygen consumption of these groups of dioestrous mice are recorded in Protocol V. and distribution graph (4).

The average rate of oxygen consumption during 'early dioestrus'(group I) was 10.39 c.c. per gram per hour with a standard deviation of 1.2; whereas during'late dioestrus' (group II) it was nearly twice this figure, namely 21.92 c.c. per gram per hour with a standard deviation of 4.5. The average dry weight in early dioestrus was 4.4 mgm. and in late dioestrus 2.4 mgm.

The question at once arises whether this remarkable difference in oxygen consumption is due to differences in the diameter of the uterus affecting the diffusion of oxygen. My calculations and also those of David (20) agree however in showing that the diameter of the uterus in dioestrus is within the critical limits of Hill's formula. This is clear from the following calculations.

$$ro = \sqrt{\frac{4.K.Yo}{a}}$$

where/

where ro = radius of the cylindrical tissue which can receive oxygen effieiently by diffusion.

K = Krogh's constant. This is 1.4×10^{-5} for muscle at 20°C. and since it rises 1 per cent. per 1°C. rise in temperature, it is about 1.64×10^{-5} at 37°C.

Yo = the concentration of oxygen in atmospheres

a = the oxygen usage observed in c.c. of oxygen per gram of wet weight per minute.

In the case of early dioestrous uteri the observed oxygen consumption is on an average 10.34 c.c. per gram of dry weight per hour or 0.032 c.c. per gram of wet weight per minute. The critical radius would therefore be

$$ro = \frac{4 \times 1.64 \times 10^{-5}}{0.032}$$

$= 0.045$ cm. and the critical diameter 0.090 cm.

The diameter of the uterus of the mouse may be approximately calculated from the length and weight of the uterus. The assumptions are made that the uterus is cylindrical and the specific gravity unity. The diameter is then $2\sqrt{\frac{W}{\pi L}}$ where W is the weight in grams and L the length in cm.

In this way the diameters of the uteri in the early stage of dioestrus were measured and were found/

found to average 0.09 cm. Since this figure lies within the limits of that calculated from Hill's formula, it was assumed that the rate of diffusion of oxygen was sufficient to supply the oxygen need of the uterus under the conditions of the experiment.

The diameters of the uteri in the late stage of dioestrus were less than that of the early dioestrus uterus and were on an average 0.07 cm. The critical diameter calculated from Hill's formula was 0.078 cm. and therefore the uterus of the late as well as that of the early stage of dioestrus receives an efficient supply of oxygen by means of diffusion.

Hence the remarkable difference in oxygen consumption of the uterus during early and late dioestrus cannot be explained on the grounds of deficiency of oxygen diffusion in the case of the early dioestrous uterus where the oxygen consumption was low. It seems therefore likely that in the intact animal some substance which increases in uterine metabolism is periodically secreted and that it produces its effect about the end of the dioestrous phase.

Parkes (58) states during dioestrus the uterus is/

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 same author also states: "During the stage of pro-oestrus the leucocytes disappear altogether from the vaginal contents, and the smear is made up entirely of lightly staining nucleated epithelial cells."

The stage of late dioestrus at which I observed the remarkable rise in oxygen consumption is clearly antecedent to this pro-oestrous stage and also antecedent to ovulation, since its most obvious characteristic was the small size of the uterus.

(2) Oxygen Consumption of the Uterus of the Adult Mouse in Oestrus.

In the same way mice with regular cycles were killed when their vaginal smears showed the characteristics of full oestrus. The results of 8 experiments are recorded in Protocol VI and distribution graph (5). The oxygen consumption varied from 7.5 c.c. to/

to 11.2 c.c. per gram per hour with a mean of 8.39 c.c., and a standard deviation of 1.197.

The results of these experiments show that the isolated uterus of the adult mouse in oestrus consumes slightly less oxygen than in early dioestrus and much less oxygen than in late dioestrus.

(3) Oxygen Consumption of the Isolated Uterus of the Adult Castrated Mouse.

Mice were castrated in the manner already described. As in rats at least 14 days were allowed to elapse between their castration and use in experiments. During that interval the vaginal smear did not show any signs of cornification. Protocol VII and distribution graph (6) show the oxygen consumption of 16 castrated mice. The oxygen consumption varied from 4.9 to 12.9 c.c. per gram per hour with an average of 8,3 c.c. and a standard deviation of 2.1.

Another series of controls were made about 6 months later on a second batch of castrated mice. These results are shown in Protocol VIII and graph (7). In this case the dry weight was calculated from the general average of 20.3 per cent. of the wet/

wet weight, which is given in Table II. The oxygen consumption varied from 3.94 c.c. to 9.21 c.c. with an average of 5.83 c.c. per gram per hour, and a standard deviation of 1.52.

These results show that the average oxygen consumption of the second batch of castrated mice was somewhat lower than that of the first batch, but this difference is not sufficient to constitute a serious experimental error.

The average figures for the oxygen consumption of the isolated uterus for the castrated and non-castrated mice are summarised in Table V.

Table V. /

Table V.

The average rate of oxygen consumption of the mouse's uterus at the various stages of the oestrous cycle.

Condition of the Mouse.	No. of animals	Av. wet wt. in mgm.	Av. dry wt. in mgm.	Av. O_2 consumption per gram per hour at N.T.P.
Adult mice in oestrus	8	68.3	10.6	8.4 c.c.
do. in dioestrus (early)	7	23.4	4.4	10.4 c.c.
(late)	5	12.1	2.4	21.9 c.c.
Adult castrated mice (batches 1 and 2)	25	17.5	3.7	6.8 c.c.

Two conclusions may be drawn from the results of these experiments:

1. The ovary secretes a substance which causes increase in the oxygen consumption of the uterus, since the oxygen consumption of the uterus of castrated animals is definitely less than that of non-castrated animals at any stage of the oestrous cycle.

2. /

2. This substance is probably secreted towards the end of the dioestrous phase since the highest values for oxygen consumption were found at the end of that period.

(4) Oxygen Consumption of the Isolated Uterus of the Immature Mouse.

The mice used were between 3-4 weeks old, their vaginal orifices were not yet established and their weights varied from 8-12 grams with an average of 9.5 grams. They were therefore definitely immature. The oxygen consumption of 36 such mice is recorded in Protocol IX and distribution graph (8). The oxygen consumption varied from 4.2 c.c. to 19.1 c.c. with a mean value of 9.43 c.c. per gram per hour, and a standard deviation of 4.7 . Distribution graph (8) shows the variation in oxygen consumption rate in this group of animals.

These results show that there is great individual variation in the rate of oxygen consumption of the immature mouse's uterus. Since the diameter of these uteri falls definitely within the limits of Hill's formula, it is probable that the great variation is due to different stages of sexual development.

SUMMARY.

The averages of my results are shown in Table VI. For comparison I have also included David's (19) results. He calculated his results as oxygen used per gram of wet weight of uterus and I have converted his figures to dry weight values on the basis of the figures given in Table II.

Table VI.

Average oxygen consumption of uterus of the mouse in c.c. per gram dry weight per hour at N.T.P.

Condition of Mice	Author's Expts.			David's experiments.	
	No. of mice	Av. O_2 consumpt.	Stand. Dev.	No. of mice.	Av. O_2 consumption.
Immature	36	9.4	4.7	55	8.0
Adult castrate (batch 1 and 2)	25	6.8	2.28	29	7.5
Oestrus	8	8.4	1.2	8	11.0
Dioestrus: Total	12	15.2	6.5	16	9.2
Group I (early)	7	10.4	1.2		
Group II (late)	5	21.9	4.5		

The/

The average of my results shows no striking difference between the oxygen consumption of the uterus of mice, immature, castrated, in oestrus, and the majority (Group I) of mice in dioestrus, but shows that during the latter period of dioestrus (Group II) the oxygen consumption is doubled. The only important difference between David's and my results occurs in the figures for the oxygen consumption of the uterus of dioestrous mice. David's figures agree with mine when mice in early dioestrus are considered, but not in the case of mice in late dioestrus. The difference may be explained by the use of different criteria of oestrus and dioestrus. The methods used by David and myself to measure the oxygen consumption were different, and the general agreement between most of our figures is satisfactory.

As regards variation it is noteworthy that castrated animals give consistently more uniform results than do immature animals; this fact is of importance in regard to the experiments described in the second part of this work.

The results obtained with rats are shown in Table VII, which is the same as Table IV mentioned on page 42.

Table VII.

Average rate of oxygen consumption of the rat's uterus at the various stages of the oestrous cycle.

Condition of rats	No. of animals	Av. wet wt. in mgm.	Av. dry wt. in mgm.	Average O_2 consumption per gram dry wt. per hour at N.T.P.
Adult castrated rats	17	50.1	9.84	3.43 c.c.
Rats in dioestrus with (a-) small uteri	4	30.88	6.40	10.1
(b) large uteri	8	109.46	22.80	4.8
Rats in oestrus	6	196.08	37.34	5.2

The /

The diameter of the ovarectomised rat's uterus falls within the limits of the critical diameter calculated from Hill's formula and in such cases the uterus was assumed to receive an adequate supply of oxygen by diffusion. The oxygen consumption of the castrated rat's uterus was found, however, to be definitely less than that of the non-castrated animal at any stage of the oestrous cycle. Since these results are corroborated by those obtained with mice, it is concluded that the ovary secretes a substance which causes a rise in the oxygen consumption of the uterus.

The results of the experiments with rats and mice are compared in Table VIII.

Table VIII /

Table VIII.

Comparison between the oxygen consumption of the uterus of rats and mice at corresponding phases of the oestrous cycle.

Condition of animal.	RATS		MICE	
	Av. dry Wt. in mgm.	Av. O_2 consumpt. per gram per hour at N.T.P.	Av. dry wt. in mgm.	Av. O_2 consumption per gram per hour at N.T.P.
Adult castrates	9.8	3.4	3.7	6.8
Dioestrous uteri:				
a. small	6.4	10,1	2.4	21.9
b. large	22.8	4.8	4.4	10.4
Oestrous uteri	37.3	5.2	10.6	8.4

This table shows that the oxygen consumption of both rats and mice varies throughout the course of the oestrous cycle in an almost identical manner. In both rats and mice the variations are small except in the case of the small dioestrous uteri, where the rate is doubled in both types of animals. It has been shown that in the case of mice, this high oxygen consumption rate occurred at the end of the dioestrous phase and was coincident with a low weight of the uterus. It is probable therefore that the low weight and coincident high oxygen consumption rate in the case of rat's dioestrous uterus occur at a similar period, namely the later part of dioestrus. If this is the case the results with rats and mice confirm each other exactly.

It is interesting to note that the oxygen consumption rate per gram per hour of the isolated uterus of the mouse is almost exactly double that of the rate in the case of the rat, at each corresponding phase of the oestrous cycle. The same ratio is observed between the oxygen consumption per unit weight of intact mice and rats. The relationship of the dry weight of the mouse's uteri with those of the rat's uteri is 1:3 at the corresponding phases of the oestrous cycle with the exception of the large dioestrous uteri, where the relationship is 1:5. Comparison of the total/

total body weight of mice and rats gives the relationship of about 1:9.

CONCLUSIONS.

The most important conclusions which can be drawn from these experiments are:-

1. The oxygen consumption per unit weight of uterus rises sharply during the last stage of dioestrus.
2. The oxygen consumption of the uterus remains nearly constant during the rest of the oestrous cycle.
3. The level of oxygen consumption is higher in immature than in castrated animals.

Protocol I.

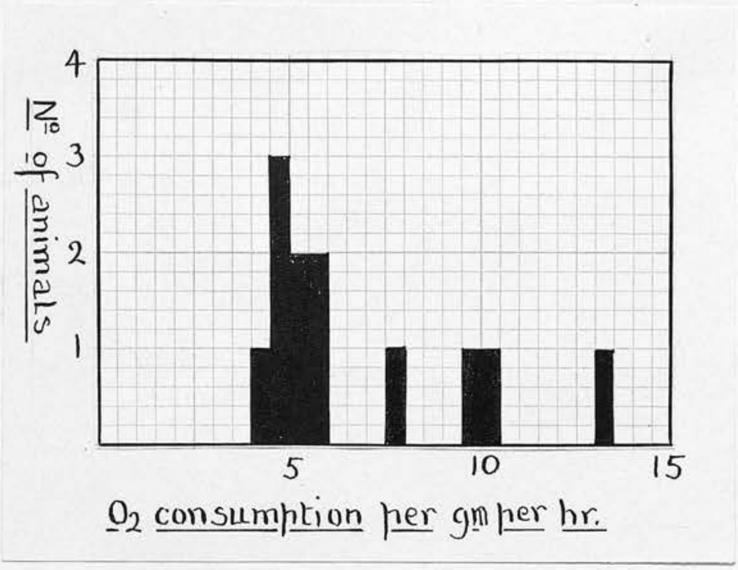
Oxygen Consumption of the Uterus of the Rat in Ringer and in Serum.

