A THESIS PRESENTED FOR

THE DEGREE OF M.D.

BY

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STUDIES ON THE REPRODUCTIVE SYSTEM IN THE GUINEA-PIG: INTRAVITAM STAINING; FAT PRODUCTION; INFLUENCE OF HORMONES.

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C O N T E N T S.

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ACKNOWLEDGEMENTS.

It gives me very great pleasure to Acknowledge throughout the course of this research the stimulation and generous advice for which I am indebted to Professor Bryce, F.R.S. He has also made all the microscopic drawings which are used to illustrate this work.

I have also to thank Professor Crew and his Department for much valuable information regarding the manner in which the experiments involving the use of hormones should be carried out.

STUDIES ON THE REPRODUCTIVE SYSTEM IN THE GUINEA-PIG: INTRAVITAM STAINING; FAT PRODUCTION; INFLUENCE OF HORMONES.

I N T R O D U C T I O N.

This research was begun in 1929, and a preliminary account of some of the results of the investigation was published in the Journal of Anatomy, January 1932. The following brief summary indicates the contents of the preliminary paper. A series of unimpregnated animals after parturition were injected with trypan blue, and killed at different intervals of time after parturition. Each animal was treated with similar amounts of the dye according to a weight basis, and received the same number of injections before being killed, the technique of preparation of the sections being the same in all cases. It was recorded that during the first half of the oestrous cycle, which is normally one of about 16 days, only a very small amount of dye appeared in the mucous membrane of the uterus, but that during the second half of the cycle dye appeared in increasing amount in the endometrium up to the time of "heat" on the 16th day post partum. The intense staining with the dye was for the most part subepithelial and practically confined to the stratum compactum; and the dye-carrying cells were large mononuclears, some of which were irregular in outline and appeared to be shedding the dye. A striking feature was that the intensity of the dye was most marked around the anti-mesometrial half of the uterine lumen, and especially at the anti-mesometrial side, i.e. over the implantation zone.

In a short series of virgin animals, the series being established by determining the date of heat by the vaginal smear method (Stockard and Papanicolaou, 1917), practically identical results were obtained.

No dye reaction was observed in the mucosa of the Fallopian

tube at any time in the cycle. Furthermore, no intense dye reaction was found in sexually immature animals.

In regard to the nature of these dye-carrying cells, the following questions were formulated:-

1. Are they enlarged endometrial cells laden with some chemical substance destined after implantation of the blastocyst for the supply of nourishment to the embryo?
2. In view of the enormous amount of destruction which takes place immediately after implantation, are these cells related rather to destructive than to constructive processes, e.g., enzyme formation responsible for tissue destruction or digestion?
3. Are they cells which wander, like the large mononuclear cells found in great numbers in the resolution phases of peritoneal inflammation, and which experimentally have been shown to take up vital dye, and which generally are regarded as macrophages (Cappell, 1930)?

No definite conclusion could be made regarding these points, nor could an explanation be given for the anti-mesometrial incidence of the dye-carrying cells, but it was hoped that further experimental work and the finding of what the reaction was in the very early stages of implantation might throw some light on the interpretation of the results.

Further work revealed the fact that animals apparently varied in the temporal incidence of the dye in the cycle, more than allowed for in the earlier researches. The question was were these differences due to normal variations in the length of the sexual cycle, to prolongation by the dye of an otherwise regular cycle, or to influences from the ovaries? These difficulties led to an independent series of studies which were published by the Royal Society of Edinburgh (1933;1934)*.

From the point of view of the trypan blue research it was

* See references to literature.

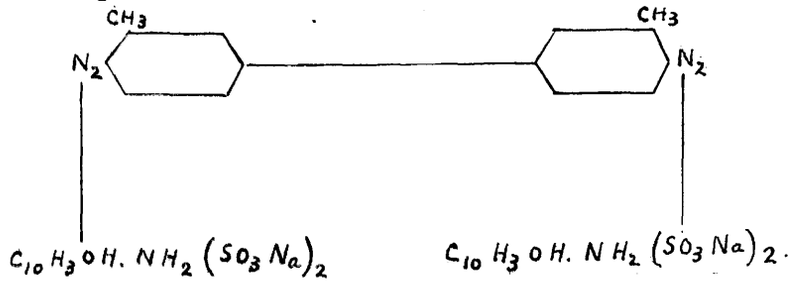
absolutely necessary, in order to arrive at results which could be depended upon regarding the temporal incidence in the oestrous cycle of the vitally stained cells, to have a series of animals with cycles known to be perfectly regular. In pursuing the research, in order to obtain still greater certainty, the appearances in the ovaries were studied and correlated with those in the uterus.

In the course of the work the problem of fat distribution in the endometrium and its relation to the trypan blue dye arose and was also studied. Further, the influence of hormones on the presence of dye and fat in the uterus was investigated experimentally. Lastly the appearances in a series of pregnant animals have also been examined and described.

DOSAGE AND MODE OF ADMINISTRATION OF TRYPAN BLUE.

Trypan blue belongs to the group of soluble acid rapidly absorbed dyes. It is a sulphonated dis-azo dye numbered C.I.No.477 (colour index of the Society of Dyers and Colourists, 1924).

The dye was obtained from the British Drug Houses, Ltd., London, and they state that each batch of trypan blue is tested chemically and also physiologically in order to ensure that its toxicity does not exceed a certain arbitrary limit, the sample used being stated to have been non-toxic to rabbits in doses of from 0.08 gram to 0.1 gram per kilogram body weight. They give the formula of trypan blue as being



In these experiments on the guinea-pig a 1.0 per cent solution of the dye in distilled water was used, the dosage for each injection being 0.8 cc of this solution per 100 grams body weight. The solution is sterilised by boiling for one minute, filtered, boiled for another minute, and allowed to cool to blood heat before injecting. The vessels for preparing the solution have previously been sterilised by boiling. The injections were given suboutaneously into the abdominal

wall*, the skin being first treated with spirit.

The site of injection should then be massaged gently to spread the dye through the tissues and facilitate absorption. Different parts of the abdominal wall were as far as possible used for each injection.

Unless otherwise stated each animal received six doses, the injection being given once daily always at the same hour, and the animal being killed by chloroform at that hour on the seventh day.

FIXATIVES AND STAINING REAGENTS.

Trypan blue is readily fixed in the tissues by most of the ordinary fixatives**, but formalin and corrosive sublimate was found to be most suitable. The sections were prepared by the paraffin method and cut at 10 microns, as the dye once fixed is relatively resistant to decolorisation by alcohol.

Dilute carbol fuchsin and carmalum were used as staining reagents, and weak eosin alone was found suitable for emphasising the topographical distribution of the dye. It is important to note that staining with carbol fuchsin should not be too intense in order that there may be clear differentiation between the dense and paler nuclei in the endometrium, afterwards to be described. Other staining reagents were unsatisfactory as they obscured the trypan blue appearances.

THE ANATOMY OF THE REPRODUCTIVE SYSTEM IN THE GUINEA-PIG.

In order to facilitate the description of the appearances in the intravitally stained animals a short account of the anatomy of the reproductive system will first be given.

The uterus is composed of two horns. These fuse distally to form the body of the uterus, but the lumen of each horn remains

* The staining is always most intense at the site of injection so that in order to avoid intense local staining of the uterus the injections were not given intraperitoneally.

** 10 per cent formalin in a saturated solution of corrosive sublimate.

distinct for a considerable distance before opening into the short common cervical canal, which in turn opens into the vagina. The body of the uterus may therefore be described as consisting of a proximal or uterine part, and a distal or cervical part. Embryos implant only in the uterine horns.

Each uterine horn consists of the following layers:

1. An outer serous coat derived from the peritoneum.
2. An outer longitudinal muscle coat, well marked, and consisting of stout bundles of muscle fibres separated by fibrous septa.
3. An inner thin circular muscle coat. The muscle fibres are arranged chiefly in bundles and run in varying directions. Between and deep to them is a varying amount of areolar tissue in which run the main blood vessels of the organ, the largest vessels being near the attachment of the mesentery.
4. A thick mucous membrane or endometrium. This is characterised by a well developed muscularis mucosae composed of circular muscle. The remainder of the endometrium consists of an outer looser zone, the stratum spongiosum, and an inner more compact zone, the stratum compactum, the inner surface of which is lined by columnar or cubical epithelium enclosing the narrow slit-like uterine lumen. The lumen is beset somewhat sparingly with the orifices of the uterine glands. The glands are simple tubes bounded by a delicate basement membrane, and lined by cubical or columnar epithelium; they usually pass obliquely through the stratum compactum, becoming coiled in the stratum spongiosum, and terminating just under the muscularis mucosae.

The blood supply of each horn is derived from a mesentery attached to its lateral aspect, so that in a transverse section the endometrium may be for descriptive purposes divided into a mesometrial and anti-mesometrial half.

In the body of the uterus the fusion of the horns has occurred along the plane of the circular muscle coats, the

longitudinal muscle coat now surrounding both horns.

The endometrium of the proximal part of the uterine body is practically similar in structure to that of the uterine horns. In the distal part of the uterine body the mucosa becomes thrown into complicated villous-like processes, glands gradually disappear, and there is no evidence of division into stratum compactum and stratum spongiosum.

The cervix is similar in structure to the lower part of the uterine body, and at the bottom of the depressions between the villous processes of the mucosa small invaginations of epithelium pass for a short distance into the connective tissue, and appear to be simple mucous glands.

The vagina is lined by a stratified epithelium which also shows small gland-like invaginations into the underlying connective tissue.

Nothing need be said about the anatomy of the tubes or ovaries except regarding the relation of the ovary to the ovarian sac which surrounds it. The sac communicates with the peritoneal cavity except at oestrus, when it is closed over the ovary. The condition then becomes in principle the same as that of the closed ovarian sac of the rat and mouse (Allen, 1932).

GENERAL REMARKS.

Twentyfour hours after the first injection of dye there is generalised vital staining. Apart from this most of the animals seem to live quite normally, although some may show a special susceptibility to the dye given in the doses stated above (page 3) and develop toxic symptoms. This will be referred to in more detail later.

If an animal has received six suitable subcutaneous doses of a one per cent solution of trypan blue the tissues of the body are everywhere of a bluish colour, the depth of the colour, however, varying widely in different organs. Cappell (1929) has given a full

account of the appearances in the mouse after several doses of trypan blue. He states that the blue coloration is due to two factors (1) a diffuse staining of certain structures, e.g. the epidermis and the connective tissues; and (2) the accumulation of dye within certain cells in the form of granules and "vacuoles", the intensity of the coloration depending chiefly on the second factor. The diffuse staining is not recognisable microscopically, and is held by most writers to be a simple absorption which is determined by the physical characters of the structure stained. Generally, it may be stated that granular staining is found chiefly in the cells of the reticulo-endothelial system, and in order to store the dye the cell must be living. No stain, unless the cell is dying, is ever found in the nucleus. The dying cell, however, stains diffusely, and the nucleus becomes uniformly stained.

INTRAVITAM STAINING IN THE REPRODUCTIVE SYSTEM DURING THE OESTROUS CYCLE.

The normal oestrous cycle in the guinea-pig is usually one of 16-18 days (Stockard and Papanicolaou 1917; Selle 1922; Nicol 1933), and to maintain the oestrous rhythm the animals must be healthy, well fed and under uniform environmental conditions (Allen 1922). At oestrus the vagina opens and ovulation occurs. In the uterus the endometrium becomes oedematous, and the epithelium is invaded by leucocytes, cast off, and rapidly regenerated in the space of ten hours. In the vagina the epithelium becomes much thickened, and cornification, invasion by leucocytes, and desquamation occurs. In this paper oestrus is taken as the first day of the cycle.* The first part of the cycle may be called the luteal phase (Loeb 1932), as the changes in the uterus are dominated by the corpus luteum hormone. During this period characteristic appearances occur in the endometrium consisting chiefly of enlargement and proliferation

* For a detailed description of the uterine changes in the oestrous cycle see Appendix II.

of the glands, which become elongated and feathery in appearance. The endometrium also becomes thicker. These changes appear to be in preparation for the possible occurrence of a fertilised ovum, which, if present, becomes implanted in the antimesometrial part of the endometrium of the uterine horn on the 7th day.

The second part of the cycle may be called the follicular phase (Loeb 1932), as the changes in the uterus are dominated by the follicular hormone, chiefly produced by the large follicles which are now present in the ovaries. During this period growth takes place in the endometrium, but the changes in the guinea-pig are not so obvious as in other animals, e.g. the dog. In the later part of this phase the vaginal epithelium becomes much thicker, and the end of the follicular phase is succeeded by oestrus.

The animals chosen for the investigation of the vital staining appearances during the cycle were animals in which, previous to injection, the last six consecutive cycles were perfectly regular, or if any variation did occur it was not more than the normal of one day in any cycle (Nicol 1933).

To obtain these animals a colony of fifty to sixty virgin animals was kept, and the animals were examined once daily for their oestrous condition, always about the same hour, during several months. When the vagina was open a daily smear of the vaginal content was taken until the vaginal orifice became occluded by the reforming "closure membrane". In taking the smears the technique of Stockard and Papanicolaou was followed. Haematoxylin and eosin were used as stains. For each animal a chart was made on which were noted the day of examination, the presence or absence of the "vaginal closure membrane"; if the vagina was open and a smear therefore taken, the amount of the smear and the nature of its cells were also noted.* In this way it was possible to separate the animals which had regular cycles from those which showed variations in their oestrous rhythm and were therefore unsuitable for experiment. The animals belonged to a strain of animals bred in the laboratory. Great care was taken to avoid in-breeding. The animal house is commodious, well ventilated, and thermostatically kept at a uniform summer temperature throughout the winter months.

Twentyeight animals were injected, each receiving one injection daily usually for six days, and then killed. Out of this group seven died, the details concerning these being shown in Table I.

* For an account of the vaginal closure membrane and the normal appearances in the vaginal smear see Appendix I.

Table 1. Records of Seven Virgin Guinea-Pigs which died after Intravital Injection with Trypan Blue.

Index Number of Animal.	Length of Normal Cycle in Days.	Number of Injections and Dosage of Trypan Blue. 1% Solution.	Time in Cycle when first Injection given.	Time in Cycle when last Injection given.	Time in Cycle when Animal Died.	State of Vagina
16	18	3 of 4 ccs.	15th Day.	17th Day.	17th Day.	Remained Closed.
15	16	6 of 7 "	10th Day.	15th Day.	16th Day.	Do.
13	17	6 of 6.5"	9th Day.	14th Day.	17th Day.	Do.
26	17	6 of 5.4"	10th Day.	15th Day.	20th Day.	Do.
27	17	6 of 6.5"	11th Day.	16th Day.	20th Day.	Do.
40	17	6 of 5 "	14th Day.	19th Day.	20th Day.	Opened slightly on 17th Day.
28.	17	6 of 5 "	13th Day.	18th Day.	50th Day.	Remained closed & animal gradually lost weight.

The seven animals which died were injected to show the appearances about the time of "heat". It may be seen (Table I) that the first animal died on the day before its expected "heat", the next two on the day of expected "heat", the next three about three days after "heat" should have occurred, and the last gradually became emaciated. In all of these the vagina remained closed. In view of this these animals were not killed on the day following the last injection as was the usual procedure, but were kept alive in the hope that oestrous signs might become manifest later. The normal mortality in our animals over a period of at least six months was not more than one in fifty, so that this unusually high death rate seems definitely to be associated with administration of the dye.* It is also perhaps not without significance that all the deaths occurred about the time of "heat". None of the animals

* The above death rate agrees closely with the death rate of 24.5% shown in the mortality table of vitally stained animals, page 64.

experimented upon showed toxic symptoms at any other time in the cycle, although the same sample of trypan blue was used. The criticism might be advanced that the dosage was too high in these fatal cases. Against this, however, it should be noted that animal No. 16 received only three injections and that in the group of sexually immature animals, afterwards to be described, and which received the same relative dosage, no evidence of toxicity was observed.

The remaining twentyone injected animals in the series appeared to live normally, and the appearances in the genital tract will now be described. The details concerning these animals are shown in Table II.

It may be seen (Table II) that for the last five animals, in three the appearance of oestrus was delayed for two days, and in two oestrus was missed or suppressed, the vagina then remaining closed. Moreover, in animals Nos. 42, 9 and 50 (Table II), although oestrus seemed to occur normally during the period of the injections, as proved by microscopic examination of vaginal smears, serial examination of both ovaries in each animal showed that ovulation had not occurred. In view of these facts, whether or not they are due to the dye, it seemed that the stage in the cycle at which an animal was killed might not be truly represented by the number of days since the last oestrus. An independent confirmation of the stage in the cycle at which each animal was killed was therefore sought by correlating the ovarian with the uterine and vaginal appearances.* Full data are given for each animal in Appendix III, and from these data it may be seen that the times in the cycle at which the animals were killed (Table II) are as near as possible correct.

* Such a correlation seemed justifiable as it has been shown that during the cycle typically corresponding changes are occurring in the ovaries and uterus (Loeb 1911 and 1914; Stockard and Papanicolaou 1917), cervix (Hartmann and Olbers 1931), and vagina (Stockard and Papanicolaou 1917; Selle 1922), a given stage in one of these organs accompanying parallel stages in the other three.
For an account of the associated uterine, vaginal and ovarian appearances during the oestrous cycle, see Appendix II).

TABLE II.

Records of Twenty-one Virgin Guinea-Pigs injected Intravitally with Trypan Blue and which showed no Evidence of Toxic Symptoms.

	Index Number of Animal.	Length of Normal Cycle in Days.	Number of Injections and Dosage of Trypan Blue 1% Soln.	Time in Cycle when 1st Injection Given.	Time in Cycle when Last Injection Given.	Time in Cycle when Killed.	Remarks.
Group I.	32	16	3 of 5 ccs.	14th day	16th day	1st day of new cycle. Verified by smear.	Killed before new ovulation should have occurred.
	34	17	4 of 4 ccs.	14th day	17th day	1st day of new cycle. Verified by smear.	Ditto.
	109	16	6 of 4 ccs.	11th day	16th day	1st day of new cycle. Verified by smear.	Ditto.
	98	16	6 of 4 ccs.	11th day	16th day	1st day of new cycle. Verified by smear.	Killed soon after new ovulation.
Group II.	42	17	6 of 6.5 ccs.	14th day	2nd day of new cycle.	3rd day of new cycle. Definite oestrus verified by smear occurred on 18th day as expected.	New ovulation failed to occur.
	9	17	6 of 6.5 ccs.	15th day	3rd day of new cycle.	4th day of new cycle. Definite oestrus verified by smear occurred on 18th day as expected.	Ditto.
	50	17	6 of 5 ccs.	16th day	4th day of new cycle.	5th day of new cycle. Previous oestrus appeared normally.	Ditto.
Group III.	22	17	6 of 5.4 ccs.	1st day of new cycle.	6th day	6th/7th day.	Oestrus and ovulation occurred normally.
	23	17	6 of 4 ccs.	1st day of new cycle.	6th day	6th/7th day.	Ditto.
Group IV.	43	18	6 of 5.8 ccs.	3rd day	8th day	9th day.	Ditto.
	49	16	6 of 4.5 ccs.	3rd day	8th day	9th day.	Ditto.
	4	17	6 of 5.6 ccs.	4th day	9th day	9th/10th day.	Ditto.
Group V.	29	16	6 of 6 ccs.	6th day	11th day	12th day.	Ditto.
	28	16	6 of 5.8 ccs.	6th day	11th day	12th day.	Ditto.
	47	17	6 of 4.4 ccs.	7th day	12th day	13th day.	Ditto.
Group VI.	52	17	6 of 4 ccs.	9th day	14th day	15th day.	Ditto.
	41	17	6 of 5 ccs.	13th day	18th day	20th day. Actually 1st day of new cycle as verified by smear.	Oestrus delayed for 2 days. Animal killed before new ovulation should have occurred.
	24	17	6 of 6.5 ccs.	13th day	18th day	20th day. Actually 1st day of new cycle as verified by smear.	Ditto.
	31	17	6 of 5 ccs.	11th day	16th day	20th day. Actually 1st day of new cycle as verified by smear.	Ditto.
Group VII.	33	16	6 of 5.5 ccs.	13th day	18th day	31st day. Actually 1st day of new cycle as verified by smear.	Previous oestrus missed or suppressed. Animal killed before new ovulation should have occurred.
	51	18	4 of 4.5 ccs.	15th day	18th day	33rd day. Actually 1st day of new cycle as verified by smear.	Previous oestrus missed or suppressed. Animal killed after new ovulation has occurred.

The difficulty of obtaining animals early in the cycle after a new ovulation should be emphasised. Several animals were planned to give this stage, but with the usual dosage of dye ovulation was either prevented or oestrus delayed. No attempt was made to secure this early stage with e.g. half the number of injections of dye, firstly as it seemed most desirable for the present purpose to keep the animals (Table II) as far as possible strictly comparable with each other, and with the group of immature animals afterwards to be described; and secondly, as there was evidence that e.g. two injections of dye seemed insufficient to produce vital staining appearances, at a time when with larger doses they were found to occur.*

THE APPEARANCES IN THE UTERUS AND VAGINA.

A full description will first be given for animal No. 28 (Table II), killed at the 12th day of a 16-day cycle when the intravitam staining is most pronounced.

Uterine Horn: The endometrium shows the most striking appearances. A very large amount of dye is present. It is almost entirely confined to the stratum compactum in which it forms a zone of intensely stained vivid blue granules (fig.1.).