Wet wt. in mgm.	Dry wt. in mgm.	O_2 consumption per gram per hour of D.W. at N.T.P. in Ringer.	O_2 consumption per gram per hour of D.W. at N.T.P. in serum.
29.5	5.37	3.86 c.c.	
21.0	3.86		3.93
52.0	10.03	4.71	
67.0	12.93		4.69
27.0	4.38	4.95	
34.0	6.17		5.01
25.0	5.34	4.04	
36.0	10.70		3.91

Protocol II.

Oxygen Consumption of the Uterus of Adult Dioestrous Rats per gram dry weight per hour at N.T.P.

Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumption per gram dry weight per hour at N.T.P.	
20.0	4.35	21.77	78.23	13.30	
22.0	5.45	24.79	75.21	9.50	
35.0	6.51	18.60	81.40	10.20	
46.5	9.21	19.80	80.20	7.50	
58.2	11.54	19.80	80.20	5.40	
61.0	10.55	17.30	82.70	5.10	
64.5	12.11	18.80	81.20	5.00	
88.8	16.05	18.09	81.91	4.50	
102.5	21.43	20.98	79.02	4.50	
180.2	31.94	17.80	82.20	5.92	
196.5	35.54	18.03	81.97	4.00	
224.0	43.65	19.49	80.51	4.50	
95.2	18.09	19.0	81	6.3	Averages

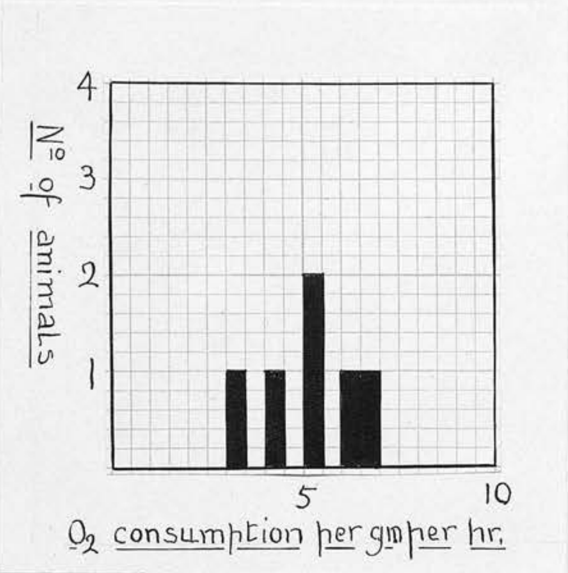


Graph 1 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult rats in dioestrus.

Protocol III.

Oxygen Consumption of the Isolated Uterus of Adult Rats in Oestrus per gram dry weight per hour at N.T.P.

Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumption per gram dry weight per hour at N.T.P.	
356.0	65.28	18.30	81.70	3.30	
193.0	34.65	17.98	82.02	6.90	
99.0	21.32	21.42	78.58	4.08	
140.5	27.90	19.85	80.15	5.10	
102.0	21.26	20.84	79.16	6.20	
286.0	53.60	18.74	81.26	5.10	
196.1	37.34	19.1	80.9	5.2	Averages

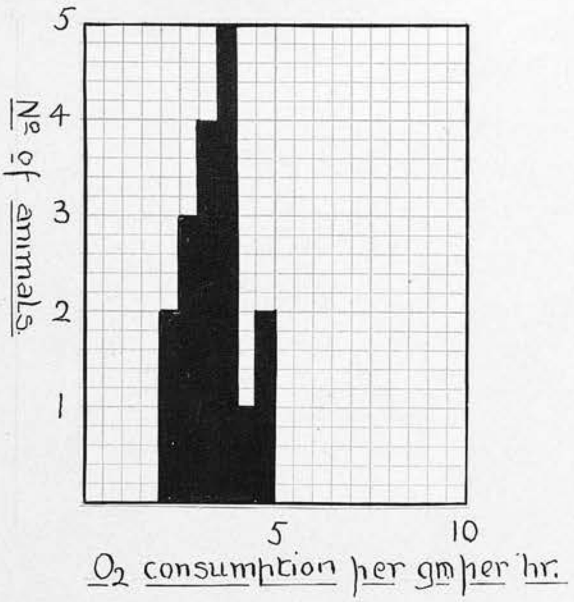


Graph 2 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult rats in oestrus.

Protocol IV.

Oxygen Consumption of the Isolated Uterus of Adult Castrated Rats per gram dry weight per hour at N.T.P.

Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumption per gram dry wt. per hour at N.T.P.	
61.0	11.30	18.50	81.50	3.72	
46.0	10.10	21.98	78.02	3.83	
75.0	15.00	20.00	80.00	2.96	
29.0	6.20	21.40	78.60	2.36	
29.0	5.30	18.27	81.73	3.07	
50.0	9.40	18.80	81.20	3.30	
40.0	7.10	17.78	82.22	3.68	
15.0	3.30	21.98	78.02	2.84	
26.0	4.60	17.70	82.30	4.60	
46.0	8.00	17.40	82.60	4.78	
82.0	16.60	20.21	79.79	2.95	
36 .0	6.60	18.32	81.68	3.22	
32.0	6.70	20.90	79.10	3.51	
67.5	12.88	19.65	80.35	4.11	
60.0	11.30	18.80	81.20	3.72	
77.0	15.70	20.40	79.60	3.38	
81.0	17.12	21.08	78.92	2.36	
50.1	9.83	19.60	80.4	3.43	Averages

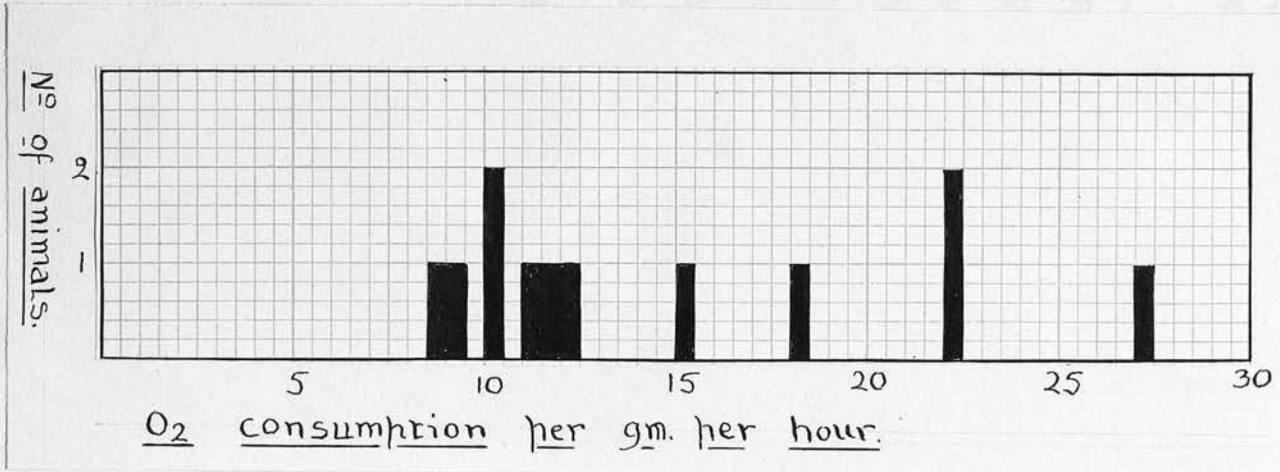


Graph 3 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated rats.

Protocol V.

Oxygen Consumption of the Isolated Uterus of Adult Mice in Dioestrus per gram of dry weight per hour at N.T.P.

Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumption per gram dry wt. per hr. at N.T.P.	
Group I					
44.0	7.23	16.40	83.60	8.50	
7.0	1.65	23,45	76,55	11.02	
21.5	4.15	19.30	80.70	11.60	
23.0	4.42	19.20	80.80	10.30	
16.5	3.46	20.98	79.02	9.10	
20.7	3.92	18.90	80.10	12.00	
31.0	6.16	19.89	80.11	10.20	
Group II					
9.0	1.66	18.49	81.51	18.30	
29.5	5.31	18.00	82.00	15.10	
6.0	1.35	22.43	77.57	27.46	
10.0	2.20	22.00	78.00	24.41	
6.0	1.49	24.82	75.18	24.42	
18.7	3.59	20.32	79.68	15.2	Averages

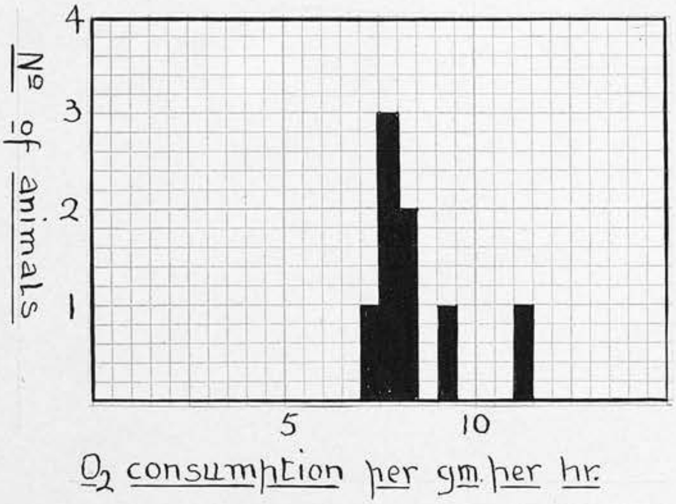


Graph 4 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult mice in dioestrus.

Protocol VI

Oxygen consumption of the isolated uterus of adult mice in oestrus per gram dry weight per hour at N.T.P.

Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumption per gram dry wt. per hr. at N.T.P.	
87.0	13.85	15.90	84.10	8.20	
39.0	6.97	17.88	82.12	7.50	
76.5	11.90	15.50	84.50	7.90	
53.0	8.89	16.75	83.25	7.70	
52.5	7.71	14.70	85.30	9.30	
102.0	14.23	13.98	86.02	8.30	
84.5	12.55	14.82	85.18	11.20	
52.0	8.48	16.30	83.70	7.30	
68 .3	17.05	15.8	84.2	8.39	Averages

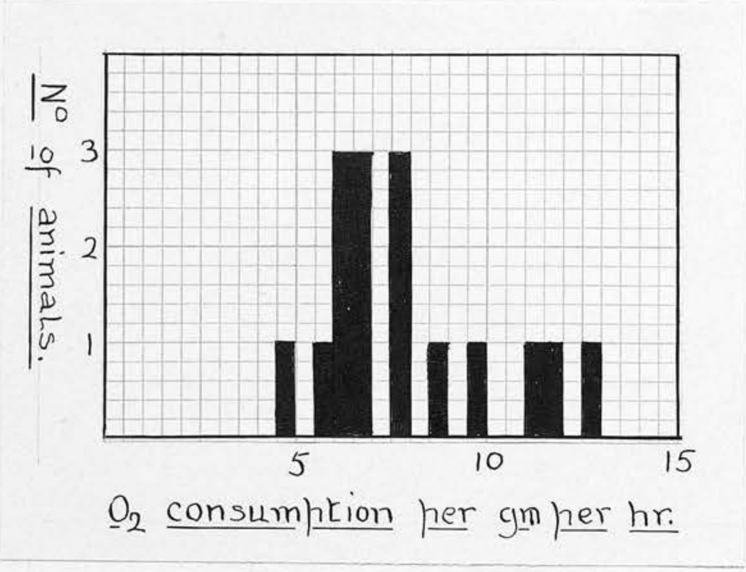


Graph 5 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult mice in oestrus.

Protocol VII.

Oxygen Consumption of the Isolated Uterus of Adult Castrated Mice, Batch I, per gram dry weight per hour at N.T.P.

Wet wt. in mgm.	Dry Wt. in mgm.	% Solids	% Water	O_2 consumption per gram dry wt. per hour at N.T.P.	
19.5	4.29	21.98	78.02	4.90	
21.0	4.38	20.81	79,09	6.38	
12.0	2.75	22.91	77.09	5.81	
14.0	3.05	21.45	78.55	6.29	
18.0	3.91	21.66	78.34	7.90	
19.5	4.26	21.81	78.19	9.54	
12.5	2.67	21.39	78.61	6.50	
19.5	4.07	20.85	79.15	11.70	
14.5	3.20	22.02	77.98	12.92	
12.5	2.90	23.20	76.80	11.35	
25.0	5.17	20.66	79.34	7.85	
6.50	1.90	29.20	70.80	6.11	
10.0	2.34	23.40	76.60	6.66	
21.5	4.21	19.60	80.40	7.94	
29.0	6.09	21.01	78.99	6.86	
22.0	4.54	20.61	79.39	8.51	
17.3	3.7	21.38	78.62	8.30	Averages

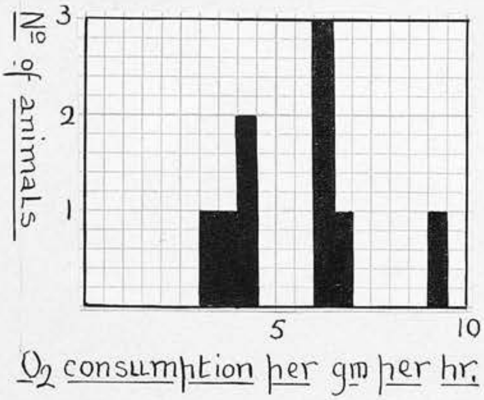


Graph 6 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice, batch I.

Protocol VIII.

Oxygen Consumption of the Isolated uterus of Adult Castrated Mice, Batch II, per gram dry weight per hour at N.T.P.

Wet wt. in mgm.	Dry wt. in mgm.	O_2 consumption per gram per hour at N.T.P.	
16.0	Calculated from Table II	6.06	
16.0		6.74	
12.0		4.95	
11.0		6.06	
18.0	3.48	6.06	
5.0		9.21	
28.0		4.95	
39.0		4.48	
15.5		3.94	
17.8		5.83	Averages

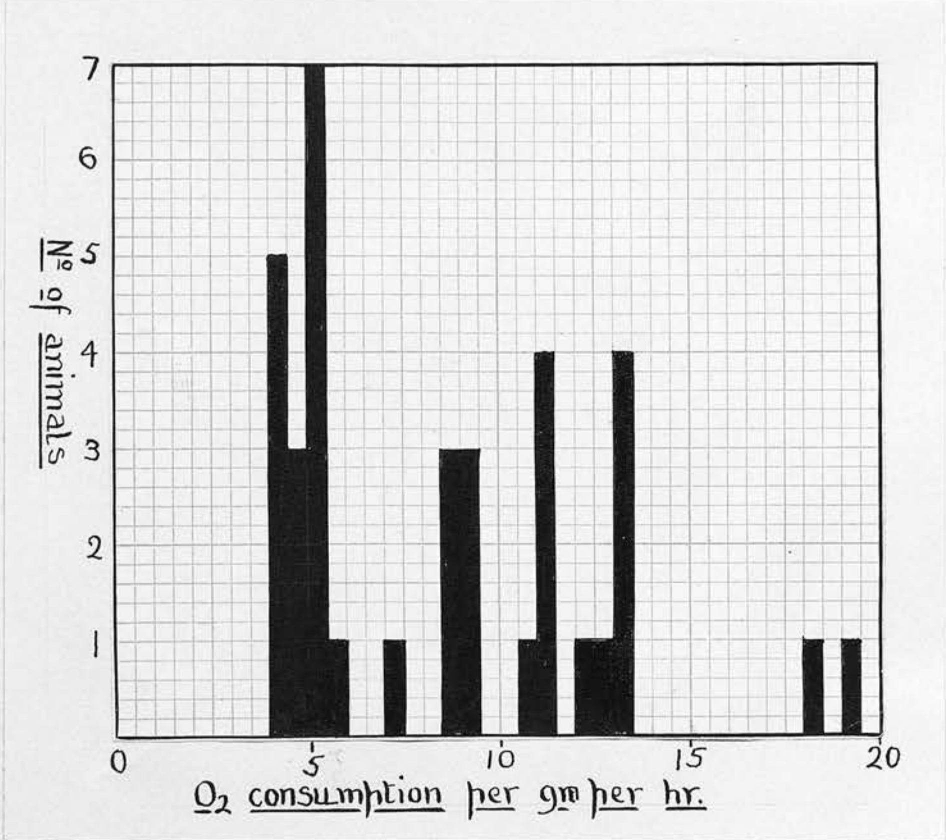


Graph 7 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice, batch II.

Protocol IX.

Oxygen Consumption of the Isolated Uterus of Immature Mice per gram of dry weight per hour at N.T.P.

Wet wt. in mgm.	Dry Wt. in mgm.	% Solids	% Water	O_2 consumption per gram dry wt. per hour at N.T.P.	
3.50	0.82	23.40	76.60	10.90	
4.00	0.78	19.50	80.50	12.30	
3.00	0.78	26.00	74.00	5.69	
6.50	1.48	22.80	77.20	4.20	
7.50	1.75	23.18	76.82	5.05	
6.50	1.35	20.78	79.22	4.30	
6.50	1.61	24.78	75.22	19.10	
6.50	1.39	21.40	78.60	18.31	
2.0	0.40	20.00	80.00	9.86	
28.0 (3 uteri)	7.41	26.50	73.50	4.1	
20.5(do.)	4.63	22.60	77.40	5.1	
15.0(do.)	3.05	20.30	79.70	9.0	
22.5(do.)	4.20	18.70	81.30	8.65	
28.0(do.)	6.49	23.10	76.90	4.80	
16.0(do.)	3.63	22.70	77.30	5.10	
22.5(4 uteri)	5.61	24.90	75.10	11.05	
17.5(do.)	3.98	22.80	77.20	14.20	
6.5	1.07	16.10	83.90	12.50	
6.36	1.43	22.5	77.5	9.43	Averages



Graph 8 showing the distribution of the various rates of oxygen consumption of the isolated uterus of immature mice.

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PART II.

Part II. The Estimation of the Oxygen Consumption of the Isolated Uterus in Animals to which the Various Sex Hormones had been Administered.

(a) Review of the Literature.

The experiments described in Part I of this paper showed that a sudden rise of oxygen consumption occurred at the last stage of dioestrus. The rate of uterine oxygen consumption during the rest of dioestrus and during oestrus is not markedly affected by the removal of the ovary and therefore presumably is not influenced by the ovary, but it is possible and even probable that the late dioestrous rise in oxygen consumption is caused by an ovarian secretion.

The following information is available concerning the action of ovarian secretions on metabolism.

Loewe and Richter (19) found a diminution of 10-20 per cent. in the oxygen consumption and CO_2 production of a castrated dog and bitch while at rest. On feeding the animals on ovarian substance, normal animals did not react at all, while a

castrated/

castrated bitch responded by an increase in metabolism of 30-35 per cent. which however sank slowly to normal after cessation of ovarian administration. Lüthji (21) was unable to confirm these results. Laqueur, Hart and de Jongh (18) made gasometric measurements of the CO_2 evolved and oxygen consumed for six mice simultaneously. Investigations were pursued daily for 3 weeks. It was found that an increase in basal metabolism resulted from the injection of female sex hormone, menformon, into the female castrates.

Zondek and Bernhardt (29) found an increased basal metabolism of about 12.4 per cent. after treating a woman castrated two years previously by freshly dried pig's ovaries.

Giest, Goldberger, Reiss and Landi (12) could not confirm this result. They made a careful study in which 48 human female subjects were investigated before and after castration, and found that there was no consistent effect on the basal metabolism. McLenden and Burr(26) reported a slight rise in the basal metabolic rate of a woman who had apparently been devoid of ovarian function since birth when 1000/

1000 mouse units or more of follicular hormone were injected. McLenden, Myrrick, Conklin and Wilson (27) produced a rise in the basal metabolism of a woman of 35, with a primary amenorrhoea, by the administration of 30,000 mouse units of an oil soluble oestrin supplied by Laqueur.

Bugbee and Simond (6) found that injection of oestrin caused a decline in body weight of normal and gonadectomised animals of both sexes. Brouha and Simonnet (5) and Allen and Doisy (1), however, found no effect on growth of the immature animal.

Laqueur (16) found that addition of potent female hormone extracts produced rapid increase in growth of tadpoles, while Kaufman ~~(15)~~, Muller and Mühlbock (15) found that extracts of the graafian follicles free from folliculin caused an increase in the rate of metamorphosis in tadpoles, but retarded their growth. Estes and Burge (11) stated that solutions of paramoccium to which dried ovarian substance is added, show an increase in carbohydrate metabolism and in growth.

Loewe and Voss (20), and Laqueur (17) attempted to explain two components of the female sex hormone: (1) growth stimulating "oil", and (2) an oestrus producing component.

Raab (28) stated that the ovarian hormone

must/

must play an important role in carbohydrate metabolism. He could not however explain the mode of action. Kaufmann (14) studied the effect of the female hormones on the fat metabolism. He found that injection of follicular fluid with a high oestrin content produced in 1-2 hours an increase in the blood fat content of 15-20 per cent. No such result was obtained after the injection of a non-specific albumin such as aolan, nor with extracts of pregnancy urine with the same oestrin content as the follicular fluid. He therefore suggested the presence in the follicular fluid of a metabolic hormone other than oestrin. In his address he mentioned the investigations of Kochmann and Wagner who found a specific metabolic stimulant in the follicular fluid, that is thermolabile and is not oestrin. They called it "oobolin".

Arvay (4) stated that the typical action on the oestrous cycle of normal and sp~~l~~ayed rats due to administration of ovarian preparations is an increase in basal metabolism. He also found that extracts of the anterior pituitary gland have a similar action in a normal female, but not in à spayed or male rat. He therefore concluded that a liberation of the ovarian hormone occurs under the influence of the anterior/

anterior pituitary extracts.

David(9) found an increase in the oxygen consumption of the uterus in artificial oestrus produced in immature mice by the injection of 'Ciba' Female Hormone, but not in adult castrated animals.

This review of the investigations on the action of ovarian preparations on the various metabolic processes affords no definite conclusions as to their mode of action. The following experiments were therefore carried out in order to investigate the action of different ovarian preparations on the oxygen consumption of the isolated uterus of rats and mice.

(b) Action of Female Hormone Ciba.

Oil-soluble extracts of the ovary were kindly supplied by the Clayton Aniline Company, who stated that their extraction method was based on that of Hermann and Fraenkel (13). These extracts were in three forms:-

(1) Female hormone, which was labelled		40 R.U. per c.c.
(2). Female hormone	do.	50 R.U. per c.c.
(3) Sistomensin	do.	5 R.U. per c.c.

To study the potency of each of these preparations as/

as regards the oestrin content, 12 test animals - 6 castrated and 6 immature mice - were injected with 5 R.U. in a single dose subcutaneously. It was found that all the animals were brought into oestrus between 48 and 72 hours after the injection.

(1) Action of Female Hormone 40 R.U. per c.c.

This preparation was given in doses of 5 R.U. and 20 R.U. firstly to 24 mice which had been castrated at least 14 days previously, and secondly to immature mice. The animals were killed at times varying from 30 minutes to 48 hours after the injection. The results are shown in Protocols X and XI and graphs (9) and (10).

The oxygen consumption of the uterus in the case of castrated mice was found to vary from 9.37 c.c. to 25.1 c.c. per gram per hour with an average of 14.14 c.c., and a standard deviation of 4.2. The average oxygen consumption of control animals was 8.30 c.c. per gram per hour, with a standard deviation of 2.1.

In the case of immature mice the oxygen consumption of the uterus varied from 10.60 c.c. to 21.00 c.c. per gram per hour with an average of 16.18 c.c. and a standard deviation of 3.11. The average rate of oxygen consumption of control immature mice was 9.43c.c. per gram per hour with a standard/

standard deviation of 4.7.