The dye is contained in large rounded cells (fig. 2) with an oval, rounded or lobed, densely stained nucleus which measures about 4 microns (longest diameter), and is smaller than the pale oval nucleus of an endometrial cell which measures about 6 microns (longest diameter). Sometimes the dye cell appears to have two nuclei apparently fused together, giving the effect of a kidney-shaped nucleus divided by a central line. The dye granules are usually scattered throughout the cytoplasm of the cells, and are very large

* The animals in Table II which were killed at oestrus, and which received less than six injections of dye, were killed to find out if the larger doses of dye were perhaps producing at this period of the cycle a mild toxicity which was not sufficiently marked to be apparent, but which in some way was reducing the capacity of any cells present to take up the dye. No evidence of this was found.

and coarse, especially close to the uterine lumen. There they are most numerous and often form large "dye masses" in which the nucleus is completely obscured (fig.3). Some of the large "dye masses" have a sharp well-defined outline and are rounded or oval in shape, measuring 12 to 17 microns in diameter; that they are large mononuclear cells as described above can be proved by bleaching the sections with permanganate of potash and sulphurous acid, and restaining with haematoxylin and eosin. Other "dye masses" are larger and very irregular in outline, and appear to lie "free" in the tissue and to have resulted from degeneration of one or more dye-containing cells. In some of the largest dye cells the dye seems to have collected around a large vacuole, and occasionally a polymorph, or one or two very small mononuclears, may appear to be lying in the interior of one of the large dye cells.

A striking feature is that the dye is in increased amount around the antimesometrial half of the lumen, which is the site where implantation occurs (fig.1). A smaller number of large dye cells is present around the mesometrial half of the lumen, and at the mesometrial side the dye cells are still less numerous and smaller in size (fig.1). Beyond the intensely stained zone a moderate number of smaller dye cells is present all around in the deeper part of the stratum compactum. In these cells the nucleus is usually easily seen and the dye granules are smaller, less numerous, and may be confined to one part of the cell (fig.2). The dye cells lie in the stroma without any apparent relation to the glands or blood vessels.

Many of the endometrial cells of the stratum compactum contain a moderate amount of fine dye granules, especially around the antimesometrial half of the lumen, and very small granules are also seen in the uterine epithelium (fig.3).

In the stratum spongiosum only a few dye cells are present. They appear to be the same type of cell as described in the stratum compactum, but the amount of intravitam staining is much less, and

the cells are smaller. They vary in size from about 7 to 12 microns in diameter. A number of the cells lie very close to the blood vessels though none were observed in the vessels. No dye was found in the glands.

A moderate number of very small mononuclears measuring about 5 microns in diameter and polymorphs measuring 7 microns in diameter are also seen in the endometrium, and some of the polymorphs contain fine pale dye granules (fig.3).

Occasionally a large dye cell appears to be passing through the uterine epithelium into the lumen, in which groups of free dye cells are sometimes seen. The latter cells are rounded or oval, and vary in size from 9 to 14 microns in diameter. Numerous coarse dye granules are present throughout the cytoplasm and may obscure the nucleus, and occasionally a polymorph may appear to be lying in the interior of one of the cells. These cells closely resemble the vitally stained cells in the endometrium (fig.2) and also the free dye cells afterwards to be described as often present in the lumen of the Fallopian tube (fig.4), and it does not seem possible to say for certain whether they are derived from the tube or uterine endometrium or both.

No dye is present in the muscle fibres, but between the fibres an occasional small elongated fibrocyte with coarse dye granules and a narrow dense nucleus is seen.

In the vascular zone between the circular muscle fibres a small number of vitally stained cells is present, especially in the connective tissue round the larger blood vessels. The cells vary in size and shape, some being irregular, rounded or oval. The nucleus is oval or rounded, and densely stained. The dye granules are scattered throughout the cell, and although relatively coarse they seldom obscure the nucleus. Some of these cells, at least, appear to be similar to those described in the endometrium. Small vitally stained fibrocytes with dye granules are also seen. A few dye cells

of similar types are present in the mesentery. Moreover, occasionally a small group of large dye cells, identical with those often found in the Fallopian tube, is seen amongst a mass of red blood cells adherent to the mesentery.

It may not be without significance that scattered throughout the endometrium and in its blood vessels there is a considerable number of dense nuclei identical with the nuclei of the rounded dye cells. The cell bodies enclosing these nuclei are not very clearly defined, but they are rounded cells with clear protoplasm and measuring about 8 or 9 microns in diameter. Both the cells and the nuclei vary slightly in size. In the stratum compactum they appear scattered between the large dye cells, and a few may be seen in the uterine epithelium (fig.3). Sometimes one of the cells is seen apparently passing through the epithelium into the uterine lumen, and a few may be found free in the lumen. Moreover, numbers of these cells may be seen apparently streaming between the fundi of the glands. Occasionally a similar cell is seen in the wall of a gland or in its lumen. Small granules of dye are sometimes seen in the cytoplasm of one of these cells, especially in the stratum spongiosum, and it is not possible to say whether this cell differs from the others or not. Similar cells without dye are seen in considerable numbers around the large blood vessels, and a few are present inside the vessels.

The above vital staining appearances extend more or less equally along the whole length of the uterine horns, as proved by serial drawings, such differences as are found being clearly due to the horn being cut obliquely, or at a bend where the lumen may be cut twice. At the tubo-uterine junction the dye is more pronounced around the mesometrial half of the lumen for a distance of 0.12 mm; in the succeeding 0.2 mm of the tube the amount of dye gradually diminishes, and in the remainder of the tube no dye is present. Occasionally, however, a small elongated vitally stained fibrocyte is seen.

In the remainder of the uterus and in the vagina significant differences in the histological appearances only occur in the mucous membrane, and the descriptions will therefore be confined to this part.

Body of uterus - Proximal part: The structure is identical with the uterine horn and the vital staining appearances are also similar. Rounded cells with dense nuclei and no dye are also present as in the uterine horn, and especially in the circular muscle zone some of the cells may be seen apparently passing through the walls of the blood vessels.

Body of uterus - Distal part: The glands have practically now disappeared, and there is no division of the mucosa into stratum compactum and stratum spongiosum.

Only occasionally are dye cells now seen. They are found usually in the tips of the mucosal folds, and are equally distributed around the lumen. The cells are similar to those in the endometrium of the uterine horn, and contain a relatively small amount of dye. A considerable number of similar cells without dye is also seen, and a few vitally stained polymorphs are present. No dye is found in the lining epithelium. In the uterine lumen a few degenerated dye cells are seen together with a number of polymorphs.

The change from the numerous dye-containing cells in the uterine horn and proximal part of the uterine body to the very few dye-containing cells equally distributed around the mucosa of the distal part of uterine body is relatively abrupt.

Cervix: The appearances are similar to those described in the distal part of the uterine body.

Vagina: A moderate number of rounded cells containing a small amount of coarse dye is seen in the deeper part of the mucosa. They are similar to the dye cells in the uterine horn. Apparently similar cells without dye are also present. A few vitally stained polymorphs and fibrocytes are also sometimes seen. No dye is present in the lining epithelium.

The Origin of the Dye-Containing Cells: The appearances throughout the uterus and vagina seem to provide convincing proof that the dye-containing cells are similar to many of the rounded cells with dense nuclei and no dye, which have been described in all situations where the dye cells are found. Further, if this be so, it points to the blood as being at least one source of the dye cells, since the rounded cells containing no dye are frequently seen passing out of the capillaries in the endometrium and through the blood vessel walls in the circular muscle zone. Very occasionally a large dye cell in the stratum compactum of the uterine horn appears to be in a terminal capillary, but the nature of the tissue makes it difficult to be absolutely certain whether or not the dye cell is definitely in the blood vessel. Apart from this no dye cells except a few polymorphs are found in the blood vessels. From this it is to be inferred, therefore, that the cells do not normally take up the dye while in the blood stream, but only after they are free in the tissues, and also that some cells take up dye while others do not. The cells that take up the dye may be in a resting phase, or more probably they are already fully occupied with some other less readily demonstrable substance (Cappell 1929). It must be stated that a few of the dye cells have a less dense paler nucleus. Similar paler nuclei are however also seen in the blood vessels. It may be that some of the dye cells with the paler nuclei are derived from the connective tissue cells of the endometrium. The vitally stained cells may therefore have a double source of origin, although it should be emphasised that the microscopic appearances point very strongly to the blood as the chief source.

In non-injected animals the cells which appear to correspond to those which take up the dye in injected animals are identified only with a certain degree of difficulty. They are more easily recognised in sections carefully differentiated by carbol fuchsin, less easily in haematoxylin and eosin sections, and still less easily in sections

stained by iron haematoxylin. Many deeply stained nuclei are seen in sections stained by any of these methods, but these nuclei very frequently apparently lack a cell body. Often, however, a definite halo can be seen round the nucleus, and when the cell happens to occur free in an intercellular space, the appearances represented in Fig. 2.a can be made out. The protoplasm of the cell body is not granular, but shows a wide meshed network indicating probably that it is vacuolated. As these cells certainly lie among the proper endometrial cells it is to be expected that their presence would be masked, in sections, by the large endometrial nuclei surrounding them.

THE DISTRIBUTION OF THE DYE THROUGHOUT THE CYCLE.

Complete laboratory descriptions as in the case of animal No. 28 were made of the appearances in the entire reproductive system for each of the twentyone animals in Table II. Generally speaking, it was found for each animal that the relative distribution of the dye along the entire reproductive tract followed the plan of distribution described for animal No. 28. Significant differences were observed only in the uterine horns and proximal part of the uterine body, and these differences were in respect of the amount of dye and its distribution in the endometrium. In order to compare the animals a typical eosin-stained section of the uterine horn of each animal was projected at a magnification of 100 diameters, and the dye cells plotted. It was then found possible to divide the animals into arbitrary groups (Table II), each animal in the same group showing comparable appearances. The differences in the amount of vital staining that did exist between the animals of the same group would seem to be accounted for in the natural differences among animals, and especially, as it will be shown later, that the amount of vital staining present is in some manner related to the action of the ovarian hormones.

The dye cells in all the animals seem to have come chiefly from the blood although they do not take up dye until free in the

tissues, and apparently similar cells without dye are invariably present.

In order to avoid tedious and lengthy descriptions the appearances in the uterine horns and vagina have been arranged in tabular form (Table III), and one or more diagrams of the distribution of the rounded vitally stained cells in the uterine horn is given for each group of animals (fig.5).*

The appearances in the uterine horns may be summarised as follows:

About midway in the cycle a relatively small number of dye-containing cells is present, scattered loosely throughout the stratum compactum and in still smaller numbers in the stratum spongiosum. None of the cells are intensely stained, but the dye granules are slightly larger in the stratum compactum.

At approximately the 12th day of a 16-day cycle, i.e. in the follicular phase, great numbers of intensely stained cells are found, especially concentrated close to the uterine epithelium, and practically confined to the stratum compactum. Many of the dye cells are very large, the nucleus is usually obscured by the coarse granules, and a number of the cells appear to be bursting and shedding the dye.

Later in the cycle, about the fifteenth day, and at oestrus, the intensity of the vital staining is diminished. Considerable differences in regard to the number of vitally stained cells present were found in the different animals killed at this period of the cycle. The intensity of the staining in all the animals, however, was considerably less than at the 12th day of the cycle when the results were uniform. In practically all the cells the nucleus is visible, and the cells may be confined to the stratum compactum, or loosely scattered through both stratum compactum and stratum spongiosum. Very few examples of cells bursting and shedding the dye are seen.

* For a detailed description of the appearances in the several groups see Appendix IV.

Table III. Showing Amount and Distribution of Intravitam Staining in the Uterine Horns and Vagina of the Animals shown in Table II.

	Group I. At Oestrus. ---	Group II. 3rd/5th Day of Cycle. --- No * Ovulation.	Group III. 6th/7th Day of Cycle. --- Ovulation has occurred.	Group IV. 9th Day of Cycle. --- Ovulation has occurred.	Group V. 12th Day of Cycle. --- Ovulation has occurred.	Group VI. At Oestrus. --- Animals killed before new ovulation.	Group VII. At Oestrus. --- Dye given at previous oestrus which was suppressed.
Uterine Horns.							
Stratum Compactum.	+	+++	+	+	++++	+	+
	+	+++	+	+	++++	++	+
	+++	+++		+	++++	++	
	+++					++	
Stratum Spongiosum.	+	++	-	+	+	-	-
	+	++	-	+	+	+	-
	++	++		+	+	+	
	++					+	
Vaginal Mucosa.	+	+	+	++	++	+	+
	+	+	+	++	++	++	+
	++	+		++	++	++	
	++					++	

* The uterine, vaginal and ovarian appearances in these animals suggest that the oestrous changes had been prolonged.

\- = Absent.
\+ = Very small amount.
++ = Small amount.
+++ = Moderate amount.
++++ = Great amount.

At the 6th or 7th day of the new cycle, i.e. in the luteal phase about the time of implantation, only a small number of dye cells is present in the stratum compactum, and some of these appear to be degenerated. Practically no vitally stained cells are seen elsewhere in the uterine horn.

Fine dye granules are present in some of the endometrial cells of the stratum compactum and also in the uterine epithelium, especially during the second half of the cycle, i.e. during the follicular phase, and at oestrus.

In practically all the animals, except perhaps those killed at oestrus, the vital staining is more pronounced around the antimesometrial half of the uterine lumen. This is especially pronounced about the 12th day of the cycle. Moreover, it should be emphasised that embryos only implant in the uterine horns, and that the intense vital staining is confined to the uterine horn and proximal part of the uterine body, which has a similar structure. The cervix and vagina show relatively few vitally stained cells.

No definite cyclic distribution of the dye could be made out for the cervix and vagina, which at no period of the cycle showed the striking appearances found in the uterine horns at the 12th day of the cycle. At the time of oestrus no very apparent increase in the number of vitally stained cells was noted in the vaginal mucosa, and no dye cells were found in the vaginal epithelium.

Around the large blood vessels in the circular muscle zone of the uterine horns varying numbers of rounded dye cells are present irregularly throughout the cycle.

In some of the animals occasional dye cells appear to be migrating through the uterine epithelium into the lumen, and in the lumen of the uterus, cervix and vagina free dye cells were often found. These cells seem to have come chiefly from the peritoneal cavity via the tube, as will be described later, but some of them are undoubtedly derived from the uterine endometrium.

From the above results in controlled animals it may therefore be said that intense vital staining is confined to the uterus and is not haphazard, but that the intensity of the staining and the number and distribution of the vitally stained cells present varies with the different phases of the cycle.

INTRAVITAM STAINING IN THE UTERUS OF SEXUALLY IMMATURE ANIMALS.

The previous results during the cycle suggested that the appearances should also be examined in sexually immature animals.

The ten animals in this series were aged from two to two and a half months.* The reproductive tract and ovaries were cut and examined in the same manner as for the mature females in Table II, and sexual immaturity verified by the absence of corpora lutea.** The appearances are summarised in Table IV. The sizes of the largest normal and atretic follicles in the ovaries of these animals are also included in Table IV to serve as a possible index of the stage of sexual immaturity.

The first six animals show similar appearances, although larger follicles are present in the ovaries of some than in others. Only very occasionally is a rounded cell with a few dye granules found in the endometrium close to the uterine lumen. The cell is similar to the vitally stained cells seen in the mature animals. Around the blood vessels in the circular muscle zone a small number of similar vitally stained cells may be present, and also in the mesentery. No dye is present in the mucosa of the cervix. In the vaginal mucosa, an occasional rounded vitally stained cell with a small amount of dye is present, especially opposite a large blood vessel, and a varying

* The female guinea-pig is sexually mature at about three months old (Stockard and Papanicolaou 1917).

** The above series originally consisted of eleven animals. In one of these corpora lutea were found in the ovaries, and this fact, together with the uterine appearances, indicated that the animal was sexually mature. Intense vital staining was present in the uterine horns, practically confined to the stratum compactum, and the ovarian and uterine appearances accurately resembled those of the animals (Table II) killed approximately at the 12th day of a 16-day cycle.

number of small dye-containing fibrocytes may also be observed.

The last four animals are slightly older than the others, and show a very small number of rounded vitally stained cells in the endometrium of the uterine horns. The few cells present contain a small number of coarse dye granules and are found in the stratum compactum, chiefly opposite the middle of the uterine lumen. A few fine granules of dye may also be seen in some of the endometrial cells. Occasionally a dye cell appears to be passing through the epithelium into the uterine lumen. No dye is seen in the glandular, uterine or vaginal epithelium.

In all the animals dense nuclei without any related dye, and similar to the nuclei of the vitally stained cells, are present throughout the endometrium and in its blood vessels, as in the mature animals. They are usually most numerous in the stratum compactum. Similar nuclei are present in the mucosa of the cervix and vagina. The cell bodies enclosing the nuclei are rounded and only seen with difficulty, as in the older animals.

A few large rounded free dye cells showing a small number of coarse dye granules may be present in the lumen of the uterine horn.

In the lumen of the distal half of the uterine body, cervix and vagina, large masses of dye-containing cells are present in some of the animals. They seem to be of two types - rounded cells of varying size with dense oval nuclei and a varying number of coarse dark dye granules, similar to the free dye cells afterwards to be described in the Fallopian tube; and larger oval cells with finer dye granules. The latter cells are sometimes in great numbers and leucocytes may be included in them; they very closely resemble the small protozoa which are known to occur in the canals of various animals. Among the dye cells a number of smaller cells with dense nuclei and no dye is seen. These appear to be similar to the rounded cells without dye found in the endometrium.

SUMMARY OF UTERINE APPEARANCES IN SEXUALLY IMMATURE ANIMALS.

The results show that in sexually immature animals very few intravitally stained cells are present in the endometrium of the uterine horns, although large follicles may be present in the ovaries. Dense nuclei without any related dye and similar in appearance to the nuclei of the dye-containing cells are, however, present as in the mature animals.

Table IV. Records of Ten Sexually Immature Female Guinea-Pigs Injected Intravitally with Trypan Blue, and which showed no evidence of Toxic Symptoms.

Index Number of Animal.	Number of Injections and dosage of Trypan Blue 1% Soln.	Dye in Mucosa of uterine horn.	Size of largest* normal follicle in ovary.	Size of largest* atretic follicle in ovary.
203	6 of 1.5 ccs.	Practically no dye.	390 microns,	570 microns.
207	6 of 2.25 ccs.	"	500 "	750 "
205	6 of 2.25 ccs.	"	500 "	790 "
208	6 of 2.25 ccs.	"	500 "	800 "
206	6 of 2.25 ccs.	"	600 "	800 "
202	6 of 1.5 ccs.	"	500 "	1000 "
204	6 of 2.25 ccs.	Very small amount in stratum compactum.	500 "	1020 "
115	6 of 2.25 ccs.	"	790 "	600 "
116	6 of 2.25 ccs.	"	500 "	560 "
88	6 of 2.5 ccs.	"	400 "	800 "

INTRAVITAM STAINING IN THE UTERUS IMMEDIATELY AFTER PARTURITION.

Three animals were injected and killed soon after parturition to examine the vital staining appearances, if any, at this period, and determine whether or not they might aid in the interpretation of the appearances already described in the non-

* Several follicles of the size recorded were present in each ovary, together with a varying number of smaller follicles.

pregnant animals.

In view of the uncertain date of parturition it was not possible to arrange that each animal would receive the same number of injections of the dye. The animals were killed on the day after the last injection.

The following are the appearances in the uterine horns of the three animals.

Animal No. 189. Received nine injections of 5 ccs trypan blue 1% solution. Littered two, born dead. Killed 30 hours post partum. New ovulation had not yet occurred.

The uterus is large, the endometrium very narrow, and the epithelium is in places being cast off. Fine dye granules are present in some of the old epithelial cells still attached to the stratum compactum. Many rounded vitally stained cells similar to those in the virgin animals are present in the endometrium, more or less all round the lumen. They are most numerous in the stratum compactum in which they are loosely and irregularly scattered. At the mesometrial side of the lumen near the placental site the cells are often concentrated in groups close to the uterine epithelium. A considerable number of the cells have no visible nucleus and appear to be in a state of degeneration. In other cells the nucleus is clearly seen. Occasionally a dye cell is seen in the uterine epithelium. A smaller number of dye cells is present in the stratum spongiosum, and in these the granules are finer and fewer in number. A considerable number of apparently similar cells without dye is scattered throughout the endometrium and in its blood vessels, as in the virgin animals. The endometrial cells tend to form fibrillae, and in these fine dye granules may be observed. In the circular muscle zone the vital staining appearances are very striking. Great numbers of large rounded dye cells are present, especially in groups around the blood vessels, and also in the mesentery. They appear to be similar to the vitally stained cells in the endometrium, but

contain slightly less dye. A number of apparently similar cells without dye is also present. No vitally stained cells are present in the blood vessels, although cells with similar nuclei are seen there. No dye appears to be present in the muscle fibres themselves, but many rounded dye cells and vitally stained fibrocytes are found in the connective tissue septa between the muscle fibres, and also under the serous coat. A number of rounded dye cells similar to those in the endometrium is also seen adherent to the outer surface of the serous coat. They have apparently come from the peritoneal cavity. Fine dye granules may be seen in some of the polymorphs present both in the endometrium and in the vessels.

Animal No. 192. Received two injections of 5 ccs trypan blue 1% solution. Killed 32 hours post partum. Littered three, born alive. New ovulation has occurred.

The uterus is at the same stage of involution as in the previous animal. A very small amount of vital staining is, however, seen. Many dense nuclei similar to the nuclei of the large dye cells in the previous animal are scattered throughout the uterine wall. They are seen in great numbers in the stratum compactum, but the cell outlines are not easily defined. Around the large blood vessels they are present in great numbers, and the rounded cell bodies are more easily seen. They are also present under the serous coat. Careful examination shows that many of the cells around the large vessels and under the serous coat contain a small number of fine dye granules, and that they are undoubtedly the same cells as were found in this situation, intensely stained with the dye, in the previous animal. Only very occasionally was any dye found in the rounded cells in the endometrium.

The histological appearances are therefore very similar in both cases, and in this animal the very small amount of vital staining suggests that an insufficient amount of dye was administered, and too little time had elapsed to allow of the cells becoming more

intensely stained.

Animal No. 279. Received three injections of 5 ccs trypan blue 1% solution. Killed 50 hours post partum. Littered two, born dead. New ovulation has failed to occur.

The stage of involution is not much further advanced than in the previous animals, and great numbers of similar rounded cells with dense nuclei are present throughout the uterine horns. The intravitam staining is definitely more intense than in animal No. 192, and in distribution resembles animal No. 189. Coarse dye granules are, however, only present in a few cells in the endometrium, and in a few cells around the large blood vessels. It should be noted that groups of the vitally stained cells are definitely inside large vascular or lymphatic channels with a thin endothelial lining.

SUMMARY OF RESULTS IN THE POST-PARTUM UTERUS.

Involution and repair of the uterus therefore seems to be accompanied by great activity in the cells which have the capacity to take up the dye. It should be noted, however, that if enough dye is given to produce marked vital staining as in animal No. 189, the intensity of the staining is nevertheless not so pronounced as in the virgin animal at the 12th day of a 16-day cycle, when very large almost solid masses of very coarse dye are present close to the uterine lumen. Furthermore, whereas in the latter animal the intense vital staining is practically confined to the stratum compactum, in the post partum animal it extends throughout the entire thickness of the uterine horns. The post-partum animals also seem to provide further evidence that many of the dye cells are derived from the blood, and perhaps also from the connective tissue around the large vessels.

THE DISTRIBUTION OF FAT IN THE UTERUS DURING THE OESTROUS CYCLE.

Goldmann (1912) pointed out that there is a close relationship between vitally stained substance and fat. In view of

this it seemed desirable to compare the distribution of fat in the uterus during the cycle with the vital staining results previously described. For this purpose ten animals were killed at different periods of the cycle. The details concerning these are shown in Table V, in which the animals are divided into groups for the purpose of description.

For each animal the last six consecutive cycles were known to be perfectly regular as in the case of the animals in Table II. Fixation was in 10% Formalin and the sections were stained for twelve hours with Grübler's Sudan III. It should be emphasised that, compared with the above method, the ordinary Sudan III technique and the Osmic Acid method used for the same tissue gave very poor results which were quite unsuitable for comparative study.

The first eight animals were not intravitally stained, in order to obtain results in animals which had not been subjected to experiment.

It was found that in the uterus fat may be present in the uterine and glandular epithelium, and in the connective tissue of the endometrium, but varies in amount. The results in the uterine and glandular epithelium have been arranged in tabular form (Table VI).*

Table VI. Showing Amount and Distribution of Fat in the Uterine and Glandular Epithelium during the Oestrous Cycle.

	Group I. At Oestrus.	Group II. 6th Day of Cycle.	Group III. 9th Day of Cycle.	Group IV. 13th Day of Cycle.	Group V. 15th Day of Cycle.
Uterine Epithelium.	+	++	+++	++	
	+	+++	++++	+++	+
Epithelium of Gland Ducts.	+	+	++	++	
	+	+	++++	++	+
Epithelium of Gland Fundi.	++	++	+++	+++	
	++	++	+++	+++	++

\+ = Very Small Amount.
++ = Small Amount.
+++ = Moderate Amount.
++++ = Great Amount.

* The full details of the appearances in the several groups will be found in Appendix V.

It may be seen (Table VI) that at "heat" the amount of fat in the uterine and glandular epithelium is small, and seems to increase up to about the midpoint of the cycle or a little later, then becomes diminished. The curve of distribution bears a close resemblance to that of the activity of the corpus luteum, which is fully formed at the fifth day of the cycle, and begins to regress about the 11th day, or soon after.

In the connective tissue of the stratum compactum fat is present in varying amount in the form of small "masses". These vary in size up to the size of the largest dye-containing cell described in the vitally stained animals, and sometimes even larger. The "fat masses" are irregular in shape and often appear somewhat granular, and to be composed of a number of droplets grouped together. An occasional mass of fat is seen in the stratum spongiosum between the glands. The fat is presumably in cells in the endometrium, but the cell outline cannot usually be easily made out. A few of the "fat masses" seem to be in capillaries, and small particles of fat are clearly seen in some of the larger vessels. The distribution of the "fat masses" in the stratum compactum at certain periods of the cycle resembles that of the vitally stained cells. In view of this, the fat in the endometrium was plotted for each animal with the projection apparatus at a magnification of 100 diameters. The results are shown in fig. 6. They indicate that at "heat" a relatively small amount of fat is present. In the early part of the cycle the amount becomes increased, and the increase is more or less maintained until about the 13th day of a 17-day cycle, after which the amount present becomes reduced. It can be seen, however, that the curve of fat distribution in the connective tissue of the endometrium does not coincide with that of the vitally stained cells.

THE PRESENCE OF FAT AND DYE IN THE ENDOMETRIUM.

Animals Nos. 47 and 52 were intravitally stained (compare

Table II) and parts of one uterine horn of each animal were stained with Sudan III for fat. It has been recorded in the description of the appearances during the cycle that much more dye is present in the endometrium of animal No. 47 than in animal No. 52. In both animals, however, fat is clearly seen in a considerable number of the dye-containing cells. In some of the dye cells only a few isolated particles of fat are seen, in others the fat forms an irregular solid mass amongst the dye granules (fig.7), and in many dye cells no fat is visible. Moreover, isolated masses of fat not related to dye cells are present in the connective tissue of the endometrium, and no dye appears to be related to the small particles of fat in the uterine epithelium.

It may therefore be said that in the endometrium fat is present in some of the vitally stained cells although absent in others, and also that fat is present without any apparent relation to the dye.

Table V. Records of Ten Animals Killed to show the Distribution of Fat in the Uterus during the Oestrous Cycle.

Index Number of Animal.	Length of Normal Cycle in Days.	Time in Cycle when killed.	Trypan Blue Injected.
66	17	1st Day. Verified by Vaginal Smear.	No.
67	16	Do.	No.
45	17	6th Day.	No.
54	17	6th Day.	No.
53	17	9th Day.	No.
65	17	9th Day.	No.
55	17	13th Day.	No.
46	18	14th Day.	No.
47	17	13th Day.	Yes - Compare Table II.
52	17	15th Day.	Yes - Compare Table II.

THE RELATION OF ANTERIOR PITUITARY AND THE OVARIAN HORMONES TO THE PRESENCE OF TRYPAN BLUE AND FAT IN THE UTERUS OF DOUBLE OVARIECTOMISED ANIMALS.

The findings previously described in this memoir strongly suggested that the present research would be incomplete without also examining the relation, if any, of the anterior pituitary and the ovarian hormones to the presence of dye and fat in the endometrium of ovariectomised animals.

Both ovaries were therefore removed from a series of young sexually mature females, care being taken to exclude the possibility of accessory ovaries both at the time of operation and when the animals were killed.

The following experiments were carried out after sufficient time had elapsed to exclude the action of the ovarian hormones.

The dye was injected once a day as in the case of the previous intravitally stained animals described in this paper, and the animal was killed on the day following the last injection of the dye. The dosage of the dye was according to the weight of the animal - page 3. For each animal one uterine horn was fixed in 10% Formalin and stained for fat with Grübler's Sudan III. The other uterine horn, body of uterus, and vagina were fixed in Formalin and corrosive sublimate and stained to study the distribution of the dye. The examination for fat was carried out in the intravitally stained animals, as the dye if present does not appear to affect the staining of fat.

With regard to the presence or absence of dye, the comparative study of the results in the following experiments was made in sections stained with weak eosin, or stained for fat with Grübler's Sudan III and not counterstained. The character of the cells was verified in sections stained with carbol fuchsin.

Experiment No. 1. Animals given Dye only.

Five animals were injected with trypan blue in order to examine for the presence of dye and fat in the uterus which has been released from the action of the ovarian hormones. The details relating to these animals are shown in Table VII.

Table VII. Records of Five Ovariectomised Females which received Dye only.

Index Number of Animal.	Time after Ovariectomy when Dye Injections Commenced.	Number of Injections and dosage of Trypan Blue, 1% Solution.
11.	5 weeks.	4 of 5 ccs.
280.	5 weeks.	4 of 3.5 ccs.
281.	4 weeks.	4 of 3.5 ccs.
166.	4 weeks.	6 of 5 ccs.
181.	12 weeks.	6 of 5.5 ccs.

Result: No dye in endometrium of uterine horns.

Fat - Small amount in one animal only.

In all the animals the stratum compactum is much narrowed, and appears to have atrophied at the expense of the stratum spongiosum in which the glands are dilated.

Dye: The vital staining appearances are similar in all the animals. No dye cells are present in the endometrium of the uterine horns, but occasionally a small vitally stained fibrocyte is seen around a large blood vessel in the circular muscle zone.

In the mucosa of the cervix and vagina a relatively small rounded dye cell is very occasionally observed. A few vitally stained polymorphs may also be seen.

In the lumen of the cervix and vagina a small group of rounded dye cells of varying size and containing a moderate number of dye granules may be seen. From the appearances in the uterus and vagina it seems clear that these cells have come from the peritoneal cavity via the tube.

Fat: The uterine horns of the first three animals only (Table VII) were stained for fat. In two of these animals no fat was visible in the uterine epithelium, glands, or in the connective tissue of the endometrium. In the third animal a small number of fat masses was

present in the connective tissue of the stratum compactum, and very occasionally a small fat droplet was observed in one of the gland fundi.

Experiment No. 2. Animals given Dye and Oestrin (Follicular Extract).

Six animals were injected subcutaneously with trypan blue and oestrin to test the effect of intense oestrin action on the presence of dye and fat in the uterus. Two different samples of oestrin were used. The first three animals received dye and ketohydroxy oestrin, the other three animals received dye and 'Progynon'*. The details relating to these animals are given in Table VIII.

Table VIII. Records of Six Double Ovariectomised Females which received Dye and Oestrin.

Index Number of Animal.	Time after Ovariectomy when Injections commenced.	Number of Injections and Dosage of Trypan Blue and Oestrin.
37	4 weeks.	0.01 mg. ketohydroxy oestrin** two-hourly for 12 hours plus 4 ccs trypan blue 0.1% solution at 2 p.m. - for 2 days. For next 4 days same dosage of oestrin continued plus 4 ccs trypan blue 1% soln daily at 2 p.m. Animal killed on 7th day.
56	4 weeks.	Same as previous animal No. 37.
282	4 weeks.	Same as animal No. 37.
169	10 weeks.	15 mouse units of 'Progynon' morning and evening plus 5 ccs trypan blue 1% soln at 2 p.m. - for 3 days. On 4th day 30 mouse units of 'Progynon' morning and evening plus 5 ccs trypan blue 1% soln at 2 p.m. Animal killed on 5th day.
180	10 weeks.	25 mouse units of 'Progynon' morning and evening plus 5 ccs trypan blue 1% soln at 2 p.m. for 6 days. Animal killed on 7th day.
182	12 weeks.	30 mouse units of 'Progynon' morning and evening plus 5 ccs trypan blue 1% soln at 2 pm for 6 days. Animal killed on 7th day.

* Supplied by Messrs Schering, London.

** A solution in absolute alcohol in the strength of 0.1 milligramme of oestrin per cc was used. The dose was therefore 0.1 cc and the injections were made with a tuberculin syringe.

The vagina opened e.g. in the first three animals (Table VIII) about the third day of the oestrin injections. Oestrus was verified by the presence of cornified cells in the vaginal smear. No dye was observed, however, in the cells in the smears.

Result: A considerable number of rounded dye-containing cells is present, especially in the stratum compactum of the uterine horns.

Fat - moderate amount present.

Dye: The vital staining appearances are comparable in the six animals, but vary in intensity. The first four animals (Table VIII) show less vital staining than the last two.

Uterine horn: In the last two animals a considerable number of rounded dye-containing cells of varying size is seen in the stratum compactum, and a few in the stratum spongiosum. The cells are similar to the vitally stained cells present during the oestrous cycle. The granules are coarse in some of the cells and fine in others, and are scattered throughout the cytoplasm. The nucleus is clearly visible, and none of the cells are stained intensely enough to be bursting and shedding the dye. A number of polymorphs containing fine granules of pale blue dye is also seen. Around the large blood vessels in the circular muscle zone a moderate number of rounded dye cells and small narrow dye-containing fibrocytes is present.

In the first four animals a smaller number of dye cells is present in the stratum compactum, and the dye granules in the cells are mostly fine.

Similar appearances are found in the proximal half of the uterine body.

Cervix: In the last two animals a few rounded dye cells may be seen only in some of the mucosal folds, and also a small number of vitally stained fibrocytes. In the other animals practically no vitally stained cells are visible.

Similar appearances are present in the distal half of the

uterine body.

Vagina: In animal No. 180 a moderate number of rounded dye cells with a small amount of coarse dye is present in some of the mucosal folds. A moderate number of vitally stained fibrocytes is also seen. In the other five animals very few vitally stained cells are visible.

In all the animals, however, there is present throughout the walls of the uterine horns, uterine body and vagina, a considerable number of dense nuclei without any related dye, and many of which are similar to the nuclei of the rounded dye-containing cells.

No dye is seen in the uterine, cervical or vaginal epithelium.

It is important to note from the above results that, although in some of the animals the staining is not intense, the vitally stained cells are always most abundant in the endometrium of the uterine horns, and fewer in number or absent in the vagina, as was described in the study of the oestrous cycle. The intensity of the intravitam staining in these animals is however much less than e.g. at the 12th day of a 16-day cycle, but compares with the appearances in some of the females (Table II) killed at the time of oestrus. It was not ascertained whether larger doses of oestrin* produced more intense intravitam staining. The above results nevertheless serve to prove that oestrin influences the appearance of the dye-containing cells in the uterus, and also indicate that this influence is exerted chiefly on the endometrium of the uterine horns.

Fat: The uterine horns of the first three animals only (Table VIII) were stained for fat.

A small number of fine fat particles is present in the uterine epithelium in two animals, and a considerable amount of relatively coarse fat irregularly distributed is seen in the uterine epithelium

* I am obliged to Dr A.S.Parkes, F.R.S., London, for advice on the dosage of 'Progynon'. I am indebted to Dr J.M.Robson, Institute of Animal Genetics, University of Edinburgh, for suggesting the procedure and dosage of ketohydroxy oestrin, corpus luteum hormone, and anterior pituitary hormone in these experiments.

of the third animal.

In the epithelium of some of the gland ducts a considerable amount of relatively coarse fat is seen, though absent in others.

In the epithelium of the gland fundi fat may be absent or a very small number of fine fat particles may be seen, and in one animal a considerable amount of relatively coarse fat is present in the epithelium of a few of the gland fundi.

In the connective tissue of the endometrium a variable number of fat masses is present - a small number in one animal, a moderate number in the second, and a considerable number in the third.

Fat particles of varying size are seen in many of the dye cells in the stratum compactum, and in similar dye cells around the large blood vessels in the circular muscle zone.

Experiment No. 3. Animals given Dye and Corpus Luteum Hormone,* the uterus previously having been sensitised by small doses of oestrin.

Three animals were injected. This experiment was carried out to test the effect of corpus luteum hormone on the presence of dye and fat in the uterus. The animals were given small doses of oestrin for six days previous to the injection of the dye and corpus luteum hormone, as it is now known that the luteal hormone does not exert its action on the uterus in the ovariectomised animal, unless the latter has been previously subjected to the action of oestrin (Robson 1934).

The details concerning these animals are shown in Table IX.

* The corpus luteum hormone used was Progestin Organon, supplied by Organon Laboratories, London, and stated by them in a private communication to be oestrin-free.

Table IX. Records of Three Ovariectomised Females which received Dye and Corpus Luteum Hormone - uterus previously sensitised by Oestrin.

Index No. of Animal.	Time after Ovariectomy when injections commenced.	Treatment.
57.	4 weeks.	0.01 mg. ketohydroxy oestrin subcutaneously morning and evening for 6 days. Then two rabbit units of Progestin * intramuscularly morning and evening plus 3.5 ccs Trypan Blue 1% solution subcutaneously at 2 p.m. daily for 4 days. Animal killed the following day.
283.	4 weeks.	Ditto.
284.	4 weeks.	Ditto.

The vagina opened about the fourth day of the oestrin injections, and remained open during the injections of Progestin and dye.

Result: Dye - Moderate number of rounded dye cells in endometrium.

Fat - Intense very coarse fat staining in uterine epithelium.

The three animals present practically identical appearances.

Dye: A moderate number of faintly stained rounded dye cells is seen in the stratum compactum, and in some of these cells small particles of fat are visible.

Around the large blood vessels in the circular muscle zone a moderate or small number of rounded dye cells and vitally stained fibrocytes is present.

Fat: The fat staining is very striking and intense (fig.8). Abundant very coarse fat droplets are present in the uterine epithelium, and occasionally in the termination of a gland duct. No fat is seen in the glands except occasionally a very small particle. In the connective tissue of the endometrium a small number of fat

* One rabbit unit into chest wall and one rabbit unit into vastus internus.

masses is seen.

Experiment No. 4. Animals given Dye and Corpus Luteum Hormone - no Oestrin previously injected.

Three animals were injected. This experiment was carried out to test the effect of corpus luteum hormone on the presence of dye and fat, when the uterus had not been previously sensitised by oestrin.

The details relating to these animals are given in Table X.

Table X. Records of Three Ovariectomised Females which received Dye and Corpus Luteum Hormone - Uterus not previously sensitised by Oestrin.

Index No. of Animal.	Time after Ovariectomy when Injections commenced.	Treatment.
19.	4 weeks.	Two rabbit units of Progestin intramuscularly morning and evening plus 4 ccs Trypan Blue 1% Solution at 2 p.m. daily for 4 days. Killed the following day.
285.	5 weeks.	Two rabbit units of Progestin intramuscularly morning and evening plus 3.5 ccs Trypan Blue 1% solution at 2 p.m. daily for 4 days. Killed the following day.
286.	5 weeks.	Ditto.

Result: Dye - absent or very occasional dye cell seen in endometrium.

Fat - absent or small amount.

Dye: The three animals show similar vital staining appearances. No dye is visible in the endometrium except a very occasional faintly stained cell near the uterine epithelium. In the circular muscle zone a few rounded dye cells and vitally stained fibrocytes may be seen around the large blood vessels, and in some of the rounded cells a small particle of fat may be present.

Fat - In the first animal a moderate number of fat masses is present in the connective tissue of the stratum compactum, and in the second animal a moderate amount of fat is seen in the uterine epithelium confined to the extreme antimesometrial side of the lumen. No other

fat is visible in these animals, and none in the third animal.

Experiment No. 5. Animals given dye only - uterus previously sensitised by oestrin.

Three animals were injected. This experiment was carried out to ascertain if the moderate number of vitally stained cells present in the animals which received corpus luteum and dye, the uterus previously having been s ensitised by oestrin (experiment No. 3), could be attributed to the previous action of the oestrin.

The details concerning these animals are shown in Table XI.

Table XI. Records of Three Females given Dye only - Uterus previously sensitised by oestrin.

Index No. of Animal.	Time after Ovariectomy when injections commenced.	Treatment.
288.	2 weeks.	0.01 mg. of ketohydroxy oestrin subcutaneously morning and evening for 6 days. Then 3.6 ccs Trypan Blue 1% solution at 2 p.m. for next 4 days. Animal killed the following day.
289.	2 weeks.	Ditto.
290.	2 weeks.	Ditto.

Result: The three animals show practically identical appearances.

Dye - A moderate number of rounded dye cells is present in the stratum compactum of the uterine horns, the vital staining appearances being identical with those in experiment No. 3. No vitally stained cells are seen in the vaginal mucosa. It is therefore reasonable to conclude that the dye cells present in the endometrium in experiment No. 3 are the result of the previous action of the oestrin.

Fat - Absent in uterine epithelium and glands. Very small number of small fat particles in the connective tissue of stratum compactum.

Experiment No. 6. Animals given Dye and Anterior Pituitary.

Three animals were injected. The experiment was carried out to test the effect, if any, of anterior pituitary on the presence of dye and fat in the uterus.

Anterior pituitary gland powder acetone dried was obtained from British Drug Houses, London. The powder was then ground in a mortar and passed through an 80 wire mesh sieve. The resultant very fine powder was injected suspended in sterilised normal saline solution.

The details concerning these animals are given in Table XII.

Table XII. Record of Three Females injected with Dye and Anterior Pituitary.

Index No. of Animal.	Time after Ovariectomy when injections commenced.	Treatment.
25.	4 weeks.	50 mg. of Anterior Pituitary powder suspended in 2 ccs normal saline injected intramuscularly at 10 a.m. plus 4 ccs Trypan Blue 0.1% soln. subcutaneously at 2 p.m. for 2 days. Same dosage of Anterior Pituitary at 10 a.m. plus 4 ccs Trypan Blue 1% soln. for next 4 days. Animal killed the following day.
287.	4 weeks.	Ditto.
63.	4 weeks.	Ditto.

Result: No Dye in endometrium.

Fat - variable amount in connective tissue of endometrium; very small amount in some glands.

Dye: The appearances with regard to the dye are similar in the three animals. No dye is visible in the endometrium of the uterine horns, and only occasionally is a rounded dye cell or vitally stained fibrocyte present around the large blood vessels in the circular muscle zone.

Fat: In the first animal a considerable number of large fat masses is present especially in the stratum compactum, a moderate number in the second animal, and a small number in the third. No fat is

visible in the uterine epithelium, and only a small number of very fine particles is seen in some of the glands.

In all the animals used in these experiments it should be emphasised that dense oval nuclei without related dye, and similar to the nuclei of dye-containing cells, are invariably present in the endometrium and in its blood vessels, and also in the mucosa of the cervix and vagina.

SUMMARY OF RESULTS OF HORMONE EXPERIMENTS.

A summary of the combined results of the previous six experiments is given in Table XIII. Before considering these results it is necessary to point out that the first three animals in experiment No. 2 and all the animals in experiment No. 6 each received six injections of trypan blue, but the first two injections were only of the strength of 0.1% solution of the dye, although for the other four injections a 1% solution was used. This procedure was adopted in order that for all the experiments a group of at least three animals in each would receive a comparable amount of dye, according to the weight of the animal.