All the results therefore agree in showing a marked rise in the oxygen consumption above the control values. In the case of castrated mice three very high figures were obtained with doses of 20 R.U. These results (which are marked with a star) were all obtained with somewhat exceptionally small uteri. For reasons which will be explained the experiments could not be repeated on a more extended scale and therefore these results have been excluded from the averages. The remaining results both with castrated and immature mice show that three hours after the injection the oxygen consumption rose to between 15 and 20 c.c. per gram of dry weight per hour, and remained nearly at this level for 48 hours. There was no certain difference between the effects produced by 5 R.U. doses and by 20 R.U. doses.

David (9) made experiments with the same preparations and measured the oxygen consumption from 24 to 72 hours after the injection. I have corrected his results to oxygen consumption per unit of dry weight for the sake of comparison. This is shown in Table IX.

Table IX.

Oxygen consumption of uteri of injected mice in c.c. per gram dry weight per hour.

Condition of animals.	Castrated Mice				Immature Mice			
	Author		David		Author		David	
	No. of expt.	Av. O_2 consumpt.	No. of expt.	Av. O_2 consumpt.	No. of expt.	Av. O_2 consumpt.	No. of expt.	Av. O_2 consumpt.
Controls	Batch (1) 16	8.3	29	7.5	36	9.4	55	8.0
Inj. with Female Hormone 1-1.5 R.U.	-	-	-	-	-	-	100	8
5-20 R.U.								
First 6 hrs	15	13.3	-	-	6	16.5	-	-
12-72 hrs.	6	16.2	8	11	3	15.2	44	15.5

As regards the immature mice my results agree with those of David, but in the case of the castrated mice I found that the oxygen consumption was nearly doubled, whereas David found no certain increase in oxygen consumption.

The/

The results show clearly that injections of this batch of Female Hormone Ciba caused a marked rise in the oxygen consumption of the uterus and that this increase occurred about 3 hours after the injection of the hormone, long before any oestrous changes appeared, and persisted until oestrus occurred. David's results with 1-1.5 R.U. show that doses too small to produce oestrus do not increase the oxygen consumption of the uterus.

(2) Action of Sistomensin 'Ciba'

Two immature mice were injected with this preparation. The results are recorded in Protocol XII. The average oxygen consumption was 15.4 c.c. per gram per hour. The results show that this preparation caused a slight rise in oxygen consumption.

(3) Action of Female Hormone 50 R.U. per c.c.

The supply of Female Hormone 40 R.U. per c.c. became exhausted during the course of the experiments and a new supply of oily extract of the ovary was kindly supplied by the Clayton Aniline Company. This/

This was labelled Female Hormone 50 R.U. per c.c. The new preparation was tested for its oestrogenic properties and found to be active. Experiments similar to those which have been described in the case of Female Hormone 40 R.U. were carried out. 37 Castrated mice (batch II), 4 immature mice and 10 rats which had been castrated at least 14 days previously were used. The results are recorded in Protocols XIII, XIV and XV.

The oxygen consumption of the isolated uterus in the case of injected castrated mice varied from 2.84 c.c. to 13.30 c.c. per gram per hour, with an average of 6.27 c.c., and a standard deviation of 3.37. The average oxygen consumption rate of the uterus of the control castrates was 5.83 c.c., with a standard deviation of 1.52. It is therefore concluded that this preparation of the Female Hormone does not cause an appreciable rise in the oxygen consumption. The distribution of the various rates of oxygen consumption of the uterus in the injected group is recorded in graph (11).

In the case of immature mice the oxygen consumption was on an average 6.0 c.c. per gram per hour. The average rate of oxygen consumption of/

of control animals was 9.43c.c. per gram per hour with a standard deviation of 4.7. This result shows that there was no rise in the oxygen consumption of the isolated uterus of immature mice in artificial oestrus produced by this preparation of Female Hormone.

The oxygen consumption of the uterus of injected castrated rats varied from 2.92 c.c. to 5.33 c.c. per gram of dry weight per hour, with an average of 3.61 c.c. and a standard deviation of 0.82. The average oxygen consumption rate of the control castrated rats was 3.43 c.c. with a standard deviation of 0.72. The distribution of the various rates of oxygen consumption is shown in graph (12).

These results show that the Female Hormone 50 R.U. has practically no effect on the oxygen consumption of the isolated uterus of castrated and immature mice and castrated rats. Since, however, Female Hormone 50 R.U. per c.c. produced oestrus as readily as did Female Hormone 40 R.U. per c.c., the remarkable difference found in their action on the oxygen consumption of the uterus indicated that the increase in oxygen consumption was caused by a hormone distinct from the oestrous producing hormone. This/

This hormone was present in Female Hormone 40 R.U. but not in Female Hormone 50 R.U.

(c) Action of Follicular Fluid.

An ovarian substance cruder than the Female Hormone 'Ciba' was injected. This was follicular fluid derived from fresh cow's ovaries. The fluid contained about 0.5 mouse unit of oestrin per c.c. One c.c. of follicular fluid was injected subcutaneously into castrated mice (batch II), and the animals were killed from 1-3 hours after the injection. The oxygen consumption of the isolated uterus was measured at the various times. The results are recorded in Protocol XVI and graph (13).

The oxygen consumption of the injected castrate was on an average 9.51 c.c. per gram of dry weight per hour with a standard deviation of 1.87, whereas the average rate of the control castrates was 5.83 c.c. with a standard deviation of 1.52. Follicular fluid therefore causes a rise in oxygen consumption in the same way as the Female Hormone Ciba 40 R.U. per c.c.

Experiments were then carried out to find, if possible/

possible, the properties of the metabolic factor which is present in the follicular fluid.

(1) Follicular fluid was boiled, cooled and then filtered through a wet filter paper: 1 c.c. of the filtrate was injected subcutaneously into each of a group of 8 castrated mice, batch II, and the animals were killed from 1-3 hours afterwards. The oxygen consumption varied from 4.65 to 6.53 c.c. per gram per hour with an average of 5.56 c.c. and a standard deviation of 0.85. The average rate of oxygen consumption in control animals was 5.83 c.c. per gram per hour with a standard deviation of 1.52.

The results show that the filtrate of the boiled follicular fluid does not contain the stimulating factor.

(2) Preparations from follicular fluid were kindly prepared by Dr H. Tod of the Royal Infirmary, Edinburgh, for the purpose of these investigations:

(i) An olive oil extract of follicular fluid. This was prepared in the following way:- 15 c.c. follicular fluid were extracted with 3 c.c. olive oil for 12 hours at room temperature, the oil and the fluid being mixed continuously with an electric stirrer. The/

The olive oil layer was then separated and injected into 3 castrated mice, batch II. The results are shown in Protocol XVII. and Graph 14.

The oxygen consumption varied from 7.74 c.c. to 13.95 c.c., with an average of 11.70 c.c. per gram per hour as compared with an average rate of 5.83 c.c. per gram per hour with a standard deviation of 1.52 in control castrates.

(ii) An alcoholic extract of follicular fluid. This was prepared in the following manner:-

1. 45 c.c. follicular fluid were precipitated with two volumes 95 per cent. alcohol.
2. The precipitate was filtered off, and extracted in a soxhlet with alcohol.
3. The filtrate from 1 and the extract from 2 were combined and evaporated to dryness in vacuo.
4. The residue was hydrolysed with about 15 c.c. distilled water on a water bath at 50°C. for 30 minutes.
5. The product was centrifuged and gave three fractions:
    - a. oily layer.
    - b. clear aqueous layer.
    - c. greyish white sediment.

The oily layer <u>a</u> was dissolved in 4 c.c. olive oil and the sediment was suspended in distilled water. The results, on the oxygen consumption of the uterus, of injecting these fractions into castrated mice batch/

batch II are shown in Protocol XVII and Graph 14. The oxygen consumption of the isolated uterus of castrated mice (batch II) injected with the oily fraction of the alcoholic extract varied from 8.21 c.c. to 10.78 c.c. per gram per hour, with an average of 9.2 c.c. In the two castrated mice that were injected with the aqueous suspension of the sediment the average rate of oxygen consumption of the uterus was 12.19 c.c. per gram per hour.

Both these fractions therefore produced an effect which was similar to that produced by the original follicular fluid. The clear aqueous layer which was rich in oestrin did not produce, however, a rise in the oxygen consumption of the isolated uterus.

Owing to the very limited supply of follicular fluid further extractions were not possible.

These results show that a factor which increases the oxygen consumption of the uterus is present in the follicular fluid, and that it is more soluble in oil than in water, and that it is partly adsorbed on the sediment.

(d) Action of Crystalline Oestrin.

Since follicular fluid and Female Hormone 40 R.U. per c.c. caused a rise in the oxygen consumption of the isolated uterus, and since Female Hormone 50 R.U. per c.c. which was an active oestrogenic extract caused no such rise in oxygen consumption, it appeared that oestrin was not responsible for the rise in oxygen consumption. Experiments were therefore carried out with crystalline oestrin. For this purpose Dr G.F. Marrian of University College, London, kindly supplied two forms of crystalline oestrin. The chemistry of these substances may be referred to in Marrian's original papers (22, 23, 24, 25). The two compounds are:

(1) Dihydroxyoestrin 18,000 m.u. per mgm.
(2) Trihydroxyoestrin 8,000 " "

They were both water soluble and resistant to boiling in alkaline solutions. Their oestrogenic activity was tested in the same manner as the oil soluble preparations which had already been described.

Action of dihydroxyoestrin.

Dihydroxyoestrin was injected subcutaneously in doses of 6-40 M.U. into castrated mice (batch II). The/

The animals were killed 1-48 hours afterwards and the oxygen consumption of the isolated uterus was measured at the various times. The results are recorded in Protocol XVIII and distribution graph (15).

The oxygen consumption varied from 3.08 c.c. to 6.28 c.c. per gram per hour with an average of 5.01 c.c. and a standard deviation of 0.93, whereas the oxygen consumption rate in the control castrates varied from 3.9 c.c. to 9.21 c.c. per gram per hour, with an average of 5.83 c.c. and a standard deviation of 1.52.

These results show that dihydroxyoestrin does not cause any rise in the oxygen consumption of castrated mice's uteri.

Similar experiments were carried out with immature mice and castrated rats. The results are recorded in Protocols XIX and XX respectively. In the case of injected castrated rats the oxygen consumption of the isolated uterus varied from 2.92 c.c. to 4.34 c.c. per gram per hour with an average of 3.60 c.c. and a standard deviation of 0.16, whereas the average oxygen consumption rate of the control castrates was 3.43 c.c. with a standard deviation of 0.72.

In/

In the case of injected immature mice the oxygen consumption of the uterus varied from 5.8 c.c. to 9.11 c.c. per gram per hour, with an average of 6.98 c.c. and a standard deviation of 1.51, whereas the oxygen consumption rate of the uterus of the control immature animals varied from 3.86 c.c. to 19.01 c.c. per gram per hour with an average of 9.43 c.c. and a standard deviation of 4.7.

Experiments were then carried out in which oestrin was added directly to the isolated uterus of castrated mice and rats. In these experiments the oxygen consumption of the isolated uterus was first measured for 1-2 hours and then oestrin, which proved to consume negligible quantities of oxygen, was added to the Ringer's fluid bathing the uterus in doses varying from 1 to 10 M.U., and the oxygen consumption was again measured. The results are recorded in Protocol XXI.

From these experiments it is concluded that whether dihydroxyoestrin is injected into castrated or immature animals or whether added to the isolated uterus in vitro, it does not cause any rise in the oxygen consumption of the isolated uterus.

Action of Trihydroxyoestrin.

Experiments similar to those which have been described in connection with dihydroxyoestrin were carried out. The results after subcutaneous injections of trihydroxyoestrin into 14 mice which were castrated at least 14 days previously (batch II) are recorded in Protocol XXII and graph (16).
The oxygen consumption of the uterus of the injected castrated animal varied from 4.22 c.c. to 6.87 c.c. per gram per hour with an average of 5.32 c.c. and a standard deviation of 0.96, whereas the average rate of oxygen consumption of the uterus of the control castrated animal was 5.83 c.c. per gram per hour with a standard deviation of 1.52. These results show that trihydroxyoestrin does not cause any rise in the oxygen consumption of the isolated uterus of castrated mice.

Protocol XXIII records the results of injecting equal parts of dihydroxyoestrin and trihydroxyoestrin, on the oxygen consumption of the isolated uterus of castrated mice (batch II). The oxygen consumption of the uterus of the injected castrated animal was on an average 6.21 c.c. per gram per hour, with a standard/

standard deviation of 0.75.