Dye: It seems clear from the above results that oestrin is responsible for the production of conditions in the uterus which bring about the appearance of dye-containing cells in the endometrium, while in the vagina the effect is less or even absent. The vital staining is not produced by the mere injection of the dye, nor by the additional influence of the corpus luteum hormone either in the sensitised or non-sensitised uterus, nor by the anterior pituitary. It should be noted, however, that there are present in the endometrium and in its blood vessels in all the animals dense oval nuclei without related dye, and some of which at least appear to be similar to the nuclei of dye-containing cells. The previous studies in this memoir strongly indicate that some of these cells are the cells which, under suitable circumstances, take up the dye, and the above experiments prove that oestrin brings about the conditions in the uterus suitable

for this purpose. It may be also that oestrin causes, directly or indirectly, an increased production of these cells.

Fat: Practically no fat is found in the uterus of the ovariectomised animal not treated with hormones.

The most striking result is that corpus luteum hormone, acting on the uterus previously sensitised by oestrin, produces an intense accumulation of very coarse fat in the epithelium of the uterine lumen, and that this appearance does not occur in the non-sensitised uterus. Furthermore, only a very small amount of fat is present in the glands and connective tissue of the endometrium in animals treated with corpus luteum hormone.

In the animals injected with oestrin a small amount of fat is seen in the glands and uterine epithelium, but a moderate number of fat "masses" is present in the connective tissue of the endometrium.

In the animals injected with anterior pituitary no fat is seen in the uterine or gland epithelium, but a moderate number of well defined fat "masses" is present in the connective tissue of the endometrium. It is not possible to say whether or not this has any significance, but the appearances suggest that perhaps the anterior pituitary has some direct action on the endometrium.

It is important to note that in all the experiments where rounded dye cells are seen, either in the endometrium or around the large blood vessels, many of the dye cells also contain small particles of fat.

Table XIII. Summary of Combined Results of the previous six Experiments in regard to the Presence of Dye and Fat in the Endometrium of the Uterine Horns.

Experiment.	Dye in Endometrium.	Fat in		
		Uterine Epithelium.	Uterine Glands.	Connective Tissue of Endometrium.
No.1. Dye Only.	Absent.	Absent.	Absent.	Absent or very small amount.
No.2. Dye and oestrin.	Considerable number of rounded dye cells.	Small amount.	Moderate or small amount.	Moderate amount.
No.3. Dye and Corpus Luteum Hormone (Uterus previously sensitised by oestrin).	Moderate number of rounded Dye cells.	Great amount.	Practically absent.	Very small amount.
No.4. Dye and Corpus Luteum Hormone (No oestrin previously injected).	Practically absent.	Absent or small amount.	Absent.	Absent or moderate amount.
No.5. Dye only. (Uterus previously sensitised by oestrin).	Moderate number of rounded dye cells.	Absent.	Absent.	Very small amount.
No.6. Dye and Anterior Pituitary.	Absent.	Absent.	Practically absent.	Moderate amount.

INTRAVITAM STAINING IN THE PREGNANT ANIMAL.

The vital staining appearances were also investigated in a series of pregnant animals, to find out if any further help might be given towards the interpretation of the previous results and the significance of intravitam staining.

Each animal received one injection of dye per day and was

killed on the day following the last injection.

Considerable difficulty was experienced in securing examples of the early stages of development. The uterine horn must be serially sectioned from end to end, and the embryo is present only on a few sections. A complete series of several thousand sections is thus necessitated for each uterus; and the animal may be sterile.

Nine animals were obtained showing normal embryos. These will now be described.

Free Blastocyst: Age 6 days, 6 hours.

Animal No. 126. Received five injections of 5.4 ccs trypan blue 1% solution. One normal blastocyst present, free in the uterine lumen.

The blastocyst shows no evidence of dye. In the endometrium opposite the blastocyst a small number of rounded vitally stained cells is present. They show varying amounts of dye, but none of the cells are stained intensely, and they are similar to the vitally stained cells found in the virgin uterus. They are, however, not more numerous at the site of the embryo than in other parts of the uterine horn where no blastocyst is seen.

In the circular muscle zone around some of the larger vessels large groups of rounded dye cells are seen, together with a number of vitally stained fibrocytes.

Embryo Just Implanted: Age 6 days, 9 hours.

Animal No. 261. Received three injections of 4 ccs trypan blue 1% solution. In one uterine horn three embryos at same stage of development. Similar appearances are seen at the site of each embryo. No embryos in other horn.

The embryo is just implanted and the decidual cavity only measures 0.17 x 0.14 x 0.1 mm (fig. 9). The stratum compactum may be described as consisting of two zones - an inner pear-shaped compact zone around the uterine lumen, its base enclosing the embryo and decidual cavity; and an outer looser zone continuous with the stratum spongiosum. The endometrial nuclei especially in the antimesometrial part of the compact zone around the decidual cavity are pale, round and very large, measuring 9 or 10 microns in diameter, and the cells

are irregularly oval in shape (fig. 9). On the inner wall of the decidual cavity is a varying number of smaller nuclei which here and there have run together and stain intensely. A number of cells with similar nuclei is seen in the space itself, and some of these have protoplasmic strands (fig. 9).

No dye is visible in the embryo or uterine epithelium. Fine dye granules are, however, present in some of the free cells and protoplasmic strands in the decidual cavity. The endometrium itself is practically free of dye, but occasionally one of the endometrial cells with the very large pale nuclei forming part of the wall of the decidual cavity shows a few granules. Only very rarely are a few fine granules seen apparently related to an endometrial cell in any other part of the uterine wall.

Scattered throughout the entire endometrium there is a small number of rounded dye-containing cells similar to those found during the oestrous cycle. Some of these cells contain a considerable amount of coarse granules, while others show only an occasional granule.

A considerable number of apparently similar cells without dye is also present in the endometrium and in its blood vessels, as in the virgin uterus. Although most of these cells have nuclei identical with the rounded dye-containing cells, in others the nucleus is more intensely stained. Many of the cells with the very intensely stained nuclei are actively dividing, and practically all stages of mitosis are seen. No dividing cells are, however, present in the blood vessels. It is not possible to say for certain whether all these cells are of the same type, but the fact that in some of those in an active state of division a few dye granules are seen suggests that they are, and that the individual differences are due to different stages of activity.

A number of vitally stained polymorphs is also present.

In the circular muscle zone around the large blood vessels

there is a considerable number of similar dye cells with a varying amount of coarse dye. The cells vary in size and some are free of dye. None of the cells, however, show mitosis. They are similar to the vitally stained cells described in this situation in the virgin animal but more numerous, and relatively large groups of them may be seen. Similar cells without dye are present in the blood vessels. The rounded dye-containing cells are, however, much less numerous than in the animals killed about the time of parturition. A few vitally stained fibrocytes are also present.

Embryo aged 9½ days.

Animals Nos. 157 and 158. Each animal received five injections of 3.6 ccs trypan blue 1% solution. One normal embryo in animal No. 157, and one in each uterine horn of animal No. 158. Similar appearances are seen in both animals.

The embryo is larger than at the earlier stage, and the decidual cavity now measures 1.0 x 0.7 x 0.9 mm (fig. 10). The nuclei forming the main part of the wall of the decidual cavity are, as around the younger embryo, very large, pale and vesicular, and from this region outwards to the stratum spongiosum they become progressively smaller, and the cells more pointed and elongated. The enlargement of the embryo and the extension of the decidual cavity have resulted in the antimesometrial half of the uterine lumen becoming obliterated, only the mesometrial part remaining.

The decidual cavity itself contains many small cells which stain more intensely than the larger endometrial cells immediately around (fig. 10). The smaller cells occur in irregular masses or singly, and some are arranged around the termination of the blood vessels. Bryce and Maclaren (1933) regard these cells as the remains of obliterated uterine and glandular epithelium, while Sansom and Hill (1931) believe them to be trophoblast. It is not proposed to touch this question in the present memoir. The termination of the blood vessels project into the cavity but do not open into it, and no

free red blood cells are ever present.

The vital staining appearances are as follows. No dye granules are visible in the embryo or yolk sac endoderm. Some of the small cells in the decidual cavity, however, show a few dye granules. In the wall of the decidual cavity striking appearances are seen. The innermost row of large endometrial cells shows only a small amount of fine dye granules, and some of the cells are free of dye. The succeeding six or seven rows of large endometrial cells, however, show intense vital staining, and form, as it were, a "barrage" of blue cells in the wall of the decidual cavity. (A small area of this "barrage zone" is illustrated in fig. 11). The dye granules are relatively fine and collected in the cell, usually in a dense mass, to one side of the large pale nucleus. Sometimes the granules are arranged around a large vacuole in the cell. The next two or three rows of cells contain a smaller amount of dye. Beyond this no dye is present in the endometrial cells, and the vital staining ceases somewhat abruptly. The large intravitally stained endometrial cells are easily distinguished, by their large size and very large pale nuclei, from the rounded vitally stained cells with smaller dense nuclei present during the oestrous cycle. It is important to note that the above appearances extend equally around the decidual cavity, but are absent opposite the träger end of the embryo where the placenta will form. They are also confined to the site of the decidual cavity, and are absent in the wall of the uterus beyond this.

No dye is present in the endometrial cells around the remains of the uterine lumen, or in the endometrial cells in any other part of the uterine wall. The epithelium of the uterine lumen is also free of dye.

Scattered throughout the endometrium there is, however, a moderate number of rounded cells with dense nuclei, similar to those described with the younger embryo and in the virgin animal. A few of these cells are also present in the wall of the decidual cavity.

Only very occasionally are any dye granules visible in one of these cells. A greater number of similar cells is present in the narrow stratum spongiosum, and a moderate number of the cells here shows a few dye granules.

In the circular muscle zone a moderate number of rounded dye cells is found around the larger blood vessels, as in the earlier pregnancy and in the virgin animal, and a number of vitally stained fibrocytes is also seen.

A pregnant uterus of the same age of 9½ days, from an animal which had not received dye, was stained for fat with Sudan III. It was found that many of the large endometrial cells in the wall of the decidual cavity contained numerous fine fat particles. These cells, in their position, appear to be the same cells as contained the dye in the intravitally stained animals, and no fat was observed in the remainder of the endometrium.

Embryo aged 12 days.

Animal No. 81. Received five injections of 4 ccs trypan blue 1% solution. Five normal embryos in one uterine horn. No embryos in other uterine horn. Similar appearances are seen at the site of each embryo.

The embryo and decidual cavity are now much elongated and enlarged. The träger end has reached almost to the mesometrial side of the uterus, where it can be seen pushing aside and obliterating the remains of the uterine lumen. In this process the uterine epithelial cells are being compressed and separated off, and are clearly seen to be adding to the many small deeply stained cells in the decidual cavity and distributed along its inner wall. The decidual cavity also contains a loose coagulum, but no red blood cells. At the antimesometrial end of the cavity many blood vessels are seen running inwards to terminate blindly, and along its sides very long straight vessels are seen coursing parallel to the edge, and only separated from the interior by a single layer of cells.

No dye granules are visible in the embryo or developing placenta, but the cytoplasm of the yolk sac endoderm is tinged a very pale blue. A few of the smaller cells in the decidual cavity show a small amount of fine dye granules.

The endometrium forming the wall of the decidual cavity, and which in the earlier pregnancy shows intense granular vital staining, has undergone a remarkable change. This entire zone is now separating off from the surrounding endometrium, and its cells, which at the earlier stage were closely packed together, are now loosely arranged and many of them are degenerating. The cell bodies are often joined together by narrow protoplasmic processes, and in a number of the cells the nuclei are intensely stained. Moreover, many of the cells contain large vacuoles. The vital staining appearances in this zone are slightly less intense than at 9½ days, and are markedly changed in character. The most intense staining is seen at the antimesometrial side of the decidual cavity. Only a few of the cells, however, show well-defined discrete dye granules. In the majority of the cells the cytoplasm is filled with very fine dye granules which give the effect of a diffuse well-marked blue coloration, which, however, since it is clearly visible microscopically, differs from the diffuse staining seen in most of the tissues when the animal was killed. The blue appearance in the cytoplasm of the cells becomes progressively less towards the mesometric side of the decidual cavity, where it is almost absent. No trace of dye is visible in the remainder of the endometrial cells.

Scattered throughout the endometrium is a moderate number of rounded cells with dense nuclei, as in the previous pregnancies. Very occasionally a few of the cells contain fine dye granules. These cells are more numerous in the stratum spongiosum, which is now almost obliterated, and here a moderate number of them show a few coarse granules.

In the circular muscle zone around the large blood vessels

the appearances are as in the earlier pregnancies, but the number of vitally stained cells present is smaller.

Embryo aged 14 days.

Animal No. 80. Received five injections of 4 ccs trypan blue 1% solution. Three normal embryos, one in one uterine horn and two in the other. Similar appearances are present at the site of each embryo.

The embryo and decidual cavity are still more elongated and enlarged.

No dye granules are visible in the embryo - träger, ectoplacental endoderm, yolk sac endoderm, or formative knob. The decidual cavity contains fewer small cells than in the previous embryo, and no red cells. The loose endometrial zone forming the wall of the cavity is still more broken up, and narrower than in the previous pregnancy. The vital staining in this zone is diminished. A relatively small number of the cells show discrete dye granules. The cytoplasm of most of the cells in this zone, however, especially around the antimesometrial side of the cavity, is tinged blue and looks granular.

In the remainder of the endometrium no blue coloration or dye granules are present.

A small number of rounded cells with dense nuclei is scattered throughout the endometrium, but none of these show dye. Small polymorphs are also seen, and some of these contain dye granules. The stratum spongiosum has practically disappeared, except at the antimesometrial side of the uterus, where the new uterine lumen is forming from the sides. Around the new lumen a considerable number of rounded cells with dense nuclei and coarse dye granules, and a number of vitally stained fibrocytes, are present.

In the circular muscle zone the appearances are similar to those in the previous pregnancy.

Embryo aged 15 days.

Animal No. 163. Received five injections of 4 ccs trypan blue 1%

solution. Three normal embryos in one uterine horn. No embryos in other horn. Similar appearances are seen at the site of each embryo.

No dye granules are visible in the embryo, developing placenta, ectoplacental endoderm, or in the foetal syncytial strands which are beginning to invade the decidua. A few fine granules are, however, seen in the calls of the yolk sac endoderm near to the placenta.

The endometrium and uterine wall show practically similar appearances to those described in the pregnancy aged 14 days.

Embryo aged 16 days.

Animal No. 112. Received six injections of 5 ccs trypan blue 1% solution. One normal embryo in one uterine horn. No embryos in other uterine horn.

The vital staining appearances are similar to those in the previous pregnancy, except that in the decidua capsularis they are perhaps less marked. A moderate number of rounded cells with dense nuclei is scattered throughout the endometrium as in the previous animals, and they are more numerous opposite the developing placenta. A few of these cells in the decidua capsularis contain dye granules.

Embryo aged 18 days.

Animal No. 83. Received five injections of 4 ccs trypan blue 1% solution. Two normal embryos in one uterine horn. No embryos present in other horn. Similar appearances are found at the site of each embryo.

The embryo has a maximum length of 2.5 mm. The yolk sac endoderm now shows an abundant deposit of the blue dye. The staining is most intense in the dorsal third of the endoderm where the cells are columnar, the dye granules being very large and coarse, and confined chiefly to the deeper parts of the cells (fig. 12). Fat particles were also observed in these cells. As the cells become progressively flatter, the dye granules are not so numerous, but are still large and coarse (fig. 13). The intense vital staining begins

near the attachment of the yolk sac endoderm to the edge of the developing placenta, and ends a short distance from the embryo. The dye could not have come from the maternal circulation as the foetal and maternal circulations are not yet in contact, the allantois in this embryo not having reached the placental site. In this case the blue staining material can only have been absorbed from the destruction space by the endoderm cells.

No dye is present in the embryonic endoderm, or in any other part of the embryo.

The ectoplacental endoderm is apparently free of dye, although an occasional small granule is found in these cells near the attachment of the yolk sac endoderm to the edge of the developing placenta.

No granular staining is present in the developing placenta, but occasionally small foetal masses near the surface of the placental labyrinth show a pale diffuse blue coloration. In the foetal syncytial masses which invade the decidua, and form an irregular boundary between the foetal and maternal tissues, commonly referred to as the central zone or "Umlagerungszone", no granular or diffuse staining is visible.

The decidua capsularis is still further broken up, and the vital staining still less marked. The cells opposite the intensely stained yolk sac endoderm show most dye. A number of these may show definite dye granules, or the dye collected around a large vacuole in the cell, but many of them show only a blue coloration of the cytoplasm. A small number of fine granules is present in some of the adjoining peripheral endometrial cells. The vital staining is still less marked and practically absent opposite the placenta.

In the circular muscle zone the appearances are as in the earlier pregnancies.

Wislocki (1921) working on older embryos of a minimum length of 15 mm in which the foetal and maternal circulations are in contact

also found the yolk sac endoderm stained, but no dye in the ecto-placental endoderm. He states that the dye was stored to a slight extent by the ectoderm covering the chorionic villi of the placental labyrinth, but was not taken up by the interlobular syncytium, or by that part of the ectoderm composed of giant cells and syncytium which occupies the central zone (umlagerungszone). He also found the dye in the form of fine granules in the layer of giant cells of Duval situated beneath the ectoplacental endoderm. He states with reference to no dye being found in the central zone "this is surprising, since in the injected mouse and rat these cells are filled with blue pigment throughout gestation. Possibly the foetal syncytium stains vitally in the guinea-pig in the younger stages, when the ectoplacental cone is sending out syncytial roots and is burrowing its way into the decidua." The appearances in this and in the younger embryos show, however, that no dye is found in the foetal syncytium even in the earliest stages of its development.

INTRAVITAM STAINING WITH TRYPAN BLUE AND THE INJECTION OF INDIA INK IN PREGNANT ANIMALS.

This experiment was done to ascertain if any of the vitally stained cells in the decidua capsularis would also take up ink particles.

Three pregnant animals each received four injections of trypan blue subcutaneously, and on the fifth day 6 ccs of 10 per cent solution of india ink into the blood stream. The animals were killed thirtysix hours later.

The operation of injecting the ink was as follows: The left common carotid artery was tied about the middle of the neck, and the ink injected downwards via this vessel into the aorta. The jugular veins were found to be too delicate for this purpose.

The embryos were aged about fifteen days. One animal contained normal embryos, but in the other two animals the embryos showed early pathological change. No ink was recognised in any of the decidual cells, or in the decidual cavity. It may be noted, however,

that suspensoid preparations, e.g. india ink, possess no powers of diffusion, and when injected into the blood stream are stored only in cells which line the blood channels, as well as in a proportion of the circulating monocytes (Cappell 1929). The absence of ink particles in the decidual cells may therefore be related to the fact that in the guinea-pig the blood vessels do not open directly into the decidual cavity.

INTRAVITAM STAINING IN THE UNFERTILE UTERINE HORN AND IN THE FERTILE HORN AT SOME DISTANCE FROM THE EMBRYO.

In the previous series of normal pregnancies both the unfertile horn, and the fertile horn at some distance from the embryo, show similar vital staining appearances to those present in the mature virgin animals at a comparable stage in the oestrous cycle. The amount and intensity of the staining is, however, slightly diminished. The appearances must not be strictly compared to those found, e.g. at definite stages of a 16-day oestrous cycle, but in the pregnant animal are more irregular. This is explained by the work of Evans and Swezy (1931) who showed that cyclic growth and regression of the follicles goes on during pregnancy, as in the virgin animal, although ovulation does not occur, but this "follicular cycle" is more irregular in its length than in the non-pregnant animal.

INTRAVITAM STAINING AROUND AN OLD PLACENTAL SITE.

The vital staining appearances were also examined at the old placental sites in a group of four animals killed about six or seven days post partum. All showed similar appearances. Great numbers of rounded vitally stained cells with dense nuclei are seen around the placental remains, and extending for some distance into the surrounding endometrium. A number of the cells close to the placental site are very large. The staining is intense, but the dye granules are much finer than e.g. at the 12th day of a 16-day cycle in the virgin animal. These appearances are confined to the vicinity of the placental remains, and in the endometrium beyond this, e.g. in animal

No. 126 which shows a normal free blastocyst, only a small number of vitally stained cells is present, scattered loosely in the stratum compactum and stratum spongiosum.

INTRAVITAM STAINING AND THE OCCURRENCE OF PATHOLOGICAL EMBRYOS.

Out of eighteen pregnant animals, in nine the embryos were normal and have been described. In the other nine animals all the embryos showed varying degrees of pathological change, and it seemed desirable also to examine the vital staining appearances associated with this condition. The details relating to the latter animals are shown in Table XIV.

Table XIV. Records of Nine Intravitally Stained Pregnant Animals in which the Embryos were Pathological.

Index No. of Animal.	Number of Pathological Embryos.	Maximum Age of Embryos.	Number of Injections and Dosage of Trypan Blue 1% Solution.
130.	1	7½ Days.	7 of 3.8 ccs.
155.	1	7½ "	7 of 3.6 ccs.
82.	1	14 "	6 of 4 ccs.
168.	1	15 "	4 of 4.5 ccs.
125.	3	16½ "	8 of 3.8 ccs.
161.	2	19 "	6 of 3.5 ccs.
184.	2	20½ "	4 of 5 ccs.
122.	1	22 "	6 of 3.9 ccs.
90.	3	25 "	5 of 4 ccs.

It may be seen (Table XIV) that the animals received a varying number of injections of the dye.