These negative results with dihydroxyoestrin and trihydroxyoestrin furnish direct proof that the substance present in follicular fluid and Female Hormone Ciba 40 R.U. per c.c. which increased uterine metabolism, is not the oestrogenic hormone.

(e) Action of Corpus Luteum.

Since oestrin is not the constituent of the ovary which is responsible for the rise in oxygen consumption, experiments were carried out to investigate the action of the luteal hormone on the oxygen consumption of the isolated uterus. A proprietary preparation labelled Luteolipex was kindly supplied by Professor H. Knaus for this purpose. It is an oil soluble extract of the corpus luteum of which 2 c.c. contain 1 Knaus unit. The results of 11 experiments in which the preparation was injected subcutaneously into castrated mice (batch II) are recorded in Protocol XXIV. The variation in the different rates of oxygen consumption are recorded graphically in graph (17).

The average rate of oxygen consumption of the isolated/

isolated uterus of the injected castrate was 6.31 c.c. per gram per hour, with a standard deviation of 2.45, whereas that of the control castrate was 5.83 c.c. per gram per hour with a standard deviation of 1.52.

It is concluded from these results that the luteal hormone does not cause an appreciable rise in the oxygen consumption of the isolated uterus and cannot therefore be the responsible factor in the crude ovarian extract, Female Hormone 40 R.U. and in the follicular fluid, that caused the rise in oxygen consumption.

(f) The Action of Human Serum.

Experiments were carried out to find if human serum contained the metabolic stimulating factor, which had been discovered in the crude ovarian preparations. Two types were used:

1. Serum from pregnant females during the 8th month of pregnancy.
2. Serum from males.

Protocol XXV records the results after the subcutaneous injection of the pregnant serum to castrated mice (batch II). The oxygen consumption was on an average 5.9 c.c. per gram per hour with a standard deviation/

deviation of 0.70 . There was therefore no rise in oxygen consumption. This serum induced oestrus in castrated mice in 48 hours.

Protocol XXVI records the results obtained when male serum was used. The average rate of oxygen consumption was 5.07 c.c. per gram per hour with a standard deviation of 0.785. Since the average rate of oxygen consumption in the control castrates was 5.83 c.c. per gram per hour with a standard deviation of 1.52, it was concluded that neither types of serum contained the metabolic stimulating factor in measurable concentration.

(g) Action of Thyroxine.

Carter (8) in studying the action of thyroxine on the oxygen consumption of the spermatozoa of Echinus miliaris, found that this drug in concentrations of 1 in 45,000 to 1 in 75,000 produced effects which were analogous to and apparently of the same nature as the effects of secretions of the eggs of the same species on the oxygen consumption, but that the effects of the egg secretions were greater in extent. He therefore suggested that the/

the egg secretions contain, in addition to other known constituents, either a body of which the physiological effect was similar to that of thyroxine, and which was perhaps similar to it in chemical structure, or some constituent from which such a body was built, when it was absorbed by the spermatozoon.

Since the crude ovarian extracts and follicular fluid proved to contain a metabolic stimulating factor which is neither oestrin nor corpus luteum hormone, it may be argued in the light of Carter's experiments, that that factor is a thyroxine-like substance. Experiments were therefore carried out to investigate the action of subcutaneous injections of thyroxine in a concentration of 1:25,000 into castrated mice, on the oxygen consumption of the isolated uterus. The results of 11 experiments are recorded in Protocol XXVII. The oxygen consumption rate varied from 5.25 c.c. to 11.07 c.c. per gram per hour with an average of 8.03 c.c. and a standard deviation of 2.13. The rate of oxygen consumption in the control castrates varied from 3.94 c.c. to 9.21 c.c. per gram per hour with an average of 5.83 c.c. and a standard deviation of 1.52. Although there was an increase of about 38 per cent. in the oxygen consumption of the isolated uterus of castrated mice which had been injected with a large dose of thyroxine, this increase was considerably/

considerably less than that which was produced by the injection of follicular fluid or Female Hormone 40 R.U. per c.c. It was concluded therefore that thyroxine was not the responsible factor in the crude ovarian preparations which caused the big rise in the oxygen consumption of the isolated uterus.

(h) Action of Extracts of the Anterior Lobe of the Pituitary Gland.

Artundo (2) found a lower basal metabolic rate in 5 out of 8 hypophysectomised dogs. Artundo and Mazzocco (3) stated that intraperitoneal injections of 3 fresh homologous anterior lobes suspended in 1 c.c. saline, in mice, produced no uniform variation in the basal metabolic rate or hepatic and muscular glycogen.

Bungler and Erhardt (7) studied the effect of anterior lobe of the pituitary administration on the metabolism of the isolated uterus. They found that administration of the anterior lobe hormone to infantile mice caused an increase in aerobic glycolysis after the uterus had increased in size as a result of the hormone injections. Since a similar increase in aerobic glycolysis was observed, 5 hours after the injection and before any macro- or micro-scopic changes in the uterus were manifest, the authors concluded that the hormone first influences the metabolism of the uterus and that this leads accordingly to increased growth.

Arvay (4) found that anterior lobe extracts caused an increase in basal metabolism of normal female rats, but not in spayed or male rats. He therefore concluded that the rise in metabolism was due to an ovarian hormone which was liberated under the influence of the anterior lobe of the pituitary extracts.

Experiments were carried out to investigate the action of anterior lobe extracts on the oxygen consumption of the isolated uterus of the castrated mouse. Fresh saline extracts of the anterior lobe of the ox's pituitary was injected subcutaneously into castrated mice in 1 c.c. doses, and the animals were killed within 5 hours from the injection. The results are recorded in Protocol XXVIII. The distribution of the various rates of oxygen consumption is recorded in graph (18). The oxygen consumption varied from 2.68 c.c. to 8.95 c.c. per gram per hour with an average of 6.03 c.c. and a standard deviation of 2.35. The anterior lobe extract therefore did not cause any rise in the oxygen consumption of the isolated uterus of the castrated mouse.

(i) Action of the Male Sex Hormone.

Since Dodds, Greenwood and Gallimore (10) suggested that oestrin and testicular hormone were similar in their chemical properties, experiments were therefore carried out to investigate the action of testicular extracts on the oxygen consumption of the uterus.

Professor E.C.Dodds of London kindly supplied me with two forms of the male sex hormone for these investigations. The two preparations were:-

a. Water soluble testicular extract.
b. Oil soluble testicular extract.

Both of these were injected into castrated mice and their effect on the oxygen consumption of the isolated uterus was studied. The results are recorded in Protocol XXIX. The distribution of the various rates of oxygen consumption is shown in graph (19).

The oxygen consumption varied from 4.96 c.c. to 9.34 c.c. per gram per hour with an average of 5.83 c.c. and a standard deviation of 1.44. The oxygen consumption of the control castrates was on an average 5.83 c.c. per gram per hour with a standard deviation of 1.52. It is therefore concluded that testicular extracts have no effect on the oxygen consumption of the isolated uterus of castrated mice.

The averages of the results of the experiments which have been described in Part II are recorded in Tables X, XI and XII.

Table X.

Average oxygen consumption of the isolated uterus of castrated mice injected with certain hormones.

Condition of the Animal.	No. of animals	Av. wet wt. in mgm.	Av. dry wt. in mgm.	O_2 consumpt. per gram dry wt. per hour at N.T.P.
A. Normal castrated mice, batch I and II	25	17.5	3.7	6.80
Castrated mice injected with				
1. Female Hormone Ciba, 40 R.U. per c.c.	21	19.8	3.56	14.14
2. Oily extracts of follicular fluid	7	16.2	3.30	10.27
3. Follicular fluid	18	14.5	2.94	9.51
4. Female Hormone Ciba, 50 R.U. per c.c.	37	27.9	4.8	6.27
5. Dihydroxy-oestrin	12	28.8	4.33	5.01
6. Trihydroxy-oestrin	14	23.4	4.34	5.32
7. Ext. of corpus luteum	11	27.9	4.72	6.31
8. Human female pregnant serum	5	38.8	5.25	5.90

9 /

Table X contd.

Condition of the animal.	No. of animals	Av. wet wt. in mgm.	Av. dry wt. in mgm.	O_2 consumpt. per gram dry wt. per hour at N.T.P.
9. Human male serum	5	15.5	3.14	5.07
10. Thyroxine	11	17.2	3.60	8.07
11. Ext. of ant. lobe of pituitary gland	6	9.4	1.90	6.03
12. Testicular extract	8	19.4	3.73	5.83

Table XI.

Average Oxygen Consumption of the Isolated Uterus of Immature Mice injected with Certain Hormones.

Condition of the animal.	No. of animals	Av. wet wt. in mgm.	Av. dry wt. in mgm.	O_2 consumpt. per gram dry wt. per hour at N.T.P.
B. Normal immature mice	36	6.36	1.43	9.43
Immature mice injected with 1. Female Hormone Ciba, 40 R.U. per c.c.	9	11.30	2.23	16.18
2. do. 50 R.U.	4	48.50	7.88	6.00
3. Dihydroxy-oestrin	6	36.8	5.99	6.98

Table XII .

Average Oxygen Consumption of the Isolated Uterus of Castrated Rats injected with Certain Hormones.

Condition of the animal.	No. of animals	Av. wet wt. in mgm.	Av. dry wt. in mgm.	O_2 consumpt. per gram dry wt. per hour at N.T.P.
C. Normal Castrated Rats.	17	50.1	9.84	3.43
Castrated rats injected with				
1. Female Hormone Ciba 50 R.U. per c.c.	10	97.2	18.40	3.61
2. Dihydroxy-oestrin	4	65.0	12.40	3.60

These tables show that the oxygen consumption of the uterus is markedly raised only after the injection of the following substances:

1. Female Hormone Ciba, 40 R.U. per c.c.
2. Follicular Fluid.
3. Oily extracts of follicular fluid.

DISCUSSION

The most important fact observed in the course of these investigations was the increase in the oxygen consumption of the uterus of castrated and immature mice, which were treated with crude ovarian preparations.

The relatively crude oily extract from ovaries Female Hormone 40 R.U. per c.c. Ciba caused an average rise of 100 per cent. in the oxygen consumption of the uterus of injected immature and castrated mice, and in a few somewhat doubtful cases it caused 5 to 10-fold increase in the oxygen consumption over that of control animals.

Follicular fluid which is another crude ovarian derivative and oily extracts thereof produced an effect which was similar to that produced by the previous extract.

The increase in the rate of oxygen consumption began in less than 3 hours after the injection of the ovarian preparation and persisted at least for 48 hours later when the state of oestrus was fully developed.

David /

David (9) made experiments with Female Hormone Ciba 40 R.U. per c.c. and measured the oxygen consumption from 24-72 hours after the injection. As regards immature mice my results agree with those obtained by David, but in the case of castrated animals I found that the oxygen consumption was nearly doubled, whereas he found no certain increase in the oxygen consumption. He attributed the rise in the rate of oxygen consumption in the case of immature animals to the anabolic changes that are abruptly brought about in order to bring the immature uterus to an oestrous state after the injection of large doses of oestrin. But Female Hormone 40 R.U. per c.c. and also follicular fluid are relatively crude ovarian preparations, and contain besides oestrin a large number of substances, one or more of which may however have been responsible for the rise in oxygen consumption. I found that pure crystalline oestrin did not produce any rise in the oxygen consumption of the uterus of castrated mice or rats or of immature mice. It was, however, a very potent oestrogenic substance, and in suitable doses produced after 48-72 hours all the demonstrable signs/

signs of oestrus in both uterus and vagina. I concluded from these experiments that the substance which is present in the crude ovarian preparations and which causes the rise in uterine oxygen consumption is not oestrin.

This conclusion is corroborated by those of Kaufmann (14) and Kochman and Wagner of the presence in the follicular fluid of a specific metabolic stimulating factor which was not oestrin. Kaufmann found that injection of follicular fluid with a high oestrin content produced in less than 3 hours an increase in the blood fat content of 15-20 per cent., an effect which could not be obtained after similar injections with a non-specific albumen such as aolan, nor with extracts of pregnancy urine with the same oestrin content as the follicular fluid. Kochmann and Wagner called the specific metabolic stimulating factor Oobolin and stated that it was thermolabile and was not oestrin.

Since my experiments with the luteal hormone, thyroxine, anterior pituitary and testicular extracts show that none of these substances produces an effect which is in any way analogous to that produced by the crude ovarian preparations, I concluded that

the/

the factor which is responsible for the rise in uterine metabolism is probably a new hormone secreted by the ovary.

Crude extracts of the ovary show that this substance whatever its nature may be is present in oily extracts of the whole ovary and also in oily and alcoholic extracts of follicular fluid. Since it was present in the oily layer of the alcoholic extract and since it was practically absent from the clear aqueous layer of the same extract, it is suggested that the new substance X is more soluble in oil than in water. Since filtrates from boiled follicular fluid produced negative results it is also suggested that X is thermolabile. It is therefore possible that Kaufmann and Kochmann and Wagner were dealing with the same substance.

Although in my experiments a great variation occurred in the dosage of the ovarian substances which were injected, the effect on the oxygen consumption did not show a corresponding variation. Thus doubling the dose of Female Hormone 40 R.U. per c.c. did not necessarily produce a corresponding rise in the oxygen consumption of the uterus.

That the ovary secretes an unidentified hormone which/

which can increase the uterine metabolism is confirmed by the fact that the rate of oxygen consumption of the isolated uterus of castrated animals is definitely lower than that of non-castrates at any stage of the oestrous cycle. The rate of uterine oxygen consumption at the various phases of the oestrous cycle also afforded further evidence that the ovary secretes such a substance. The oxygen consumption rate undergoes very little alteration during the cycle except at the end of the dioestrous phase when the rate is nearly doubled. This sharp rise is analogous to that produced by the crude ovarian preparations, and since in such cases this effect is produced within a short time after the injection, it seems likely that in the intact animal the ovary secretes this X substance at the end of the dioestrous phase about 12-18 hours before the onset of oestrus.

The increase in cellular activity and weight of the uterus is not necessarily preceded or accompanied by increase in uterine oxygen consumption, hence such substances as crystalline oestrin and corpus luteum extracts which produce an enormous degree of cellular activity and growth of the uterus do not necessarily increase the amount of oxygen consumed per unit of weight of the uterus.

SUMMARY

1. A method has been described for the measurement of the oxygen consumption of the isolated uterus.

2. Variation in the water content of the uterus of rats and mice occurs during the oestrous cycle and under the influence of oestrin and corpus luteum hormone.

3. The oxygen consumption rate of the isolated uterus of rats and mice shows a cyclic variation. During oestrus and early dioestrus the oxygen consumption of the uterus is almost uniform. At the end of dioestrus there is a rise of about 100 per cent. in the oxygen consumption rate.

4. The uterine oxygen consumption rate of castrated and immature animals shows no cyclic variation. In these animals the rate is similar to that of uncastrated animals during oestrus and early dioestrus.

5. Subcutaneous injections of follicular fluid and certain oily extracts of the ovary produce a marked rise in the oxygen consumption of the uterus of immature and castrated mice.

6. /

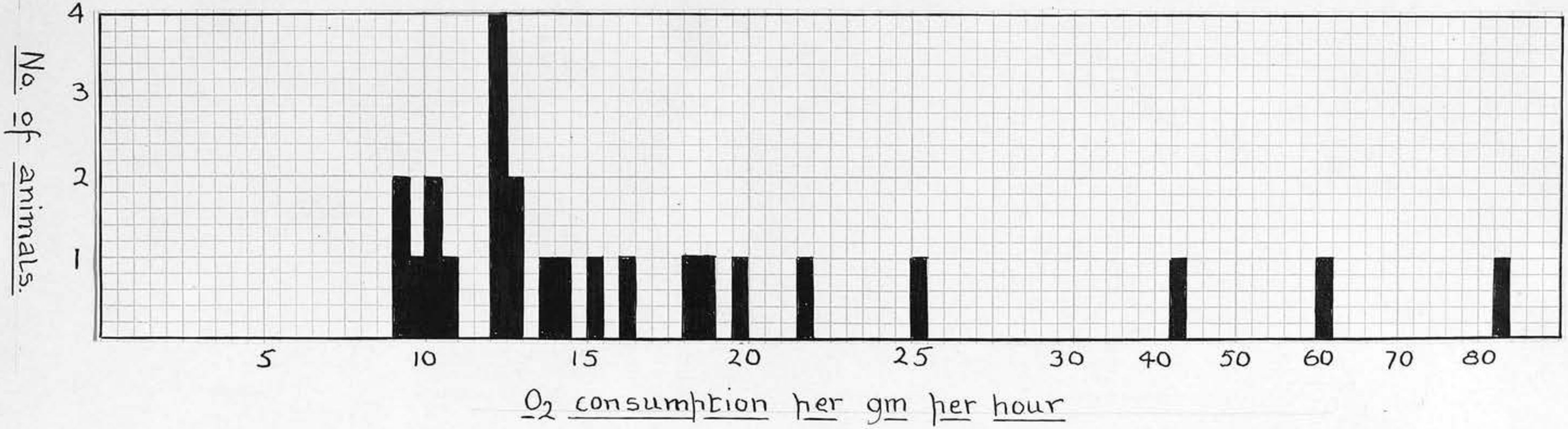
6. Crystalline oestrin, corpus luteum extract, anterior lobe of pituitary gland extract, thyroxine and male hormone produce a little or no rise in the oxygen consumption of the uterus. It is therefore concluded that the ovary secretes a metabolic stimulating factor which is none of these substances.

I am deeply grateful to Professor A.J. Clark for suggesting this work and for his constant guidance throughout its course. I am also deeply indebted to Dr C.M. Scott of the Pharmacology Department for his continual help through the course of the work. The expenses of this research have been defrayed partly by a grant from the Moray Fund of Edinburgh University and partly by a grant from the Egyptian Government.

Protocol X.

Oxygen consumption of the Isolated Uterus of Castrated Mice injected with Female Hormone Ciba 40 R.U. per c.c.

Units inj.	Time allowed in hrs.	Vaginal smear	Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumpt. per gram per hour.
5	.5	Di-oestrous	11.0	2.61	23.81	76.19	12.31
5	.5	"	20.0	4.44	22.00	78.0	9.98
5	1.	"	13.5	2.96	21.93	78.07	12.52
20	1	"	4.0	0.91	22.72	77.28	43.51*
5	2	"	7.0	1.52	21.70	78.30	18.20
5	3	"	24.5	4.48	18.25	81.75	10.63
5	3	"	20.5	3.54	17.25	82.75	13.58
20	3	"	7.5	1.30	17.35	82.65	61.40*
20	3	"	4.0	0.91	22.73	77.27	84.50*
20	3	"	11.5	2.22	19.30	80.70	12.37
20	3	"	15.0	3.01	20.01	79.99	9.37
5	4	"	24.5	4.94	20.18	79.82	9.45
5	4	"	8.0	1.62	20.21	79.79	16.24
20	4	"	15.0	2.71	18.02	81.98	12.28
20	4	"	8.0	1.67	20.85	79.15	25.10
5	5	"	19.0	3.88	20.40	79.60	10.22
20	6	"	20.5	4.26	20.73	79.27	12.79
20	6	"	14.0	3.37	24.02	75.98	14.19
20	12	"	19.5	3.93	20.16	79.84	18.71
20	12	"	18.0	3.57	19.50	80.50	12.23
20	36	"	31.5	3.98	12.80	87.20	15.20
20	36	"	57.0	8.85	15.50	84.50	10.22
20	48	Art. oestrus	20.0	2.38	11.90	88.10	21.80
20	48	"	37.0	4.92	13.30	86.70	19.55



Graph 9 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice (batch I) injected with Female Hormone Ciba 40 R.U. per c.c.

Protocol XI.

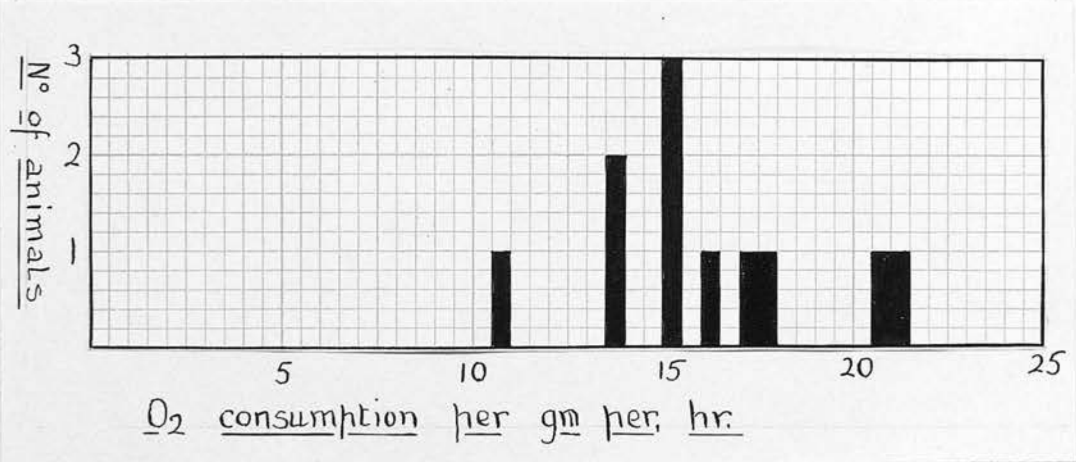
Oxygen Consumption of the Isolated Uterus of Immature Mice injected with Female Hormone Ciba 40 R.U. per c.c.

Units inj.	Time allowed in hrs.	Vaginal smear.	Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumpt. per gram pe hr.
5	3	Vagina closed	6.0	1.56	26.0	74.0	10.60
5	3	"	7.5	1.56	20.8	79.2	21.00
5	3	"	10.5	2.50	23.82	76.18	16.37
20	3	"	5.5	1.37	24.90	75.10	20.74
20	3	"	6.5	1.79	27.50	72.50	15.31
20	3	"	6.5	1.82	28.00	72.0	15.20
5	48	Artificial oestrus	12.5	2.55	20.40	79.60	15.03
5	48		22.0	3.37	15.30	84.70	17.59
5	48		25.0	3.60	14.40	85.60	13.76

Protocol XII.

Oxygen Consumption of the Isolated Uterus of Immature Mice injected with Sistomensin Ciba.

5	72	Artificial oestrus	39.5	5.36	13.60	86.40	13.79
5	72	do.	29.5	4.18	14.15	85.85	17.20



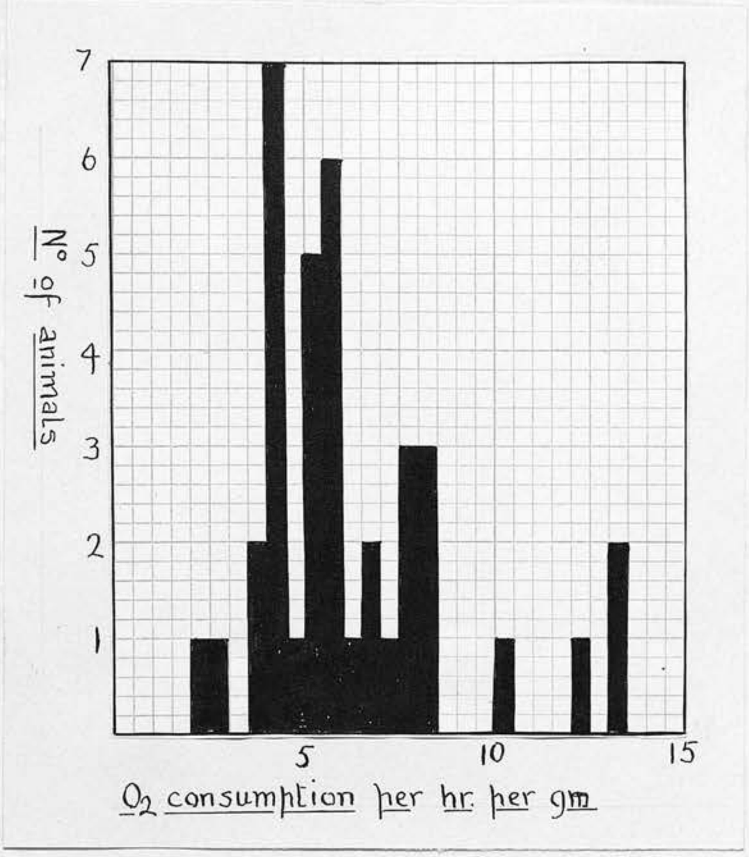
Graph 10 showing the distribution of the various rates of oxygen consumption of the isolated uterus of immature mice injected with Female Hormone Ciba 40 R.U. per c.c.