All the embryos showed varying degrees of the same pathological change. This consists of an invasion chiefly by small rounded cells with dense round nuclei of varying size. A number of the nuclei are relatively large. In some cases the embryo is converted into an indefinite mass swarming with these cells. The

nuclei are more homogeneous than the dense nuclei of the dye-containing cells present in the virgin animal during the oestrous cycle. Very occasionally a moderate amount of dye was observed in a few of the invading cells.

Among the large number of pregnant uteri from non-injected guinea-pigs examined in this Department pathological embryos were not commonly found. It seems clear, therefore, that the high percentage of pathological pregnancies recorded above must be attributed to the dye.

SUMMARY OF RESULTS IN THE PREGNANT ANIMAL.

The above studies in the pregnant animal show that no dye is present in the free blastocyst or young embryo. When the embryo has just implanted, about the 7th day post partum, a few of the endometrial cells in the wall of the decidual cavity show a small amount of dye. Later, at the 9th day post partum, the endometrial cells forming the wall of the decidual cavity show intense vital staining, and form, as it were, a "barrage" of blue cells in the wall of the cavity. Fat is also present in these cells. The vital staining is, however, absent opposite the developing placenta. About the 12th or 14th day post partum the vitally stained zone becomes involved in the destruction of the endometrium that has been proceeding from the time of implantation, and the amount of dye present gradually becomes diminished. The yolk sac endoderm then begins to take up the dye, and at the 18th day post partum it shows abundant dye, and also fat in its cells. No dye is however present in the developing placenta, or in the foetal syncytial strands invading the decidua at this site. Further, none of the decidual cells were found to take up particulate matter such as ink particles.

These results clearly indicate that the vitally stained decidual cells are providing pabulum for the growing embryo.

In the unfertile horn, and in the fertile horn at some distance from the embryo, the cyclic distribution of rounded dye

cells, as in the virgin animal, shows that the cyclic ovarian influence on the uterus continues during pregnancy, although dampened down.

Great numbers of apparently similar vitally stained cells are present around the old placental site.

The dye is toxic to some of the animals, and in 50 per cent of the pregnancies examined the embryos were pathological. Practically no vital staining is, however, associated with the pathological changes in the embryo.

INTRAVITAM STAINING IN THE OVARIES.

In view of the cyclic distribution of the dye in the uterine horns, and its relation to the action of the ovarian hormones, it seemed desirable that the ovarian appearances should also be examined in the same groups of animals.

The Appearances in the Ovaries during the Oestrous Cycle.

Table XV illustrates by comparison the chief appearances in the ovaries during the cycle, the animals being most of those detailed in Table II. The examples described seem to be sufficient to emphasise the main features.

Normal follicles. No dye was observed in normal growing follicles or ova. Very occasionally, however, a small narrow fibrocyte in the theca externa showed a few fine granules.

Atretic follicles. No dye is present in the many small follicles in which the degenerating ovum still persists, and in which the follicular cavity is practically obliterated by proliferated cells, possibly of the theca interna.

In the large atretic follicles no dye is usually seen at the stage when the granulosa cells are undergoing dissolution, and the follicular cavity contains numerous very small mononuclears and polymorphs. In the centre of some of the large follicles, however, a few dye cells are occasionally seen. The dye cells are rounded or oval in shape, and often very large. They commonly measure about 16

microns in diameter, but vary in size. Two examples of these cells are shown in fig. 14. The dye granules are coarse and scattered throughout the cell. The nucleus is usually dense, oval or rounded in shape, and generally situated to one side of the cell. In some of the cells the nucleus is obscured or absent, and in the latter the cell appears to be in a state of degeneration, and to have been converted into a large "vacuole" containing coarse dye granules. Very small mononuclear cells and polymorphs are sometimes seen apparently lying in the interior of the vitally stained cells, both in cells with a healthy nucleus and in those which appear degenerated. Moreover, masses of "free" dye granules may be seen permeated with small mononuclears and polymorphs. These are probably the remains of dye-containing cells which have completely degenerated, or burst and shed the dye. It should be noted that the large vitally stained cells are only found in the follicular cavity. They may be altered granulosa cells, but on the other hand they are similar in appearance, both in regard to size and nuclear constitution, to some of the free dye-containing cells present inside and outside the ovarian sac, and also found free in the lumen of the Fallopian tube.

No dye was observed in the granulosa layer of cells. In the theca interna and theca externa a few vitally stained fibrocytes with coarse dye granules may be seen. Occasionally in the theca interna there is present a rounded dye cell which resembles but is slightly smaller than the large dye cells in the follicular cavity. It should be mentioned, however, that scattered throughout the follicular wall and especially in the granulosa, there is a number of apparently similar rounded cells with no dye.

The most abundant dye is seen in follicles in which the centre is being filled with young connective tissue, and in which few or no small mononuclears or polymorphs are present. In the centre of some of these follicles numerous large dye-containing cells are seen. They are similar in appearance to the large vitally stained cells

already described, but the dye granules are coarser, and the nucleus more often obscured. Many of the cells contain so much dye that they appear to have become irregular in shape, and some seem to have burst and shed the dye. A moderate number of smaller cells with coarse dye granules and similar nuclei is scattered throughout the more external parts of the follicle, and a few small vitally stained fibrocytes may also be observed there.

It should be noted that dye may be present or absent in follicles which appear to be at the same stage of atresia, and no explanation of this seems obvious from an examination of the sections. Moreover, in ovaries in which dye is present the vital staining varies in degree; if it is abundant in some follicles it is moderate in amount in others, scanty in others, and in many follicles is absent. It may be of interest to note that in animal No. 51 abundant dye is still present in some of the atretic follicles, although the dye was injected about the time of the previous expected oestrus, which was missed or suppressed.

Generally speaking there is most dye in ovaries showing much follicular atresia, but it may be seen (Table XV) that there is no definite cyclic distribution of the amount of dye present in the follicles.

Corpora lutea. No dye was observed in very young corpora. In fully formed corpora about the 7th day of a 17-day cycle a small granule of dye may occasionally be seen in some of the luteal cells. Numerous large dye-containing cells are however seen in the loose reticulum which occupies the centre of the corpus (fig.15). The dye-containing cells vary in size. Many are large rounded cells about 12 to 14 microns in diameter, others are smaller and more irregular in shape. The dye granules are coarse and spherical, or rod-like, and may be scattered throughout the cell or concentrated in one part of it. The nucleus is oval, round, irregular, lobed, or appears to consist of two fused nuclei. It is usually dense, but may be pale, and in some cells

is obscured by the dye granules. The largest of the dye cells seem to be similar in appearance to those described as occurring in the follicular cavity of atretic follicles. Similar dense or paler nuclei without dye are seen scattered throughout the corpus and in its blood vessels. Occasionally one of these nuclei in the corpus has a few dye granules related to it, but no dye was observed with the cells in the blood vessels. A moderate number of small vitally stained fibrocytes with coarse granules is present, especially in the centre of the corpus, and a few may be found in the capsule.

About midway in the cycle the corpora show similar appearances, although perhaps the dye-containing cells in the centre are slightly less numerous.

At the 12th day of a 16-day cycle a moderate amount of fine granular dye is seen in many luteal cells scattered throughout the corpus, the dye usually being placed in the cell to one side of the nucleus. In other luteal cells smaller amounts of dye are seen. In the centre of the corpus, which is now practically solid, a smaller number of dye-containing cells is seen, and the cells are smaller in size than in the younger corpora. At this time also small masses of "free" dye granules are observed, especially near the centre of the corpus, and appear to be the remains of vitally stained cells which have degenerated.

About the time of oestrus no dye is usually seen in the luteal cells of the regressing corpus, though in certain animals a very small amount is seen in some of the luteal cells. A considerable number of large rounded spaces or "vacuoles" may, however, be observed scattered throughout the corpus, and containing a small number of fine dye granules. In some corpora the "vacuoles" seem to be most numerous near the periphery. No nucleus is usually seen in the"vacuoles", but if present it is oval and dense, or oval and pale like that of a luteal cell. Some of the dye-containing vacuoles are undoubtedly degenerated luteal cells. In the centre of the corpus a

few rounded dye cells may be seen, but they are smaller than the vitally stained cells in the younger corpora.

In still older corpora no dye was present in the luteal cells, but occasionally rounded cells with dense nuclei and containing a small amount of coarse dye were observed. No vitally stained cells were seen in the blood vessels of the corpora.

From these observations it may therefore be said that in the early part of the cycle a considerable number of rounded dye cells is present in the centre of the corpus. Later in the cycle when the centre has become organised the dye cells present are smaller in size, less numerous, less intensely stained, and some appear to have degenerated. In the luteal cells dye is only definitely visible in some of the cells about the 12th day of the cycle, when the corpus is beginning to regress. In still older corpora showing advanced degeneration no dye is present in the luteal cells.

Germinal epithelium. Dye is practically always present in the germinal epithelium in the infra-nuclear part of the cells, but the amount varies irregularly throughout the cycle (Table XV).

The most striking feature is the increased amount of dye in the epithelium covering the most prominent part of the fully developed corpus, present only if the corpus reaches practically to the surface. The dye may be moderate in amount, or abundant and coarse (fig.16), and is usually most intense over the extreme projecting part of the corpus as proved by serial sections. At the edge of the projecting part of the corpus the increase in the amount of dye in the epithelium usually ceases abruptly. A lesser degree of this is present at oestrus if the regressing corpora still project on the surface. No increase in the amount of dye is found over the surface of a very young corpus.

A small or moderate amount of dye is present in the remainder of the germinal epithelium, and although usually evenly distributed, sometimes small areas are more intensely stained.

Practically no dye, or only a few fine granules, are observed in the flattened mesothelial cells.

Interstitial tissue. Occasionally a few rounded dye cells measuring about 12 microns in diameter are seen. A few vitally stained fibrocytes are also present.

Free dye cells around ovary, and in lumen of Fallopian tube. Large groups of free dye cells which have undoubtedly come from the peritoneal cavity may be found outside and inside the ovarian sac, between the coils of the Fallopian tube, or in its lumen. They may not be found in all these situations in the same animal, and it appears from Table XV that they occur irregularly at any time in the cycle, as would be expected. When related to the ovarian sac they are usually very numerous, and are always found there in company with numerous red blood cells and a considerable number of small mononuclears and polymorphs. In the tube they are found in much smaller numbers. They are rounded in shape, and measure usually from 12 to 16 microns in diameter, but they vary in size, and dye-containing cells of 7 microns are also seen. The nucleus is usually dense, but paler nuclei are also seen; in size it seems to vary relatively with the cell; in shape it is most commonly oval, and some of the cells appear to have two small fused nuclei. The dye granules are very coarse and large, and are scattered throughout the cell, in many cases completely obscuring the nucleus. A group of these cells is shown in fig.4. Occasionally a polymorph appears to be lying inside a "dye" cell.

Fallopian tube. No rounded dye cells were observed in the mucosa of the tube at any time in the cycle. Occasionally, however, a small vitally stained fibrocyte with fine dye granules is present.

Connective tissue between the coils of Fallopian tube. Numerous vitally stained cells are present. The most obvious types of these are 1) elongated tapering cells with narrow dense nuclei and a considerable number of coarse dye granules; 2) large branching cells with dense oval nuclei and the cytoplasm packed with coarse dye

granules; and 3) large rounded or oval cells of varying size with oval dense nuclei, or oval paler nuclei, and numerous coarse dye granules in the cytoplasm. The large rounded vitally stained cells are similar in appearance to the dye-containing cells in the endometrium. Similar cells without dye are also present.

(TABLE XV).

The Appearances in the Ovaries of Sexually Immature Animals.

The appearances are summarised in Table XVI. Apart from the absence of corpora lutea, they closely resemble the appearances in the mature animals. Varying amounts of dye are present in the atretic follicles and germinal epithelium, and no dye in the mucosa of the tube.

In some of the animals rounded vitally stained cells with varying amounts of dye are found adherent to the peritoneal surface of the ovary, free in the ovarian sac, and in the lumen of the tube, as in the mature animals.

(TABLE XVI).

TABLE XV.

Intravitam Staining in the Ovaries and Fallopian Tubes during the Oestrous Cycle.

Index Number of Animal.	Length of Normal Cycle in Days.	Time in Cycle when Killed.	Dye in Atretic Follicles.	Dye in Young Corpora Lutea.	Dye in Old Corpora Lutea.	Dye in Germinal Epithelium.	Free Dye Cells around Ovary.	Free Dye Cells in Lumen of Tube.	Dye in Mucosa of Tube.
32	16	1st day of new cycle.	Small amount and only in some follicles.	Animal killed before new ovulation should have occurred.	Average mean diameter of "old" corpora 1.25 mm. Luteal cells—No dye. Scattered through corpus is a considerable number of "vacuoles" containing a small amount of fine granular dye and usually no nucleus. In centre of corpus a few rounded cells containing a small amount of coarse dye are seen.	Small amount unequally distributed. Slightly increased over projecting corpus.	Present	None observed	No rounded dye cells. Occasionally a small vitally stained fibrocyte
34	17	1st day of new cycle.	Ditto.	Ditto.	Average mean diameter of "old" corpora 1.2 mm. Luteal cells—no dye. In centre of corpus occasional rounded cells containing a few coarse dye granules.	Small amount. Slightly increased over projecting corpus.	None observed.	Present.	Ditto.
109	16	1st day of new cycle.	Abundant in some follicles; moderate amount in others; absent in many	Ditto.	Average mean diameter of "old" corpora 1.5 mm. Luteal cells—very small amount of fine dye in a few cells. Other appearances similar to those in Animal No. 32.	Very small amount.	None observed.	None observed.	Ditto.
98	16	1st day of new cycle.	Ditto.	Average mean diameter of new corpora 0.89 mm. No. Dye.	Average mean diameter of old corpora 1.2 mm. Similar appearances to those in previous animal, No. 109, but slightly less dye.	Moderate amount unequally distributed. No increase of dye over new corpus.	Numerous inside ovarian sac.	Present.	Ditto.
42	17	3rd day of new cycle.	Ditto.	No young corpora present—ovulation failed to occur.	Average mean diameter of old corpora 1 mm. Luteal cells—no dye. Occasional small rounded cells containing a small number of relatively coarse dye granules.	Very small amount.	None observed.	Present.	Ditto.
9	17	4th day of new cycle.	Ditto.	Ditto.	Ditto.	Moderate amount.	Numerous	Present.	Ditto.
50	17	5th day of new cycle.	Ditto.	Ditto.	Ditto.	Small amount.	None observed.	None observed.	Ditto.
22	17	6th/7th day	Practically absent although atretic follicles present.	Average mean diameter of corpora 1.4 mm. Luteal cells — a small granule of dye in some cells. In centre or corpus numerous large rounded cells containing a large amount of coarse dye.	Average mean diameter of corpora 0.7 mm. Similar appearances to those in Animal No. 42.—	Moderately intense over most prominent part of young corpus, and only if corpus reaches practically to the surface. Practically no dye in remainder of germinal epithelium.	None observed.	None observed.	Ditto.

TABLE XV.—(*continued*).

Index Number of Animal.	Length of Normal Cycle in Days.	Time in Cycle when Killed.	Dye in Atretic Follicles.	Dye in Young Corpora Lutea.	Dye in Old Corpora Lutea.	Dye in Germinal Epithelium.	Free Dye Cells around Ovary.	Free Dye Cells in Lumen of Tube.	Dye in Mucosa of Tube.
23	17	6th/7th day	Ditto.	Ditto.	Average mean diameter of corpora 0.8 mm. Similar appearances to those in Animal No. 42.	Small amount over projecting young corpus. Practically no dye in remainder of germinal epithelium.	Present.	None observed.	Ditto.
43	18	9th day	Very small amount and only in some follicles.	Average mean diameter or corpora 1.6 mm. Similar appearances to those in Animal No. 22.	Ditto.	Moderate amount over projecting young corpus, and more over some corpora than others. No dye in remainder of germinal epithelium.	Present.	None observed.	Ditto.
49	16	9th day	Relatively abundant in a few follicles. Absent in many.	Average mean diameter of corpora 1.6 mm. Luteal cells—very small granules of dye in some cells, situated to one side of nucleus. In centre of corpus numerous large rounded cells containing a considerable amount of coarse dye.	Average mean diameter of corpora 0.85 mm. Similar appearances to those in Animal No. 42.	Abundant over projecting young corpus. Moderate amount in remainder of germinal epithelium. Less in one ovary than in other.	None observed.	Present.	Ditto.
4	17	9th/10th day	Small amount and only in some follicles.	Average mean diameter of corpora 1.6 mm. Luteal cells—very occasionally a small dye granule seen. Centre of corpus practically solid and only a few relatively small rounded dye—containing cells seen.	Ditto.	Moderate amount over projecting young corpus. Small amount in remainder of germinal epithelium. No increase of dye over old corpus.	Numerous	None observed.	Ditto.
29	16	12th day	Moderate amount and only in some follicles.	Average mean diameter of corpora 1.66 mm. Luteal cells — moderate amount of fine granular dye in many cells, situated to one side of nucleus. In centre of corpus a moderate number of rounded cells containing a small amount of coarse dye. A few of these cells scattered through corpus. Occasional small masses of "free" dye granules in centre of corpus.	Average mean diameter of corpora 0.65 mm. No visible dye.	Moderate amount over projecting young corpus. Very small amount in remainder of germinal epithelium.	None observed.	None observed.	Ditto.
28	16	12th day	Ditto.	Average mean diameter of corpora 1.7 mm. Similar appearances to those in previous animal, No. 29.	Average mean diameter of corpora 0.7 mm. No visible dye.	Abundant over projecting young corpus. Very small amount unequally distributed in remainder of germinal epithelium.	Numerous	Present.	Ditto.

TABLE XV.—(*continued*).

Index Number of Animal.	Length of Normal Cycle in Days.	Time in Cycle when Killed.	Dye in Atretic Follicles.	Dye in Young Corpora Lutea.	Dye in Old Corpora Lutea.	Dye in Germinal Epithelium.	Free Dye Cells around Ovary.	Free Dye Cells in Lumen of Tube.	Dye in Mucosa of Tube.
47	17	13th day	Small amount and only in some follicles.	Average mean diameter of corpora 1.6 mm. Luteal cells–small amount of fine granular dye in many cells, situated usually to one side of nucleus. In centre of corpus a small number of rounded cells containing a small amount of coarse granular dye. A few of these cells scattered through corpus.	Average mean diameter of corpora 0.8 mm. No visible dye.	Moderate amount over projecting young corpus. Very small amount unequally distributed in remainder of germinal epithelium.	Present.	Present.	Ditto.
52	17	15th day	Abundant in a few; moderate amount in a few; absent in most.	Average mean diameter of corpora 1.5 mm. Luteal cells—very small amount of fine dye in some cells. In centre of corpus a small number of rounded cells containing a few coarse dye granules.	Average mean diameter of corpora 0.5 mm. No visible dye.	Small amount unequally distributed.	None observed.	None observed.	Ditto.
41	17	1st day of new cycle.	Ditto.	Animal killed before new ovulation should have occurred.	Average mean diameter of "old" corpora 1 mm. Luteal cells—no dye. Moderate amount of scattered "free" dye granules. Occasional rounded cells containing a few coarse granules.	Very small amount.	Numerous inside and outside ovarian sac.	Present.	Ditto.
24	17	1st day of new cycle.	Moderate amount in some follicles. Small amount or absent in others.	Ditto.	Average mean diameter of "old" corpora 1.1 mm. Luteal cells—very small amount of dye. Large rounded spaces usually with no nucleus, and containing a moderate amount of fine dye, present especially near centre of corpus.	Small amount unequally distributed. Slightly increased over projecting corpus.	Numerous inside and outside ovarian sac.	Present.	Ditto.
51	18	1st day of new cycle. Dye given at time of previous oestrus, which was suppressed.	Abundant in some; moderate amount in others; absent in many.	Average mean diameter of new corpora 0.8 mm. No dye.	No. dye.	Small amount. No increase of dye over new corpus.	None observed.	None observed.	Ditto.

Table XVI. Intravitam Staining in the Ovaries of Sexually Immature Animals. *

Index No. of Animal.	Dye in atretic follicles.	Dye in germinal epithelium.	Free Dye cells around ovary.	Free Dye cells in lumen of tube.	Dye in mucosa of tube.
203.	Present.	No Dye.	Absent.	Present.	No Dye.
207.	"	Abundant in one small area. Small amount in remainder of germinal epithelium.	Absent.	"	"
205.	"	No Dye.	Absent.	"	"
208.	"	Small amount unequally distributed in one ovary. No dye in other ovary.	Present.	"	"
206.	"	No Dye.	Absent.	"	"
202.	"	No Dye.	Present.	"	"
204.	"	No Dye.	Present.	"	"
115.	"	Small amount in one ovary. No Dye in other ovary.	Present.	"	"
116.	"	Small amount unequally distributed.	Absent.	"	"
88.	"	Small amount.	Absent.	"	"

* The ovaries are from the animals in Table IV. Each animal received the same number of injections of dye.

THE PRESENCE OF FAT AND DYE IN THE OVARIES.

The ovaries of the intravitally injected mature animals Nos. 47 and 52 (Table II) were stained with Sudan III to compare the distribution of fat and dye, and to ascertain whether any of the large vitally stained cells in the ovaries contained fat particles, as was observed in the endometrium.

Practically the entire ovary is loaded with very coarse fat

droplets, and so intense is the staining that the structure of its different parts is very much obscured. The appearances may be summarised as follows:

Normal follicles. In follicles which appear to be definitely normal fine droplets of fat are present in the periphery of the ovum, and more concentrated at one pole than at the other. No fat seems to be present in the granulosa cells, but fine particles are seen in the tunica interna and externa.

Atretic follicles. The entire wall of the follicle, especially the organising type, is loaded with coarse fat particles, and if the degenerating ovum is seen it contains still larger and coarser droplets of fat. In some of these follicles large dye-containing cells are seen in the follicular cavity, and fine fat particles are present in most of the dye cells, irregularly scattered among the dye granules.

Corpora lutea. Coarse fat is present throughout the entire thickness of the corpus except in the centre, where only a very small amount of fine fat is visible. In some corpora the fat is irregularly distributed, in others it is slightly more marked at the periphery. A few small fat particles are seen in the capsule of the corpus. Occasionally a small particle of fat is seen in the rounded intravitally stained cells in the centre of the corpus.

Germinal epithelium. Very occasionally a small droplet is observed. The dye is definitely increased over the most projecting part of the corpus, but no fat was observed in the cells there.

Interstitial tissue and blood vessels. A considerable amount of very coarse fat is irregularly distributed in the interstitial tissue, and in the blood vessels an occasional small fat droplet was observed.

SUMMARY OF RESULTS IN THE OVARIES.

From the above studies in the ovaries, it should be emphasised in relation to intravitam staining that large rounded dye cells were found in organising atretic follicles, in the organising

centres of the growing corpora lutea, free around the ovary and in the tube, and in the connective tissue between the coils of the tube. In all these situations the cells vary in size and in the amount of dye contained, and generally speaking the smaller the cell the less dye present. The nuclei appear to be similar, and the same variations in nuclear constitution are seen. Moreover, cells with similar nuclei and without any related dye are invariably present. Further, fine fat particles have been demonstrated in the dye cells in the atretic follicles and in the corpora. A comparison of figs. 14, 15 and 4, showing examples of the dye cells in the follicles, corpora, and free in the tube, suggests strongly that these cells all belong to the same class, and also that they are similar to the dye cells present in the endometrium of the virgin animals during the cycle (compare fig. 2).

In the luteal cells both fat and dye were found, although the large amount of fat is out of all proportion to the very small amount of fine dye granules present in the cells. In the walls of the atretic follicles the abundant fat seen is not accompanied by vital staining of the cells.

MORTALITY IN ANIMALS INTRAVITALLY STAINED WITH TRYPAN BLUE.

It may be of interest to include in this memoir a table showing the mortality in the intravitally stained animals.

Number of Animals Injected.. 117 (Excluding Animals in Table II).

Number of Deaths..... 21 (" " " Table I).

which represents a death rate of 24.5 per cent.

The details relating to the above twentyone animals which died are shown in Table XVII.

Table XVII. Records of Twentyone Deaths of Animals Intravitally Stained with Trypan Blue.

Index No. of Animal.	Number of Injections and Dosage of Trypan Blue (1% Soln.).	Remarks.
129.	1 of 3.6 ccs.	Non-Pregnant.
133.	1 of 3.6 ccs.	
128.	1 of 4 ccs.	
131.	2 of 3.6 ccs.	
132.	2 of 3.6 ccs.	
170.	2 of 5.5 ccs.	
16.	3 of 4 ccs.	
71.	4 of 5 ccs.	
85.	5 of 4.5 ccs.	
182.	6 of 5 ccs.	
48.	6 of 5 ccs.	
259.	6 of 5.1 ccs.	
256.	6 of 5.4 ccs.	
250.	6 of 6.7 ccs.	
247.	6 of 6.9 ccs.	
251.	6 of 7 ccs.	
151.	1 of 3.6 ccs.	Pregnant.
163.	4 of 4 ccs.	
164.	4 of 4 ccs.	
72.	4 of 5 ccs.	
74.	10 of 4.5 ccs.	

The above data together with some of the previous results in this paper confirm that the dye is definitely toxic to a number of the animals.

GENERAL SUMMARY OF CHIEF RESULTS.

It has been shown that in the uterus there is a cyclic and topographical distribution of vitally stained rounded cells. The staining is at the minimum about the midpoint of the cycle, when the cells are sparsely scattered throughout the endometrium. About the 12th day, i.e. during the follicular phase, the maximum intensity is reached, and the cells are concentrated in the stratum compactum, especially around the antimesometrial half of the uterine lumen. From this point to oestrus, and during the first part of the cycle or luteal phase, the number of cells and the intensity of the staining diminishes. No cyclic distribution was found in the vagina,

where the cells are fewer in number or may be absent, and no vitally stained rounded cells were found at any time in the cycle in the wall of the Fallopian tube. In sexually immature animals vitally stained cells are practically absent. In ovariectomised females the vitally stained cells were proved to be under the control of the follicular hormone oestrin, and it was found that the chief influence is exerted on the uterine horn, as was observed also during the oestrous cycle in normal animals.

There is also a cyclic distribution of fat, both in the epithelium and in the connective tissue of the endometrium. The fat cycle does not coincide, however, with that of the vitally stained cells, but corresponds with the period of activity of the corpus luteum. In ovariectomised animals in which the uterus was previously sensitised by small doses of oestrin, the corpus luteum hormone was proved to influence the production of fat, especially in the uterine epithelium.

No vitally stained cells resulted from the injection of anterior pituitary in ovariectomised females, but the results suggested that perhaps the anterior pituitary might in some way influence the appearance of fat in the connective tissue of the endometrium.

In the involuting uterus soon after parturition a large number of vitally stained cells was found, scattered loosely throughout the entire thickness of the uterus, and especially around the placental site.

In the pregnant uterus a few days after implantation the endometrial cells in the wall of the decidual cavity show intense vital staining. No dye is however present opposite the developing placenta or in the embryo. Later the wall of the decidual cavity where the vitally staining cells are concentrated breaks down to provide pabulum for the growing embryo, and the yolk sac endoderm then takes up the dye. No dye is present at this stage in the

developing placenta, or in the endometrium which is being invaded by the placental offshoots. In the unfertile uterine horn, and in the fertile horn at some distance from the embryo, rounded dye cells are found, as at a comparable stage in the oestrous cycle in the virgin animals. The staining is, however, less in amount.

Practically no dye is seen in the small cells which invade pathological embryos.

With regard to the ovaries no cyclic distribution of vitally stained cells was found. Large dye cells were present especially in organising atretic follicles, and in the organising centres of the growing corpora, and over the projecting part of the corpus the germinal epithelium was usually intensely stained. Vital staining is usually absent in regressing and in old corpora lutea.

Groups of rounded dye cells from the peritoneal cavity were often found free in the lumen of the tube and uterus.

The vitally stained decidual cells are clearly different from the rounded dye cells in the endometrium in the virgin animal. The latter cells, however, seem to belong to the same class as the vitally stained cells in the atretic follicles, corpora, and in the lumen of the tube. The blood appears to be the chief source of these cells, although they only take up the dye when in the tissues, and similar cells without dye are invariably present.

Lastly, it should be noted that small fat particles were found in many of the rounded dye cells in practically all situations, and also in the vitally stained decidual cells and yolk sac endoderm.

DISCUSSION.

The present research concerns the reaction to intravitam staining with trypan blue exhibited by the tissues of the reproductive system in the guinea-pig in the course of their normal physiological activities.

These activities are intimately controlled by the ovarian

hormones, and the present state of our knowledge in this connection must first be defined. A vast amount of evidence relating to this subject has appeared in recent years, which has been carefully surveyed by Parkes (1929), Allen (1932) and Robson (1934). In the light of these reviews the present position may be stated as follows - The ovarian hormones, secreted at various stages of the ovarian activity, act upon the uterine endometrium and other sex organs, and bring about the alterations which are typical of the different stages of the uterine cycle. The ovarian activity, however, does not occur spontaneously and independently, but is definitely regulated by the anterior lobe of the pituitary. Two hormones - Prolan A and B, are believed to be secreted by the anterior pituitary. Prolan A is responsible for follicular maturation in the ovaries and stimulates the secretion of oestrin, while Prolan B brings about luteinisation followed by the secretion of the luteal hormone. Leonard (1931) has advanced evidence that ovulation may be due to a third pituitary hormone, but this view still awaits confirmation.

Oestrin* is therefore secreted chiefly during the follicular phase of the cycle. It is present not only in the fluid of the Graafian follicles, but also in the remainder of the ovarian substance. In the lower rodents oestrin produces growth of the uterus and thickening and cornification of the vagina, and brings about the morphological picture of full oestrus, including a stimulation of the reflexes which constitute mating. It should be noted that in the uterine endometrium oestrin causes a proliferation which is definitely different from that brought about by the corpus luteum hormone (Robson 1934).

The luteal phase of the cycle may be called the secretory phase. It is under the control of the corpus luteum hormone, and characterised especially by glandular proliferation (progestational proliferation). The changes are presumably in preparation for the

* Other names have been applied to this substance, e.g. theelin, folliculin, menformon, etc.

possible occurrence of a fertilised ovum.

It may also be mentioned that in the luteal phase, about the time of implantation, both in pregnant and non-pregnant animals, the endometrium possesses a very important physiological property, namely, the power of forming decidual cells. This phenomenon was first demonstrated by Loeb in 1908. By cutting the endometrium in guinea-pigs he found that large blocks of cells were formed within a few days at the site of the incision, but this reaction was only possible between the 3rd and 9th day following ovulation (Loeb 1932). Later authors have demonstrated the same phenomenon in a number of animals. The above conditions in the luteal phase of the cycle have been proved to be under the control of the corpus luteum hormone, but the latter is only capable of acting on an endometrium, which has a short time previously been subjected to the action of oestrin.*

The results of vital staining in ovariectomised guinea-pigs in the present research agree closely with the above statements, regarding the difference in action of the anterior pituitary, follicular, and corpus luteum hormones. No direct action on the uterus could be made out for the anterior pituitary. On the other hand oestrin and corpus luteum hormone produced totally different results. Oestrin was proved to influence the appearance of vitally stained cells in the endometrium, and this was associated with growth and enlargement of the uterus. Corpus luteum hormone, acting on a uterus previously sensitised by oestrin, caused proliferation of the glands and enlargement of the organ along with the appearance of a smaller number of vitally stained cells in the endometrium. The vital staining was, however, proved to be due to the previous action of the oestrin, since the luteal hormone itself did not produce this result. Further, vitally stained cells were absent in the uterus of animals not treated with hormones. In all the animals, however, whether treated with hormones or not, apparently similar cells

* It should be noted that in the lower animals oestrin is also present in the corpus luteum, but in no appreciable quantity.

showing no vital staining were invariably present.

While the present work was in progress Fluhmann (1928 and 1932) independently investigated in the rabbit a number of similar problems after intravitam staining with trypan blue. He found the presence of small numbers of vitally stained cells in the rabbit's uterus and vagina to be a fairly constant phenomenon. He confined himself, however, chiefly to the reaction of these cells in aseptic inflammation, and to the response to injection of ovarian hormones in the normal and traumatised uterus in both normal and spayed animals. In the uterus of the spayed rabbit he showed that oestrin stimulation was associated with the appearance of large numbers of these cells. In the normal or the spayed animal the insertion of a silk ligature through the uterus resulted in the appearance of many vitally stained cells, but this reaction was purely a local one. If, however, oestrin was also injected, or a progestational proliferation induced by pregnancy urine, the response was tremendously increased and was apparent throughout the whole uterus. In view of the present findings in the guinea-pig, the increase in the number of vitally stained cells noted by Fluhmann when progestational proliferation was induced, would seem to be due to the oestrin present in pregnancy urine. The vitally stained cells described by Fluhmann in the rabbit seem to be similar to those found in the guinea-pig uterus during the oestrous cycle. Fluhmann considers them in the rabbit's uterus to be macrophages, belonging to the reticulo-endothelial system, and whose function is to act as scavengers in removing tissue debris and to assist in the healing of tissue injury. He states that they are of great importance in local tissue immunity, and has discussed the possibility of stimulating these cells in pelvic inflammatory disease in the human subject by employing sex hormones.

Fluhmann also points out that a number of observations are available on the incidence of tissue macrophages in the human uterus. Motta (1927) described certain phagocytic cells containing hemosiderin

fatty pigments, and lipoids, consistently present in the connective tissues of the non-pregnant uterus, and found that they increased in number at the time of the menstrual period. He considers these cells as identical with Aschoff's reticulo-endothelial cells. They are also found during pregnancy, when they are seen throughout the uterine wall and in the broad ligament (Hormung 1924; Hofbauer 1926; Motta 1927). In his study of the involution of the uterus post partum, Teacher (1927) found fat-laden phagocytes in the connective tissue planes between the muscle, and many groups of phagocytes laden with blood pigment around the placental site. Macrophages are also at times seen in the stroma of hyperplastic endometrium, and a similar, if not identical, type of cell often occurs in the lumina of the endometrial glands (Fluhmann 1931). Further, they may be readily observed in areas of degeneration in fibromyomata during pregnancy (Fluhmann 1932) and judging from this writer's fig. 6, which illustrates macrophages from a fibromyoma uteri, these cells appear to be similar to the vitally stained cells found in the virgin uterus of the guinea-pig.

A close histological resemblance seems to exist between many of these observations and the findings in the present research. Before discussing the possible significance of the results it may be well, therefore, to define the reticulo-endothelial system and the functions attributed to it. It need only be noted here that the cells of the reticulo-endothelial system fall into three main groups - (a) cells forming the lining of blood and lymphatic channels in special situations; (b) certain cells lying free in the loose connective tissues of the body; (c) a small proportion of cells free in the blood stream (Cappell 1930).

The following functions have been attributed to the reticulo-endothelial cells in connection with infections: first, the phagocytosis and intracellular destruction of the microorganisms; second, the reception, detoxication and digestion of the waste products that are formed during the process of inflammation, including the toxic

substances liberated from the bacteria; third, the absorption of soluble toxins; and fourth, the secretion of the antibodies." (Jaffé 1927). Moreover, the importance of any factor which may stimulate the local reaction of reticulo-endothelial cells in combating local or general infection has been demonstrated by several workers, e.g. Gay and Morrison (1923) by stimulating a macrophage response in the pleura of experimental animals induced a condition which enabled them to survive infections normally rapidly fatal; and Nakahara (1925) produced a macrophage reaction in the peritoneal cavity of mice, and found that as a result they would survive multiples of the fatal doses of staphylococci and pneumococci.

There seems little doubt that the vitally stained rounded cells in the uterus of the non-pregnant guinea-pig belong to the reticulo-endothelial system. They are always present in the sexually mature endometrium, but in varying numbers. Moreover, from the previous hormone experiments the vital staining appearances at different stages of the oestrous cycle, and as between different animals, would appear to be related to the balance of action between the different hormones at that period. The appearance of the intensely stained cells at the 12th day of the cycle suggests, however, that in the uterus they may have a different function from those attributed to this system of cells generally. During this period of the cycle growth changes are occurring in the endometrium, although the nature of these changes is not so obvious in the guinea-pig as in some other animals. From the temporal incidence of the vitally stained cells, however, they do not appear to be scavengers. This is further supported by the fact that they are not increased in number at oestrus, but appear to be less active, and that in the pregnant animal the cyclic distribution of the cells is more or less continued in the unfertile horn, and in the fertile horn at some distance from the embryo. The weight of evidence indicates that these cells are elaborating food substances from the tissue fluids, and

engaged in the transference of this material to the endometrial cells around. Further evidence is afforded by the fact that the endometrial cells of the stratum compactum contain fine dye granules, especially during this phase of the cycle. During the luteal phase of the cycle, and especially about the time of implantation, the small number of vitally stained cells present seems to indicate a resting phase in the endometrium, when the changes are chiefly secretory.

The topographical distribution of the dye also seems significant. The concentration of the vitally stained cells during the follicular phase of the cycle in the stratum compactum, and especially around the antimesometrial half of the uterine lumen (i.e. the implantation zone), suggests that very specialised changes are occurring in this situation. This is further supported by the fact that at this period only a few vitally stained cells are present in the vaginal mucosa. These changes have been proved to be under the control of the follicular hormone oestrin, and in the experimental evidence the chief effect was also found to be exerted on the uterus. In regard to the antimesometrial incidence of the vital staining in the uterus, it may be of interest to contrast the appearances in the small bowel, which has a similar mesenteric blood supply. Vitally stained cells apparently similar to those in the uterus were found in the stroma of the villi, but equally distributed around the lumen. Although from the same animal, the amount and intensity of the staining in the bowel was much less than in the uterus. The intensity of the staining, however, varied along the length of the bowel, and the site of most marked staining varied in different animals, but where the staining was most marked there was definite increased vascularity. Moreover, no increase or decrease in the amount of vital staining was present at the sphincteric sites. These findings further confirm the view of the writer that the antimesometrial incidence of the dye in the uterus is related to intimate histological processes going on there. It might be suggested, perhaps, that the process of

sensitisation of the endometrium for the later action of the corpus luteum is associated with these appearances.

The possibility of the existence of predetermined areas for implantation was also considered, as it is known that, if more than one embryo is present in the uterine horn, the embryos are always equally spaced. The fact, however, that the specificity of the dye reaction around the antimesometrial half of the uterine lumen extends equally along the whole length of the uterine horns, as proved by serial drawings, indicates that this method does not demonstrate any special sites for implantation of the blastocyst.

In the involuting uterus post partum the rounded vitally stained cells are scattered everywhere, and are more numerous around the placental site. It might be suggested that they are supplying food substances for the great alterations occurring in the surrounding tissue, while on the other hand they may be acting as scavengers on this occasion.

Again the almost complete absence of vitally stained cells in or around the pathological embryos might be held to suggest that such substances as they can transfer for tissue growth are no longer required.

In the pregnant uterus the vitally stained endometrial cells seem clearly to be concerned in the supply of nourishment to the growing embryo, which later takes up the dye material. They do not appear to be a line of defence cells against e.g. the reaction of trophoblastic enzymes, and this seems to be confirmed by their absence opposite the invading offshoots from the developing placenta.

With regard to the ovaries Goldmann (1909), working on mice and rats, regards the vitally stained cells in the follicles as modified follicular cells. This may be so also in the guinea-pig, but in the latter they seem to be similar to the vitally stained cells in the centres of the growing corpora, and also to the dye-containing cells described in the endometrium of the virgin uterus.

They may occasionally be seen in atretic follicles which are being invaded by many small monomuclears and polymorphs, and some of these may appear to be lying in a vitally stained cell. On the other hand they are most numerous in follicles in which the centre is being filled with young connective tissue, and many of the vitally stained cells are then in a state of degeneration. In the same manner they are found in the organising centres of the growing corpora lutea, but practically absent in corpora which are degenerating and regressing. In both cases it might be agreed that the vitally stained cells are supplying substances necessary for the growth processes taking place, while it might also be said they may be removing débris, or perhaps performing both functions.

It is difficult to suggest an explanation for the intense staining of the germinal epithelium over the projecting part of the corpus. Might not the distension due to the growing corpus simply have stimulated these cells to greater activity, and resulted in their becoming more intensely stained with the dye?

The vitally stained cells which have come from the peritoneal cavity, and are found free in the lumen of the Fallopian tube and uterus, seem to belong to the same class as those found in the endometrium and in the ovaries, and have probably resulted from a mild irritation of the peritoneum. They also seem to be similar to the cells described by Cappell (1930) in the mouse after mild irritation of the peritoneum, and, as in the mouse, a reserve of undifferentiated smaller cells is invariably present in the guinea-pig. Cappell states that these cells in the mouse are probably derived from the cells of the adventitial sheaths and tâches laiteuses of the omentum and mesenteries.

From the above studies it may be seen that although the cells which stain vitally are most commonly of mesodermal origin, cells of other embryonic derivatives may also under certain conditions take up the dye. This has been recorded previously by several workers

including Goldmann (1909 and 1912), Cappell (1929) and Wislocki (1921).

A number of the findings in the present research pointed naturally to the question as to the nature of intravitam staining. This has been reviewed by Cappell (1929) and by later workers, but the question is still unanswered. The physical characters of the dye solutions are in many cases related to the number of sulphonic radicals present, but it cannot be said that any general law has as yet been formulated correlating chemical constitution and biological action. It is not proposed to enter this controversy in the present memoir except to state that Goldmann (1912) regarded the stained granules as evidence of secretory activity on the part of the cells in which they appeared, and sought to identify them with other known cell constituents. Goldmann emphasised a close relationship between vitally stained substance and fat, and this stimulated the fat studies in the present research.

Fat has been demonstrated in the yolk sac endoderm, in the wall of the decidual cavity and in many of the rounded vitally stained cells in all situations in the uterus. Moreover, a cyclic distribution of fat has also been demonstrated both in the epithelium and connective tissues of the endometrium, and has been found to be apparently under the control of the corpus luteum hormone. The luteal hormone, however, does not by itself influence the appearance of vitally stained cells in the endometrium. It may be mentioned that in the pig, Okey, Bloor, and Corner (1930) have described a definite but not large increase in the percentage of lecithin at the time of greatest elaboration of the endometrium. Their results were obtained by grinding the mucosa in a mortar and extracting with alcohol and ether.

In regard to the ovaries in the present investigation, fat was also found in small quantity in many of the large vitally stained cells in the follicles and corpora.

The present findings therefore confirm Goldmann's view of the

close relationship between fat and vitally stained substance, but nevertheless indicate that the two substances are distinct, since fat is often present in the endometrium, and especially in the ovaries, without any relation to vital staining. Fatty substance is, however, found normally in many cells, and also in cells in tissue culture (Woollard 1927), and it may be argued that its appearance in the vitally stained cells may be of no significance, except as an index of specialised activity. This may be so, but nevertheless it should be pointed out that fat was not present generally in the endometrial cells, at least in the same quantity. The precise nature of the fatty substance was not determined. It must be noted that the appearance of fat in cells may be either a sign of storage with a view to functional utilisation, or it may be an evidence of damage. In the present observations the fat did not occur in cells which showed signs of degeneration, or which were in course of breaking down; accordingly there is good reason for the view that the fat was related to functional activity just as is the case with capacity for vital staining.

Goldmann (1912) could not find a similar close relationship between glycogen and vitally stained substance. In this connection only two uteri were examined in the present research, but as glycogen was found in e.g. the placenta where no vital staining at a similar stage had been observed, the matter was not pursued further.

In regard to the toxicity of the dye this has been observed by many workers, but it may be noted that the present results indicate that in specially susceptible animals the toxic effects are more marked at oestrus.