Protocol XIII.

Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with Female Hormone Ciba 50 R.U. per c.c.

Results of 12 Typical Experiments.

Units inj.	Time allowed in hrs.	Vaginal smear.	Wet wt. in mgm.	Dry wt. in mg.	% Solids	% Water	O_2 Consumpt. per gram per hour.
40	12	Di-oestrous	32.0	4.73	14.80	85.20	2.86
40	12	"	31.0	5.85	18.85	81.15	4.08
25	18	"	58.0	9.66	16.70	83.30	4.20
25	18	"	37.0	6.27	16.93	83.07	5.80
25	36	"	68.0	10.47	15.39	84.61	4.40
25	36	"	43.0	6.93	16.10	83.90	5.00
40	48	Artificial oestrus	58.0	6.62	11.40	88.60	7.70
40	48		17.5	2.30	13.12	86.88	7.80
50	48	"	27.5	3.69	13.40	86.60	6.70
40	51	"	68.0	9.04	13.26	86.74	5.22
40	58	"	68.0	9.47	14.12	85.88	5.03
40	58	"	85.0	10.83	14.02	85.98	5.35



Graph 11 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice (batch II) injected with Female Hormone Ciba 50 R.U. per c.c.

Protocol XIV.

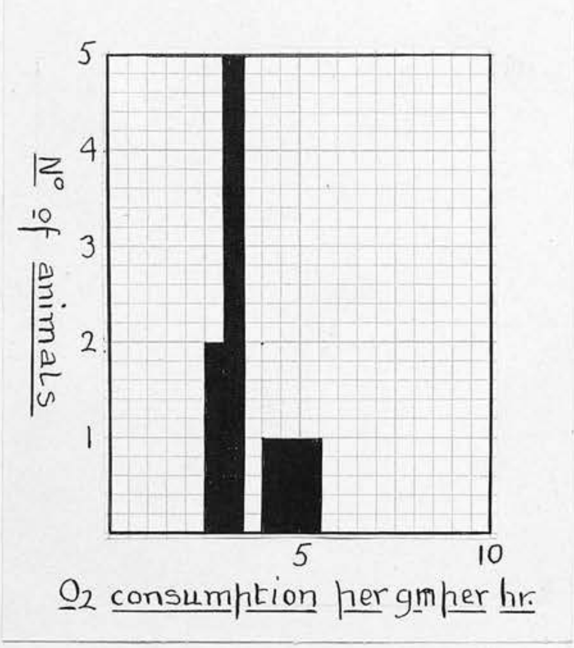
Oxygen Consumption of the Isolated Uterus of Immature Mice injected with Female Hormone Ciba 50 R.U. per c.c.

No.of uteri	Units inj.	Time allowed in hrs.	Vaginal smear	Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumpt. per gram per hour.
2	5	48	Artificial oestrous	51.0	8.11	15.90	84.10	5.80
2	5	48		46.0	7.65	16.61	83.39	6.20

Protocol XV.

Oxygen Consumption of the Isolated Uterus of Castrated Rats injected with Female Hormone Ciba 50 R.U. per c.c.

40	1	Di-oestrous	46.0	8.70	18.92	81.08	2.93
40	3	"	31.0	6.60	21.23	78.77	4.07
40	3	"	35.0	8.00	22.86	77.14	3.01
40	3	"	56.0	10.90	19.49	80.51	4.93
40	6	"	158.0	29.50	18.70	81.30	3.22
40	6	"	70.0	15.35	21.90	78.10	3.29
40	6	"	119.5	24.60	20.60	79.40	3.13
40	6	"	193.0	35.40	18.30	81.70	2.92
30	56	Artificial oestrus	138.0	23.30	16.88	83.12	5.33
30	56		125.0	21.80	17.41	82.59	3.31

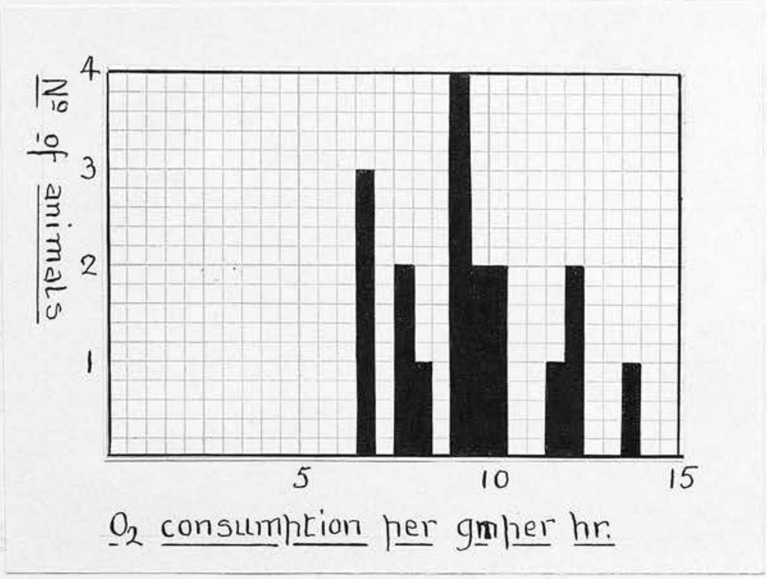


Graph 12 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated rats injected with Female Hormone Ciba 50 R.U. per c.c.

Protocol XVI.

Oxygen Consumption of the Isolated Uterus of Castrated Mice Injected with Follicular Fluid.

Dose of injection	Time allowed in hrs.	Wet wt. in mgm.	O_2 consumpt. per gram per hr. at N.T.P.	Dry weight
1 c.c.	1	6.5	11.58	This was calculated from the percentage of solids in the castrated mouse's uterus. (Table II)
1 c.c.	1	13.5	6.91	
1 c.c.	1	13.5	9.17	
1 c.c.	3	4.0	8.48	
1 c.c.	3	5.0	7.70	
1 c.c.	3	6.5	12.22	
1 c.c.	3	7.0	9.56	
1 c.c.	3	18.0	6.87	
1 c.c.	3	20.0	9.21	
1 c.c.	3	12.0	9.43	
1 c.c.	3	22.0	10.01	
1 c.c.	3	28.0	13.50	
1 c.c.	3	8.0	12.01	
1 c.c.	3	23.0	6.85	
1 c.c.	3	19.0	7.81	
1 c.c.	3	15.0	9.56	
1 c.c.	3	11.5	10.20	
1 c.c.	3	28.5	10.11	

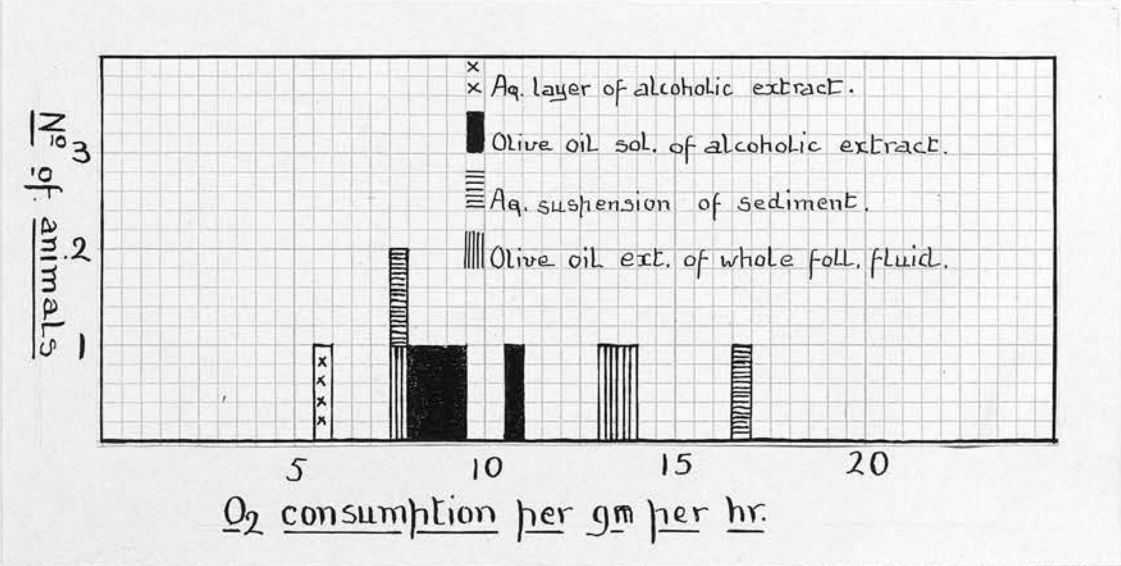


Graph 13 showing the distribution of the various rates of oxygen consumption of adult castrated mice (batch II) injected with Follicular Fluid.

Protocol XVII.

Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with Extracts of Follicular Fluid.

Fraction tested	Units inj. in c.c.	Time allowed in hrs.	Wet wt. in mgm.	Dry wt.	O_2 consumpt. per gram per hr. at N.T.P.
A. Olive Oil. extract of whole follicular fluid	1	2.5	9.5	Calc. from Table II.	13.95 c.c.
	0.45	2.5	21.0		7.74
	0.45	2.5	11.5		13.40
B. Alcoholic Fraction					
(a) Oily fraction dissolved in 4 c.c. olive oil	1	3	16.0	Calc. from Table II.	8.58
	1	3	12.0		10.78
	1	3	27.0		9.23
	1	3	16.5		8.21
(b) aqueous layer	1	3	11.0	do.	5.89
(c) aqueous suspension of sediment	1	3	10.0	do.	7.77
	1	3	5.0		16.61



Graph 14 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice (batch II) injected with alcoholic extracts of follicular fluid.

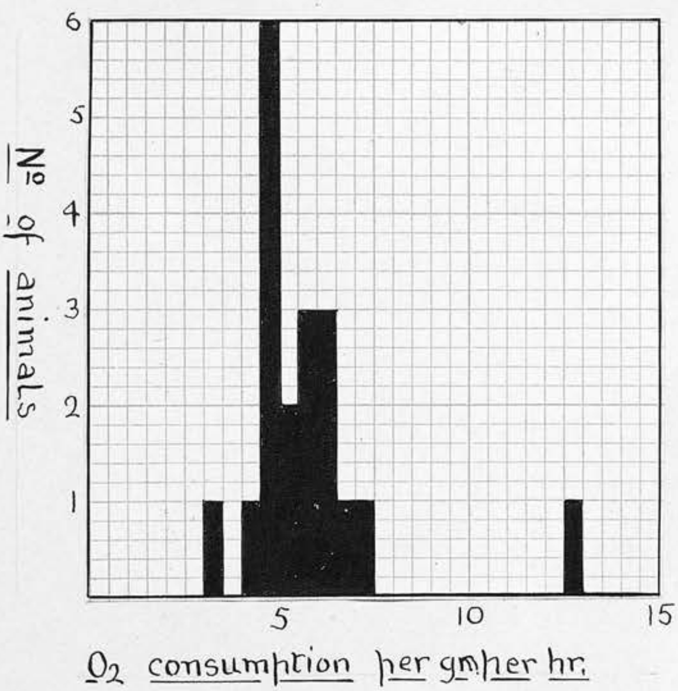
Protocol XVIII

Oxygen consumption of the Isolated Uterus of Castrated Mice injected with Dihydroxyoestrin.

Units inj.	Time allowed in hrs.	Vaginal smear	Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumpt. per gram per hr.
6[†]	1	Di-oestrus	37.0	7.73	20.91	79.09	4.56
6[†]	2	"	26.0	4.96	19.10	80.90	4.95
6[†]	3	"	11.50	2.09	17.80	82.20	4.57
20	1.5	"	11.00[*]	–	–	–	4.37
20	1.5	"	16.0[*]	–	–	–	3.08
40	48	Aft. oestrus	50.0	6.80	13.60	86.40	6.20
40	48	"	74.0	8.93	12.08	87.92	6.12
40	48	"	56.0	7.11	12.70	87.30	4.95
40	48	"	64.0	8.87	13.86	86.14	6.28

[*] In these two experiments the dry weight was calculated according to Table II.

[†] In these three experiments two uteri were suspended in each Barcroft apparatus.



Graph 15 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice (batch II) injected with dihydroxyoestrin.

Protocol XIX.

Oxygen Consumption of the Isolated Uterus of Immature Mice injected with Dihydroxyoestrin.

Units inj.	Time allowed in hrs.	Vaginal smear	Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumpt. per gram per hr.
20*	48	Art. oestrus	60	10.39	17.28	82.72	5.82
20*	48	"	80	12.73	15.90	84.10	9.11
20*	51	"	81	12.85	15.85	84.15	6.00

*In these three experiments two uteri were suspended in each Barcroft apparatus.

Protocol XX.

Oxygen Consumption of the Isolated Uterus of Castrated Rats injected with Dihydroxyoestrin.

Units inj.	Time allowed in hrs.	Vaginal smear.	Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumpt. per gram per hr.
40	1	Di-oestrus	67	12.7	18.15	81.85	3.65
40	3	"	65	12.04	18.50	81.50	4.34
40	3		46	8.02	17.45	82.55	3.50
40	3		82	16.54	20.08	79.92	2.92

Protocol XXI.

Comparison between the Oxygen Consumption of the Isolated Uterus of Castrated Mice and Rats Before and After the Addition of Oestrin to the Uterus in Vitro.

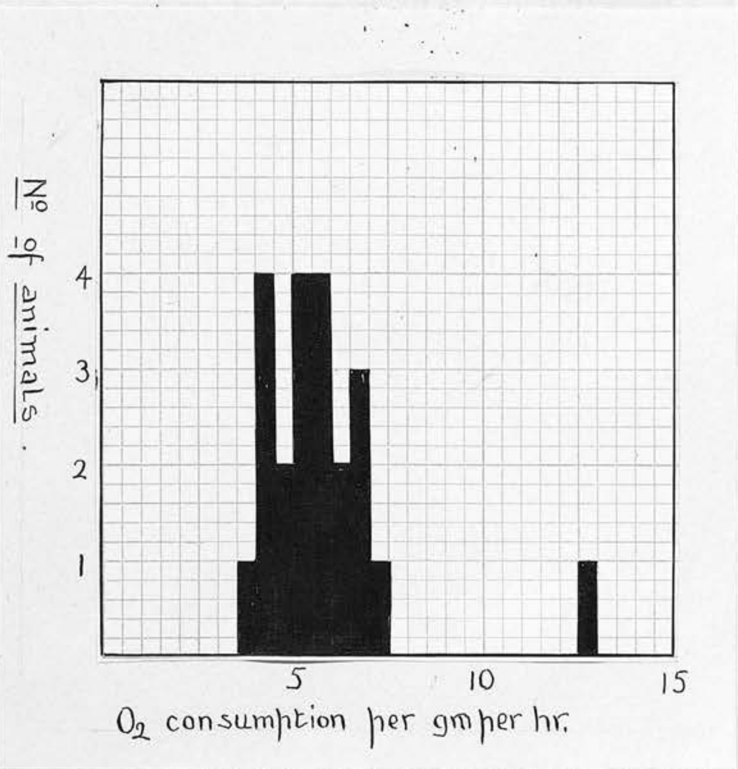
Kind of animal	Units added	Dry wt. in mgm.	O_2 consumpt. before addition of oestrin.	O_2 consumpt. after addition of oestrin.
Cast. Mouse	2	5.17	7.85	9.85
	2	1.90	6.11	8.48
	2	2.34	7.49	6.66
	4	4.21	7.93	7.96
	4	2.44	16.60	17.80
	4	4.19	6.50	5.01
Cast. Rat	4	11.31	3.72	3.36
	4	10.07	3.83	3.66
	8	12.88	4.11	2.95
	8	11.30	3.71	3.13
	10	15.70	3.38	2.58
	10	17.12	2.56	2.15

Protocol XXII.

Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with Trihydroxyoestrin.

Units inj.	Time in hrs.	Wet wt. in mgm.	Dry wt. in mgm.	O_2 consumpt.	Observations.
6*	1	58	10.58	4.22	2 + 4 U. after 30 mins.
6*	2	61	12.31	4.69	2 + 4 U. after 1 hr.
6*	3	36	6.26	6.45	2 U. at hourly intervals.
20	1.5	14	–	5.28	Calc. ratio 4.86 (Table II)
20	1.5	13	–	3.74	"
20	2.5	12	–	5.52	"
20	2.5	24	–	5.42	"
20	2.5	15	–	4.48	"
20	2.5	21	–	5.08	"
40	48	33	4.93	6.73	Art. oestrus
50	60	41	6.63	6.87	

*In these experiments two uteri were suspended in each Barcroft apparatus.



Graph 16 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice (batch II) injected with trihydroxyoestrin.

Protocol XXIII.

Oxygen consumption of the isolated uterus of immature mice injected with equal parts of di- and tri-hydroxyoestrin.

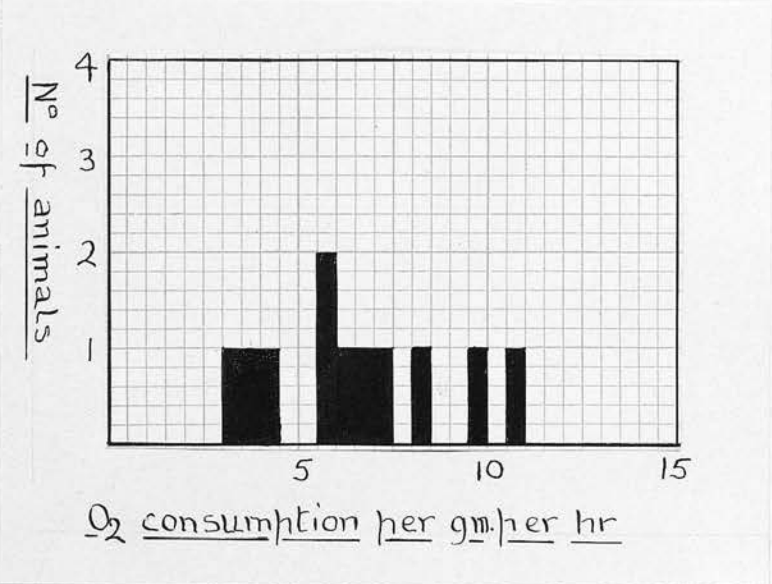
Dose inj.	Time allowed in hrs.	Vaginal smear	Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumpt. per gram per hour.
40	48	Art. oestrus	38	5.26	13.80	86.2	6.70
40	60	do.	72	9.96	13.80	86.2	5.25
50	60	do.	35	4.74	13.50	86.50	5.72
50	60	do.	52	7.29	13.98	86.02	7.17

Protocol XXIV.

Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with Extract of Corpus Luteum.

Dose in c.c.	Time allowed in hrs.	Vaginal smear	Wet wt. in mgm.	Dry wt. in mgm.	% Solids	% Water	O_2 consumpt. per gram per hr.
0.5	18	Dioestrus	50.0	7.98	15.95	84.05	5.80
0.5	18	"	14.0	2.22	15.89	84.11	6.76
0.5	36	"	29.0	4.10	14.15	85.85	10.80
0.5	36	"	43.0	6.06	14.10	85.90	8.45
1.0	48	"	50.5	8.52	16.88	83.12	3.50
1.0	48	"	33.0	4.62	14.00	86.00	6.60

Note:- 2 c.c. of extract = 1 Knaus unit.



Graph 17 showing the distribution of the various rates of oxygen consumption of the isolated uterus of castrated mice (batch II) injected with corpus luteum extract.

Protocol XXV.

Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with Serum of Pregnant Women.

Dose inj.	Time allowed in hrs.	Wet wt. in mgm.	Dry wt. in mgm.	O_2 consumpt. per gram per hour at N.T.P.
1 c.c.	3	11.0	Calc. Table III	5.65 c.c.
1 c.c.	3	7.0		5.70 c.c.
1 c.c.	3	6.0	do.	5.83 c.c.
* 1 c.c.	48	141.0	16.50	5.30 c.c.
* 1 c.c.	48	29.0	4.90	7.30 c.c.

* In these experiments the animals were in full oestrus.

Protocol XXVI.

The Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with Male Human Serum.

Dose inj. in c.c.	Time allowed in hrs.	Wet wt. in mgm.	Dry wt. in mgm.	O_2 consumpt. per gram per hr. at N.T.P.
1	3	17.0	Calc. from Table II	5.30 c.c.
1	3	14.0		6.37
1	3	18.0		4.83
1	3	16.0		3.95
1	3	12.5		4.91

Protocol XXVII.

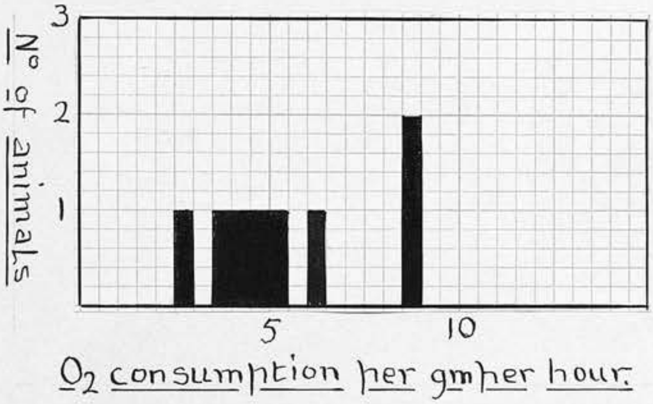
Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with Thyroxine.

Amount of 1:25,000 solution injected.	Time allowed in hrs.	Wet wt. in mgm.	Dry wt. in mgm.	O_2 consumpt. per gram per hr. at N.T.P.
0.5 c.c.	3	25.0	Calc. from Table II.	10.73
0.5	3	15.0		11.07
0.5	3	17.0		5.25
0.5	3	27.0		6.22
0.5	3	20.0		8.18
0.5	3	12.0		9.60
0.5	3	14.0		5.30
1.0	3	17.0		6.27
1.0	3	11.0		6.52
1.0	3	16.0		8.43
1.0	3	15.0		10.73

Protocol XXVIII.

Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with extract of the Anterior Lobe of the Pituitary Gland.

Units inj. in c.c.	Time allowed in hrs.	Wet wt. in mgm.	Dry wt. in mgm.	O_2 consumpt. per gram per hr. at N.T.P.
1	1	3.0	Calc. from Table II.	8.95
1	1	11.5		3.92
1	1	6.0		8.90
1	3	6.0		6.47
1	3	6.0		6.47
1	3	8.0		5.27

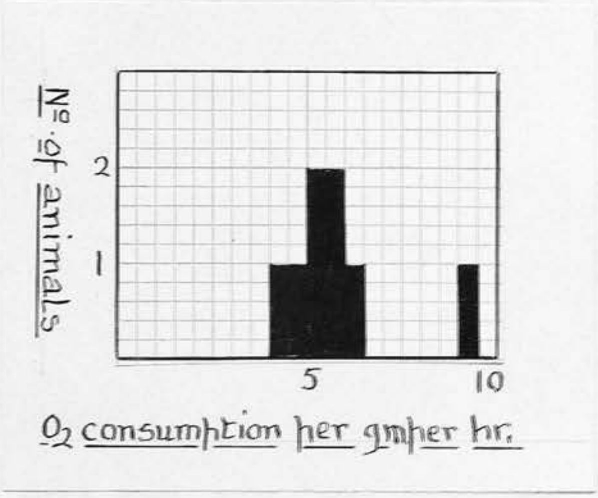


Graph 18 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice (batch II) injected with extract of the anterior lobe of the pituitary gland.

Protocol XXIX.

Oxygen Consumption of the Isolated Uterus of Castrated Mice injected with Testicular Extract.

Form of extract	Units inj. in c.c.	Time in hrs.	Wet wt. in mgm.	Dry wt. in mgm.	O_2 consumpt. per gram per hr.
Water soluble	0.5	1	23	Calc. from Table II.	5.69
	0.5	1	17		5.50
	0.5	2	7		4.96
	0.5	2	18		5.45
Oil soluble	0.5	2	35		9.34
	0.5	2	10		4.28
	0.5	6	19		5.05
	0.5	48	26	3.6	6.35



Graph 19 showing the distribution of the various rates of oxygen consumption of the isolated uterus of adult castrated mice (batch II) injected with testicular extract.

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