In conclusion it may again be noted that the above investigations are a study of the normal physiological processes in the uterus in the light of intravitam staining. The following are the findings from these researches which, as far as I am aware, have not been recorded elsewhere - 1) the cyclic distribution of vitally

stained cells in the mature endometrium and their antimesometrial incidence; 2) the absence or small number of vitally stained cells in the sexually immature uterus; 3) the stimulation of these cells in ovariectomised animals - which is produced only by oestrin injections - and the lack of response to corpus luteum hormone alone, or to anterior pituitary; 4) the cyclic distribution of fat in the uterine epithelium and in the connective tissue of the endometrium; 5) the production of abundant fat in the uterine epithelium in ovariectomised animals by injection of corpus luteum hormone, the uterus previously having been sensitised by small doses of oestrin - and the absence of this effect from the injection of the luteal hormone in the non-sensitised uterus; 6) the vital staining appearances in the very early stages of implantation; 7) the absence of cyclic vital staining appearances in the ovaries; 8) the intense vital staining of the germinal epithelium over the most projecting part of the corpus luteum; 9) the almost constant occurrence in the lumen of the tubes and uterus of vitally stained cells derived from the peritoneum; 10) the toxicity of the dye at oestrus.

In regard to the light thrown by vital staining on the activities of certain cells met with in the tissues of the reproductive system, the work as a whole suggests that one of the normal functions of the reticulo-endothelial cells under special circumstances may be to take up nutritive substances from the tissue fluids, to elaborate them and render them suitable for transference to the surrounding tissues - the term histiotrophic may be applied to these functions. It may also be that the appearances in the wall of the decidual cavity indicate that this function is not confined to the reticulo-endothelial cells. Indeed it is suggested that tissue nutrition generally is not merely a simple process of absorption from the body fluids, but is of a much more elaborate and complex nature, and is subserved by a special class of cells the histiotrophes.*

* I am indebted to Professor Bryce for the suggestion that this term may be applied in the present connection.

APPENDIX I.

THE NORMAL APPEARANCES IN THE VAGINAL SMEAR.

Between the oestrous periods the vagina is normally closed by what appears to be a thin glossy membrane.* At the time of oestrus a temporary opening is established between the vagina and the outside, the orifice remaining open for three or four days, then closing very tightly.

Stockard and Papanicolaou (1917) found the period of "heat" or oestrus to last about twenty-four hours,** during which the vagina contained an abundant quantity of fluid, and microscopic examination of smears of the vaginal content showed that the constituent elements changed in a definite manner from the beginning to the cessation of the oestrous flow, so that the period of oestrus could be divided into four stages. These are now well known, and shortly stated are:-

Stage 1. Mucous secretion. Epithelial cells (cornified).
Duration - 6 to 12 hours.

Stage 2. Vaginal fluid thick and cheesy. Great numbers of epithelial cells (nucleated).
Duration - 2 to 4 hours.

Stage 3. Fluid thinner. Epithelial cells and polymorphonuclear leucocytes. Leucocyte-containing cells strikingly typical. Epithelial cells gradually becoming less numerous. Duration - 4 to 6 hours.

Stage 4. Continuation of Stage 3. Red cells due to slight haemorrhage into uterus and vagina.
Duration - 1 to 2 hours.

Ovulation occurs at the end of Stage 2, or the beginning of Stage 3.

During dioestrus the vagina contains leucocytes and atypical epithelial cells.

* See Kelly and Papanicolaou (1927).
** The female is in "heat", strictly speaking, only 2 to 3 hours, usually near end of Stage 1 and beginning of Stage 2. (Stockard and Papanicolaou 1917 and 1919; Squier 1932).

It should be noted that no conclusions regarding the presence of a state of "heat" can be arrived at from the opening of the vagina alone, unless the vaginal content is examined microscopically.

APPENDIX II.

THE ASSOCIATED APPEARANCES IN THE OVARIES AND UTERUS DURING THE OESTROUS CYCLE.

A full description will first be given and later a summary of the points used to verify the times in the cycle at which the animals (Table II) were killed.

The ovarian and uterine appearances during the cycle were described by Loeb (1906, 1911, 1914) and by Stockard and Papanicolaou (1917). The normal follicular cycle was investigated by Evans and Swezy (1931), and Nicol (1933) recorded the ovarian appearances after parturition in a series of non-pregnant and pregnant animals.* The related observations of these investigators agree in most essentials.

Evans and Swezy (1931) state that ovogenesis in the guinea-pig and other mammals occurs rhythmically during the sexual cycle, and have called this rhythm the "follicular cycle". They point out that the relationship between the follicular cycle and the oestrous cycle is so close that the time of the oestrous cycle at which an animal was killed may be determined from the ovary with a high degree of accuracy without recourse to its history; and that this has been done repeatedly with the dog and the guinea-pig. In addition to this a valuable confirmation is to be found in the condition of the endometrium with its rhythmical growth, maturity and regression.

* Loeb gives no measurements either of follicles or corpora lutea, and groups the follicles into small, medium and large types. Evans and Swezy state that mature follicles seem to have a size variation between 900 and 1260 microns, and give tables showing the cyclic distribution of small, medium and large follicles, but no measurements indicating the range in size of the small and medium types. Nicol (1933) arbitrarily describes the large follicles as measuring from 800 to 1200 microns in diameter, the medium-sized from 500-800 microns, and the small from about 200 to 500 microns.

The ovarian and uterine appearances during the cycle may be described as follows, the description being a summation of the accounts recorded by the previous workers, and supplemented by personal observations.

At Heat.

The ovary contains follicles of all sizes, a few of which are mature or have recently ovulated, the remainder showing all stages of degeneration. Ovulation usually occurs spontaneously some time during the first day of the cycle. The degenerative changes in the follicles consist of the gfamulosa degenerating and becoming dissolved, and connective tissue growing into the follicular cavity, this process ending finally in an almost complete disappearance of these follicles. Moreover, the follicular degenerative changes affect equally both ovaries of one animal, even if a rupture of follicles should have taken place in only one of the two ovaries.

If a young corpus be present it consists of large cells with rounded or oval nuclei, irregularly arranged around a central cavity in which is found a variable number of polymorphonuclear leucocytes. Capillary vessels are beginning to grow into the corpus at the periphery.

The average mean diameter* of such a corpus is about 0.9 mm.

The regressing corpora lutea of the preceding cycle are usually present in at least one ovary. These vary in size according to their rate of regression. Vacuolisation of the luteal cells is especially seen at the periphery of the corpus, and a considerable number of double-layered blood vessels is present. Notwithstanding the degenerative processes which are apparent in these corpora lutea it is not uncommon to still find mitoses in the lutein cells within the first twenty hours after ovulation.

In the uterus when heat begins the epithelium becomes tall and columnar, and its cells become filled with mucus. The nuclei of the columnar cells appear closely packed one against the other, and are pressed into different levels in the various cells so as to give an appearance of several rows of nuclei. At the same time a large number of leucocytes begin to migrate from the capillaries through the stroma and towards the epithelium. The stroma itself is congested. These are the appearances seen during the first stage of "heat", when only epithelial cells (cornified) are found in the vaginal smear. During the second stage the leucocytes accumulate in large numbers below the epithelium, and in the third stage penetrate into the epithelium, some of them passing between the epithelial cells into the uterine lumen. The entire epithelium becomes disintegrated and vacuolated, and under the destroyed epithelium haematomata are to be seen in several places. Generally the epithelium falls off still connected with pieces of the stroma, which also seems to be destroyed to some extent every heat period. When the epithelium falls away the haematomata are uncovered, and the blood contained in them passes into the lumen of the uterus. Regeneration and the falling off of the degenerated epithelium take place at the same time, the separation starting from the necks of the uterine glands which have remained

* The recorded mean diameter of the individual corpora lutea is the product of three measurements: (1) the thickness as obtained from the number of sections; (2) the greatest vertical; and (3) the greatest transverse measurement. If more than one corpus luteum is present and the mean diameters roughly agree, the average mean diameter of the corpora lutea for a given female is recorded.

intact during the entire process of destruction. The repair of the epithelium seems to take place very quickly and is completed in about ten hours (Stockard and Papanicolaou 1917). This stage of reparation corresponds to the fourth stage of heat.

The connective tissue is cellular, but soon shows a distinct fibrillar character; its nuclei are relatively large; it is somewhat oedematous, and only rarely are mitoses seen.

The glands are short and do not show much branching and some are filled with polymorphonuclear leucocytes. The epithelium of the gland ducts is cylindrical, but that of the gland fundi is lower. Some mitoses are present in the ducts but absent in the gland fundi (Loeb 1914).

Three Days after Heat.

During the first four or five days after oestrus the final degeneration and disappearance of the follicles from the preceding follicular cycle are completed, and the beginning of the new follicular cycle is initiated. Moreover, during this time the ovary shows a smaller number of healthy follicles than at any other period of the oestrous cycle (Evans and Swezy 1931).

The ovary three days after heat contains no large atretic follicles. In my material not intravitally stained, medium-sized follicles measuring up to 760 microns in diameter and showing chiefly connective tissue atresia are still present, and a moderate number of small normal follicles of 200 to 300 microns in diameter are seen.

The cells of the young corpora lutea are now arranged in irregular columns, and the single-layered capillary vessels have penetrated to the central cavity of the corpus (Loeb 1906; Nicol 1933) The central cavity may be small or large, and is usually filled with loose connective tissue. Fresh blood may be found in the central cavity.

The regressing corpora lutea of the preceding ovulation are smaller, more vacuolated, and contain more double-layered vessels.

In the uterus the epithelium is mostly high cylindrical, but at places may be cuboidal; and usually shows no mitoses. The gland duct epithelium is high cylindrical, and the epithelium of some of the gland fundi is becoming higher. There are many mitoses present in the gland ducts, and mitoses may be found in the gland fundi which have enlarged. The connective tissue is cellular, the nuclei are vesicular, but no mitoses are as yet visible. Some polymorphonuclear leucocytes are still present in the mucosa and also several small mononuclear cells, and some of the latter may migrate into the gland and uterine epithelium.

Four Days after Heat.

In the ovary normal follicles are more numerous, and in my material varied from 300 to 500 microns in diameter. Follicles showing chiefly connective tissue atresia and measuring up to 700 microns were also found.

The young corpora lutea show practically the same appearances as at three days after heat, and the regressing corpora of the preceding ovulation are still more degenerated.

The lining epithelium of the uterus and that of the gland ducts have become lower and are now of medium height or cuboidal, but the epithelium of the gland fundi is higher. There are many mitoses present in the gland fundi, but nowhere else in the epithelium. The connective tissue layer under the uterine epithelium is now enlarged, and shows frequent mitoses especially close to the uterine lumen. The connective tissue cells have round or oval vesicular nuclei, their cytoplasm is permeated with vacuoles, and they form fibrillar processes. The tissue also appears hyperemic. The arrangement is the one typical for regenerating connective tissue as in wounds, the blood vessels running at right angles to the layers of the connective tissue,

and this appearance continues for the next few days. Between the gland fundi the intercellular fibrils are more marked; the number of nuclei is smaller and there are fewer mitoses present. The enlargement of the connective tissue causes the gland ducts to become drawn out.

Five Days after Heat.

Normal follicles are still more numerous in the ovary and vary in size from 210 to 580 microns in diameter. Connective tissue atresia is found in follicles from 400 to 560 microns in diameter and occasionally an atretic follicle measuring 700 microns is seen, but the amount of atresia is less than at four days after heat.

The young corpora lutea are now fully formed (Loeb 1906). They are a little larger than at four days after heat, and show practically similar appearances. The corpora of the previous ovulation have become still more vacuolar and degenerated.

The uterine and glandular epithelium is of medium height and almost without mitoses; a few isolated mitoses may be found in the gland fundi. The structure of the connective tissue is similar to the previous period; it contains many mitoses, and some small mononuclear and a few polymorphonuclear leucocytes are also seen.

Six Days after Heat.

Normal follicles of small and medium size are found, and some of these may measure up to 750 microns in diameter. Follicles showing connective tissue atresia are also seen, and vary from 300 to 500 microns in diameter, but are not very numerous. From about the sixth to the ninth or tenth day of the cycle the amount of degeneration taking place is less than at any other period of the cycle (Evans and Swezy 1931).

The corpora lutea appearances are as at five days after heat. Mitoses are present in the lutein cells, as well as in the endothelial cells of the capillaries.

The lining epithelium of the uterus is of medium height and the epithelium of the gland ducts and gland fundi is cuboidal. No mitoses are present in the epithelium. Mitoses are present in the connective tissue layer of the mucosa, but are probably not as frequent as at four days after heat. Beneath the uterine epithelium the connective tissue nuclei are large and vesicular and densely packed in layers. There are many small mononuclears in the connective tissue. Some polynuclear leucocytes are also present, and also some disintegrating cells, the latter being possibly mainly polynuclear leucocytes (Loeb 1914).

Seven Days after Heat.

The follicular appearances are as at six days after heat.

The young corpora lutea have now reached an average mean diameter of about 1.4 mm; the vessels are still practically all single-layered, though very occasionally a small vessel with a double-layered wall may be visible at the periphery. The old corpora are in full process of retrogression and vary in size.

The lining epithelium of the uterus and glands is cuboidal or of medium height. No mitoses are present in the epithelium. The connective tissue of the mucosa shows appearances similar to that of the previous period; there are a number of mitoses but not as many as at five days after heat. Some polynuclear leucocytes and small mononuclears are present in the mucosa, and also some disintegrating nuclei are seen under the uterine epithelium.

Eight Days after Heat.

The condition of the ovaries is practically the same as at seven days after heat, but degeneration of the granulosa has now set

in in some of the larger follicles.

The corpora lutea show similar appearances to those at seven days after heat; they grow actively about this period and frequent mitoses are seen in the lutein cells.

In the connective tissue of the mucosa no mitoses are now seen, and a number of nuclei under the uterine epithelium disintegrate, perhaps these being the nuclei of the proliferated connective tissue layer (Loeb 1914).

Nine Days after Heat.

The ovaries and uterus show practically the same appearances as are described at eight days after heat. The connective tissue of the mucosa still shows vesicular nuclei. The glands are not very large, and contain a relatively small number of coils.

Ten to Eleven Days after Heat.

Normal follicles of all sizes are present in the ovaries and some of these measure more than 800 microns in diameter (i.e. have reached the size of mature follicles). However, many of the large follicles especially now show various stages of granulosa degeneration and connective tissue ingrowth.

The young corpora are well developed and have reached their greatest size, but may now begin to show regressive changes, some of the cells at the periphery becoming vacuolated (Loeb 1911; Evans and Swezy 1931). Mitoses in the lutein cells are usually frequent and may perhaps occur also in capillary endothelial cells. Most of the vessels are still single-layered though occasionally a double-layered vessel is present at the edge of the corpus.

The corpora lutea of the previous ovulation are now small vacuolar bodies with much connective tissue and thick vessels.

The lining epithelium of the uterus and gland ducts is cylindrical of medium height. The gland fundi are smaller and lie near the uterine lumen. No mitoses are seen. The mucosa is becoming smaller and more fibrillar. Some mononuclear cells may be present.

Twelve Days after Heat.

There are many normal follicles of all sizes and a number of these have reached the size of mature follicles. Many of the follicles especially those of medium and large size, show granulosa degeneration, while others are in various stages of connective tissue atresia.

The corpora present appearances as at eleven days after heat.

The uterine epithelium and that of the glands is cuboidal, and no mitoses are seen. A few gland ducts and the adjoining uterine epithelium have high cylindrical epithelium. Some small mononuclear cells may be seen migrating through the uterine epithelium. The mucosa is narrow, the nuclei of the connective tissue cells are small, and the glands are correspondingly smaller.

Fourteen Days after Heat.

The follicular appearances are as at twelve days after heat.

In the corpora lutea vacuolisation of the peripheral cells begins more definitely, and more double-layered vessels may be seen penetrating into the corpora. Mitoses are still found in some of the lutein cells.

The uterine and gland duct epithelium is cuboidal or of medium height, and some mitoses are seen. The gland fundi are smaller. There may be some dilated glands with polynuclear leucocytes. The mucosa is narrow and its nuclei small and some oedema may be present. Some mononuclears may be seen in the mucosa.

Fifteen Days after Heat.

The ovaries present about the same appearances as at fourteen

days after heat. Most of the large follicles show degenerative changes, only a few remaining normal.

The uterine and gland duct epithelium is cuboidal or low cylindrical. Some mitoses are present in the uterine epithelium and very occasionally in a gland duct.

The mucosa is narrow and oedematous, and its nuclei are small.

Animals which failed to Ovulate at Heat.

Apart from the absence of young corpora lutea the characteristic feature of the ovaries in these animals is the persistence of very large atretic follicles measuring about 1000 microns in diameter. These should normally have disappeared about the third day of the cycle after ovulation, but where ovulation has failed to recur they persist at least up to about the seventh day of the cycle. This I found both in animals which had not received dye intravitally and in those which had.

THE CYCLIC CHANGES IN THE VAGINA AND CERVIX.

The vaginal changes were described histologically by Selle (1922). In the interval between the "heat periods" the epithelium is only one or two cells high, but towards the end of the interval it rapidly increases in height and reaches a thickness of ten to twelve cells, the most superficial cells becoming vacuolated. At the beginning of oestrus, immediately before the vagina opens, many of these superficial vacuolated cells are cast off into the lumen, while two to four cells below the surface of the epithelium cornification is involving four to six cell layers.

As the process of cornification continues the cornified cells become loosened from the underlying layers and are cast off into the lumen singly or in groups, constituting the cells present in the vaginal smear (Stage 1 of Stockard and Papanicolaou). The entire cornified layer is sometimes shed as a cast. Many of the underlying epithelial cells are then thrown off furnishing the appearances in the smear (Stage 2 of Stockard and Papanicolaou). At this time leucocytes invade the submucosa and the epithelium, and a little later are found in the lumen of the vagina (Stage 3 of Stockard and Papanicolaou), while the desquamation of epithelial cells gradually diminishes. At the end of this stage the vaginal epithelium is only two to four cells high then becomes reduced to its lowest condition of one to two cells typical of the interval between the "heat periods."

In the cervical epithelium Hartmann and Olbers (1931) found cyclical changes analogous with those in the vaginal epithelium. Between the "heat periods" the cervix is lined by a single layer of cylindrical epithelial cells with basal nuclei. Just before the beginning of "heat" the epithelium becomes several layered - six to eight cells high with nuclei now almost centrally placed, and as a result is thrown into folds. During oestrus the epithelium desquamates and is thrown off into the lumen.

For the purposes of the present research the following points from the above description were used to verify the times in the cycle at which the animals were killed.

Follicles:- At the time of ovulation a degeneration of practically all follicles present in the ovaries occurs. Within the next few days normal small follicles grow and have reached medium size at about six days after ovulation (Loeb 1911). During this period the

degeneration and disappearance of the follicles from the preceding cycle is completed, so that largish follicles showing connective tissue atresia and found in the ovaries about this time belong to the previous follicular cycle. Very large atretic follicles of the previous cycle have, however, disappeared by the third day after ovulation, except in those animals in which a new ovulation has failed to occur, when the large atretic follicles persist longer. Normal follicles have reached a large size by the ninth or tenth day. By the tenth day, also, degeneration has begun in many of the larger follicles, the amount of this increasing with each succeeding day until the end of the cycle, and normal follicles taking the place of the degenerating ones. Mature normal follicles appear first approximately eleven to thirteen days after ovulation.

Corpora lutea:- Single-layered capillary vessels have penetrated to the central cavity of the young corpus by the third day after ovulation, and by the fifth day the corpus is fully formed (Loeb 1906). At the sixth day the vessels are still practically all single-layered, but for the first time a double-layered vessel may be seen at the periphery of the corpus. The average mean diameter of the corpus at the seventh day is about 1.4 mm, and the corpus reaches its greatest size about the tenth day. At this time most of the vessels are still single-layered, though occasionally a double-layered vessel is present in the periphery of the corpus. From about the eleventh day the corpus begins to regress, a few of the peripheral cells becoming vacuolated. This is seen more definitely about the fourteenth day but is still slight, and more double-layered vessels may be seen penetrating into the corpus.

At the next ovulation which usually occurs about the sixteenth or seventeenth day the regressive changes in the corpus are still more marked, and this corpus now becomes that of the preceding cycle. Shrinkage of the corpora occurs, and three days after the new ovulation they are usually smaller than immediately after the new ovulation. Vacuolisation of the lutein cells increases, more

thick vessels appear, and the corpora gradually become small vacuolar bodies surrounded and permeated by thick connective tissue. The rate of regression of these corpora varies, and they may vary in size even in the same ovary.

Uterus:- At the time of ovulation the epithelium of the gland fundi is low, from the fourth to the sixth day it is cylindrical and active, thereafter it becomes cuboidal or of medium height. The uterine epithelium and that of the gland ducts have become cuboidal or of medium height by the fourth day of the cycle, and remain so until about the next ovulation. From the time of ovulation the mitoses migrate from the lining epithelium of the uterine lumen to the gland fundi, and disappear completely in the epithelium in five to six days. From the sixth to the twelfth day the epithelium is without mitoses; then mitoses begin to reappear in the epithelium of the uterine lumen.

In the connective tissue of the mucosa proliferation starts on the fourth day after ovulation, and ceases on the seventh day. This results in the mucosa becoming wider then and the gland ducts being drawn out, and the tissue has at this time the appearances of regenerative connective tissue as in wounds.

Vagina:- In the interval between the "heat periods" the lining epithelium is only one or two cells high. Towards the end of the interval it rapidly reaches a thickness of ten to twelve cells, and at oestrus cornification and desquamation occur.

Cervix:- Between the "heat periods" the lining epithelium consists of a single layer of cylindrical cells. Just before the beginning of oestrus it becomes several layered - six to eight cells high - and during oestrus desquamation occurs.

APPENDIX III.

Records of Associated Uterine, Vaginal and Ovarian Appearances of the Animals shown in Table II.

The picture of events in the ovaries was obtained as follows: Both ovaries of each animal were cut serially at 10 microns. The

sections were serially mounted, usually about five rows of ten sections to one slide, and each ovary occupying usually about eight to ten slides. The formidable task of counting and measuring all the follicles was not attempted, but what seemed a sufficiently reliable compromise was obtained by the following procedure.— The centre section of each row was first examined; the number of normal and atretic follicles and the type of atresia were noted, and the largest diameter of each obvious follicle measured. The other sections in the row were then examined to find if the centre section truly represented the characteristics of the row, and any obvious differences were adjusted. The same plan was carried out for all the rows in each slide. From the data obtained a chart was then prepared for each ovary showing the variation in size of the follicles. Only the measurements of the largest atretic and largest normal follicle are given for each animal, no useful purpose apparently being served by detailing the follicular charts. The corpora lutea were examined after the follicular data had been obtained. Both ovaries of the same animal showed similar appearances, except for the presence or absence of corpora lutea. The same procedure was followed for each animal so that the results might be comparable.

Animal No. 32.

Killed on 1st day of 16-day cycle.
Ovaries: Many atretic follicles, measuring up to 1070 microns in diameter. Small number of normal follicles, the largest measuring 870 microns. Several corpora lutea (average mean diameter 1.25 mm) with double-layered vessels and a number of the luteal cells in a state of degeneration.
Uterus and Vagina: Epithelium of uterine lumen tall and columnar. Epithelium of gland ducts cylindrical, and of gland fundi medium in height. Mucosa relatively narrow, fibrillar and oedematous. Glands short. Considerable number of leucocytes and mononuclears in the blood vessels and scattered throughout mucosa.
Cervical epithelium six to eight cells high, and superficial cells vacuolated and being cast off.
Vaginal epithelium ten to twelve cells thick. Superficial cells vacuolated and being cast off. Cornification present in deeper cells.
These features confirm that this animal was killed at the beginning of oestrus (before ovulation should have occurred), and agree with the appearances in the vaginal smear.

Animal No. 34.

Killed on 1st day of 17-day cycle.

Ovaries: Many atretic follicles, measuring up to 1180 microns in diameter. Small number of normal follicles, the largest measuring 680 microns. Several corpora lutea (average mean diameter 1.2 mm) with double-layered vessels and a number of the luteal cells in a state of degeneration.
Uterus and Vagina: Epithelium of uterine lumen tall and columnar. Epithelium of gland ducts cylindrical, and of gland fundi medium in height. Mucosa relatively narrow and fibrillar, congested and oedematous. Glands short and not much coiled. Considerable number of leucocytes and mononuclears are scattered throughout the mucosa. Cervical epithelium six to eight cells high, and superficial cells vacuolated and being cast off.
Vaginal epithelium ten to twelve cells thick. Superficial cells vacuolated and being cast off. Cornification present in the deeper cells.

These features confirm that this animal was killed at the beginning of oestrus before ovulation should have occurred, and agree with the appearances in the vaginal smear.

Animal No. 109.

Killed on 1st day of 16-day cycle.
Ovaries: Many atretic follicles, measuring up to 1300 microns in diameter. Small number of normal follicles, the largest measuring 1200 microns. Several corpora lutea (average mean diameter 1.25 mm) with thick vessels and many of the luteal cells in a state of degeneration.
Uterus and Vagina: Epithelium of uterine lumen tall and columnar and being invaded by leucocytes. Epithelium of gland ducts cylindrical and of gland fundi medium in height. Mucosa narrow and congested, fibrillar and oedematous. Glands short. Many leucocytes and mononuclears in the blood vessels and scattered throughout mucosa. Cervical epithelium six to eight cells high; superficial cells vacuolated and desquamating.
Vaginal epithelium ten to twelve cells thick. Superficial cells vacuolated and being cast off. Deeper cells cornified and being separated off as a cast of vagina.

These features confirm that this animal was killed at the time of oestrus, and agree with the appearances in the vaginal smear, which indicate the end of the second stage of heat, just previous to ovulation.

Animal No. 98.

Killed at 1st day of 16-day cycle.
Ovaries: Many atretic follicles, measuring up to 1190 microns in diameter. Considerable number of small normal follicles, the largest measuring 230 microns. No large normal follicles. Several newly formed corpora lutea (average mean diameter 0.9 mm). Capillaries only beginning to penetrate edge of young corpus.
Uterus and Vagina: Epithelium of uterine lumen tall and columnar, and nuclei pressed into different levels. Great numbers of polymorphs invading the epithelium. Gland duct epithelium also columnar and invaded by leucocytes. Epithelium of gland fundi of medium height. Mucosa narrow, congested and fibrillar. Glands short. Great numbers of leucocytes in blood vessels and scattered throughout mucosa. Cervical epithelium - several cells thick and desquamating.
Vaginal epithelium about six cells high-as cornified cells have been thrown off. Many polymorphs and mononuclears.

These features confirm that this animal was killed at the time of oestrus, and agree with the appearances in the vaginal smear.

Animal No. 42.

Killed at 3rd day of 17-day cycle.
Ovaries: Considerable number of atretic follicles, largest measuring 1190 microns in diameter. Moderate number of small normal follicles of 200 microns, and a few normal follicles measuring 450 microns. Several old corpora lutea (average mean diameter 1.0 mm) with thick vessels and degenerating luteal cells.
Uterus and Vagina: Epithelium of uterine lumen high cylindrical but at places cuboidal. Epithelium of gland ducts and of glands tall and columnar. Moderate number of polymorphs and mononuclears present, and several of the latter may be seen in the uterine and gland epithelium.
Cervical epithelium still desquamating.
Vaginal epithelium two to four cells high at thinnest parts, and cornified cells have separated as a cast which is still present in the lumen.
The vaginal closure membrane had almost completely reformed when the animal was killed, and a vaginal smear taken two days previously indicated oestrus.

The above data seem to confirm that this animal was killed about the third day of its new cycle, but suggest that the dye has prevented ovulation and has lessened the intensity and prolonged the duration of the oestrous changes.

Animal No. 9.

Killed at 4th day of 17-day cycle.
Ovaries: Considerable number of atretic follicles, measuring up to 1160 microns in diameter. Considerable number of small normal follicles measuring from 250 to 350 microns in diameter, and a few measuring 450 microns. Several old corpora lutea (average mean diameter 1.0 mm) with thick vessels and degenerating luteal cells.
Uterus and Vagina: Epithelium of uterine lumen cuboidal. Epithelium of gland ducts and fundi medium in height. Mucosa relatively narrow. Glands not much coiled. Nuclei of stratum compactum closely packed together and rather small. Moderate number of leucocytes and mononuclears scattered throughout mucosa.
Cervical epithelium one to two cells high at thinnest part and some desquamation still appears to be occurring.
Vaginal epithelium one to two cells high at thinnest parts.

The above data seem to confirm that this animal was killed about the fourth day of its cycle, and suggest that the dye has prevented ovulation.

Animal No. 50.

Killed at 5th day of 17-day cycle.
Ovaries: Considerable number of atretic follicles, measuring up to 1140 microns in diameter. Considerable number of normal follicles from 200 to 350 microns in size; and a few measuring 480 microns. Several old corpora lutea (average mean diameter 1.0 mm) with thick vessels and degenerating luteal cells.
Uterus and Vagina: Epithelium of uterine lumen and gland ducts cuboidal. Epithelium of gland fundi of medium height. Mucosa relatively narrow and glands not much coiled. Nuclei of stratum compactum rather small and closely packed together. Moderate number of leucocytes and mononuclears in the mucosa.
Cervical epithelium one or two cells high at thinnest parts and some desquamation still appears to be taking place.
Vaginal epithelium one to two cells high at thinnest parts.

The previous oestrus appeared normally as indicated by the vaginal smear.

The abova data seem to confirm that this animal was killed approximately at the fifth day of its cycle, but suggest that the dye has prevented ovulation.

Animal No. 22.

Killed at 7th day of 17-day cycle.
Ovaries: Moderate number of atretic follicles, measuring up to 550 microns in diameter. Considerable number of normal follicles from 200 to 420 microns in diameter. Several young corpora lutea (average mean diameter 1.6 and 1.4 mm), with vessels all single-layered and having reached centre of corpus. Several old corpora (average mean diameter 0.7 mm) with thick vessels, fibrosis and degenerated luteal cells.
Uterus and Vagina: Epithelium of uterine lumen cuboidal.
Epithelium of gland ducts cuboidal and of gland fundi low cylindrical. The mucosa is enlarged and the gland fundi much coiled. The nuclei of the stratum compactum are very numerous and closely packed together. A small number of mononuclears and polymorphs is scattered throughout mucosa.
Cervical epithelium - one cell thick at thinnest parts and cells often stain intensely and irregularly.
Vaginal epithelium - one or two cells thick at thinnest parts.
These appearances seem to confirm that this animal was killed approximately at the seventh day of its cycle.

Animal No. 23.

Killed at 7th day of 17-day cycle.
Ovaries: Moderate number of atretic follicles, measuring up to 490 microns. Considerable number of normal follicles from 200 to 400 microns in diameter. Two young corpora lutea (average mean diameter 1.4 mm) Several old corpora lutea (average mean diameter 0.8 mm) with thick vessels, fibrosis and degenerated luteal cells.
Uterus and Vagina: Epithelium of uterine lumen cuboidal or medium in height. Epithelium of gland ducts cuboidal and of gland fundi low cylindrical. Mucosa relatively wide and gland fundi much coiled. The nuclei of stratum compactum are very numerous and closely packed together. A small number of polymorphs and monomuclears is scattered throughout mucosa.
Cervical epithelium - one cell thick at thinnest parts.
Vaginal epithelium - one or two cells high at thinnest parts.
These appearances seem to confirm that this animal was killed approximately at the seventh day of its cycle.

Animal No. 43.

Killed at 9th day of 18-day cycle.
Ovaries: Moderate number of atretic follicles, measuring up to 530 microns in diameter. Considerable number of normal follicles, the largest measuring 500 microns. Several young corpora lutea (average mean diameter 1.6 mm) and with vessels chiefly single-layered and having reached centre of corpus. Several old corpora lutea (average mean diameter 0.8 mm) with thick vessels and degenerating luteal cells.
Uterus and Vagina: Epithelium of uterine lumen, gland ducts and gland fundi medium in height. Narrow uterine lumen. Very wide mucosa, especially stratum compactum, where nuclei are large and closely packed together. Gland fundi much coiled and ducts drawn out. Considerable number of leucocytes and mononuclears scattered throughout mucosa.
Cervix lined by a single layer of cylindrical epithelium with basal nuclei. Epithelial cells often very intensely stained.
Vaginal epithelium - one to two cells high at thinnest parts.

These appearances seem to confirm that this animal when killed was approximately at the ninth day of its cycle.

Animal No. 49.

Killed at 8th/9th day of 16-day cycle.
Ovaries: Moderate number of atretic follicles, measuring up to 520 microns in diameter. Considerable number of normal follicles, the largest measuring 350 microns. One young corpus luteum (average mean diameter 1.58 mm) with vessels chiefly single-layered and having penetrated to centre of corpus. Also three smaller young corpora lutea (average mean diameter 1.1 mm) each containing remains of ovum. Several old corpora (average mean diameter 0.85 mm) with thick vessels and degenerated luteal cells.
Uterus and Vagina: Epithelium of uterine lumen, gland ducts and gland fundi medium in height; narrow uterine lumen. Very wide mucosa, especially stratum compactum whose nuclei are large and closely packed together. Gland fundi much coiled and gland ducts drawn out. Moderate number of leucocytes and mononuclears scattered throughout mucosa.
Cervix lined by single layer of cylindrical epithelium with basal nuclei. Epithelial cells often intensely stained.
Vaginal epithelium - one or two cells high at thinnest parts.

These appearances seem to confirm that this animal was killed approximately at the ninth day of its cycle.

Animal No. 4.

Killed at 9th/10th day of 17-day cycle.
Ovaries: Moderate number of atretic follicles measuring up to 600 microns in diameter. Considerable number of normal follicles, the largest measuring 570 microns. Several young corpora lutea (average mean diameter 1.58 mm) and vessels chiefly single-layered; only very occasionally is a vessel with a double-layered wall seen at the periphery of corpus. Several old corpora lutea (average mean diameter 0.8 mm) with thick vessels and degenerating luteal cells.
Uterus and Vagina: Epithelium of uterine lumen and gland ducts cubical. Epithelium of gland fundi cylindrical. Wide mucosa. Gland fundi much coiled and gland ducts drawn out. Stratum compactum: cells large and very closely packed together. Considerable number of leucocytes and mononuclears scattered throughout mucosa.
Cervix lined by single layer of cylindrical epithelium with basal nuclei. Epithelial cells often intensely stained.
Vaginal epithelium - one or two cells high at thinnest parts.

These appearances seem to confirm that this animal was killed approximately at the ninth or tenth day of its cycle.

Animal No. 29.

Killed at 12th day of 16-day cycle.
Ovaries: Many atretic follicles, measuring up to 1000 microns in diameter. Considerable number of normal follicles, the largest measuring 500 microns. Four young corpora lutea (average mean diameter 1.66 mm). A number of the blood vessels in the young corpora are double-layered. Several old corpora (average mean diameter 0.65 mm) with many thick vessels.
Uterus and Vagina: Epithelium of uterine lumen cubical; epithelium of glands and gland ducts of medium height. Narrow stratum compactum with oval nuclei closely packed together. Relatively wide stratum spongiosum and nuclei more loosely arranged.
Cervix lined by single layer of deeply staining epithelium. Moderate number of mononuclears scattered throughout mucosa.
Vaginal epithelium - two or three cells high.

These features seem to confirm that this animal was killed approximately at the twelfth day of its cycle.

Animal No. 28.

Killed at 12th day of 16-day cycle.
Ovaries: Many atretic follicles, measuring up to 1250 microns in diameter. Considerable number of normal follicles, the largest measuring 950 microns. Several young corpora (average mean diameter 1.7 mm). A number of the blood vessels in the young corpora are definitely two-layered, and a few of the luteal cells appear to be degenerating. Several old corpora (average mean diameter 0.7 mm) with many thick vessels.
Uterus: Epithelium of uterine lumen cubical; epithelium of glands and gland ducts of medium height. Occasionally mitoses seen in gland epithelium. Narrow stratum compactum with oval nuclei closely packed together. Relatively wide stratum spongiosum and glands much coiled, especially around antimesometric half of lumen. Nuclei of stratum spongiosum similar in appearance to those of stratum compactum, but slightly paler and more loosely arranged. Moderate number of mononuclears scattered throughout mucosa.
Cervix lined by single layer of epithelium.
Vaginal epithelium two or three cells high.
These features seem to confirm that this animal was killed approximately at the twelfth day of its cycle.

Animal No. 47.

Killed at 13th day of 17-day cycle.
Ovaries: Many atretic follicles, measuring up to 1000 microns in diameter. Considerable number of normal follicles, the largest measuring 960 microns. Several young corpora lutea (average mean diameter 1.6 mm). A number of the blood vessels in the young corpora are two-layered, and some of the luteal cells appear to be degenerating Several old corpora (average mean diameter 0.8 mm) with many thick vessels.
Uterus and Vagina: Epithelium of uterine lumen cubical; epithelium of glands and gland ducts of medium height. Narrow stratum compactum with nuclei closely packed together. Relatively wide stratum spongiosum and glands much coiled. Moderate number of mononuclears scattered throughout mucosa.
Cervix lined by single layer of epithelium.
Vaginal epithelium two or three cells high.
These features seem to confirm that this animal was killed approximately at the thirteenth day of its cycle.

Animal No. 52.

Killed at 15th day of 17-day cycle.
Ovaries: Many atretic follicles, measuring up to 1060 microns in diameter. Considerable number of normal follicles, the largest measuring 860 microns. Several young corpora lutea (average mean diameter 1.5 mm). A number of the blood vessels in the corpora are two-layered, and several of the luteal cells are in a state of degeneration. Several old corpora (average mean diameter 0.5 mm) with many thick vessels.
Uterus and Vagina: Epithelium of uterine lumen cubical; epithelium of glands and gland ducts of medium height. Occasional mitoses seen in the epithelium. Relatively narrow stratum compactum with nuclei closely packed together. Wider stratum spongiosum with glands moderately coiled. Moderate number of mononuclears scattered throughout mucosa.
Cervical epthelium - two to four cells high, superficial cells becoming vacuolated.
Vaginal epithelium - two to four cells high at thinnest parts, superficial cells becoming vacuolated.

These features seem to confirm that this animal was killed approximately at the fifteenth day of its cycle.

Animal No. 41.

Killed at 1st day of 17-day cycle (oestrus delayed two days - compare Table II).
Ovaries: Many atretic follicles, measuring up to 1000 microns in diameter. Considerable number of normal follicles, the largest measuring 500 microns. Several corpora lutea (average mean diameter 0.9 mm) with thick blood vessels and a number of the luteal cells in a state of degeneration.
Uterus and Vagina: Epithelium of lumen columnar; epithelium of gland ducts cylindrical, and of gland fundi medium in height. Mucosa narrow. Glands short and not much coiled. Considerable number of leucocytes and mononuclears in the blood vessels and scattered throughout mucosa.
Cervical epithelium mostly single-layered and cells appear to be becoming vacuolated.
Vaginal epithelium about ten cells thick; superficial cells vacuolated and being cast off, and cornification present in deeper cells.
These features seem to confirm that this animal was killed approximately at the beginning of oestrus.

Animal No. 24.

Killed at 1st day of 17-day cycle (oestrus delayed two days - compare Table II).
Ovaries: Many atretic follicles, measuring up to 1320 microns in diameter. Considerable number of normal follicles, the largest measuring 600 microns. Several corpora lutea (average mean diameter 1.1 mm), with thick blood vessels and a number of the luteal cells in a state of degeneration.
Uterus and Vagina: Epithelium of uterine lumen tall and columnar; epithelium of gland ducts cylindrical and of gland fundi cubical. Mucosa narrow and congested. Stratum compactum appears to be oedematous and cells are more loosely arranged. Glands short and not much coiled. Large number of mononuclears and leucocytes in blood vessels and in endometrium.
Cervical epithelium six to eight cells high, and superficial cells vacuolated and desquamating.
Vaginal epithelium ten to twelve cells thick; superficial cells vacuolated and being cast off, and cornification present in deeper cells which have become loosened from the underlying cell layers.
These features confirm that the animal was killed on the 1st day of its cycle. The appearances of the vaginal smear indicate the 1st/2nd stage of "heat".

Animal No. 31.

Killed at 1st day of 17 day cycle (oestrus delayed two days - compare Table II).
Ovaries: Many atretic follicles, measuring up to 860 microns in diameter. Considerable number of normal follicles, the largest measuring 650 microns. Several corpora lutea (average mean diameter 1.5 mm) with double-layered vessels especially at periphery and a number of the luteal cells degenerating.
Uterus and Vagina: Epithelium of uterine lumen columnar. Epithelium of gland ducts cylindrical and of gland fundi medium in height. Mucosa narrow and fibrillar. Glands not much coiled. Considerable number of leucocytes and mononuclears in the blood vessels and scattered through the mucosa.
Cervical epithelium six to eight cells high, and superficial cells

vacuolated and desquamating.
Vaginal epithelium eight to ten cells thick; superficial cells vacuolated and desquamating; invaded by many leucocytes.

These features seem to confirm that this animal was killed during oestrus, and agree with the appearances in the vaginal smear.

Animal No. 33.

Killed at first day of 16-day cycle (previous oestrus missed or suppressed - compare Table II).
Ovaries: Many atretic follicles, measuring up to 1100 microns in diameter. Moderate number of normal follicles, the largest measuring 850 microns. Several corpora lutea (average mean diameter 1.2 mm) with thick blood vessels and a number of the luteal cells degenerating.
Uterus and Vagina: Epithelium of uterine lumen columnar. Epithelium of gland ducts columnar and of gland fundi medium in height. Mucosa relatively wide, and fibrillar. Many leucocytes in the blood vessels and scattered throughout the mucosa. Glands short and not much coiled.
Cervical epithelium six to eight cells high and superficial cells vacuolated and being cast off.
Vaginal epithelium ten to twelve cells thick, superficial cells vacuolated and being thrown off, and cornification present in deeper cells which have become loosened from the underlying cell layers.

These features seem to confirm that this animal was killed at the time of oestrus, and agree with the appearances in the vaginal smear.

Animal No. 51.

Killed at 1st day of 18-day cycle (previous oestrus missed or suppressed - compare Table II).
Ovaries: Many atretic follicles, measuring up to 970 microns in diameter. Small number of normal follicles, the largest measuring 200 microns. Several young corpora lutea (average mean diameter 0.8 mm) with capillary vessels only penetrating at the edge and not yet having reached centre of corpus. Several remnants of old corpora in a state of fibrosis and degeneration.
Uterus and Vagina: Epithelium of uterine lumen tall and columnar. Epithelium of gland ducts columnar, and of gland fundi medium in height. Mucosa narrow and fibrillar. Glands short and not much coiled. Many leucocytes and mononuclears scattered throughout mucosa.
Cervical epithelium six to eight cells high and desquamating. Vaginal epithelium five to six cells thick as cornified cells have separated off. Many leucocytes and mononuclears.

The above features confirm that this animal was killed during oestrus, and agree with the appearances in the vaginal smear.

APPENDIX IV.

THE DISTRIBUTION OF THE DYE THROUGHOUT THE OESTROUS CYCLE - DESCRIPTION OF APPEARANCES IN THE UTERUS AND VAGINA IN THE ANIMALS SHOWN IN TABLE II.

Group 1.

The four animals in this group were killed at the time of oestrus, the first three before ovulation should have occurred, and

the fourth soon after the new ovulation. In none of these animals is the vital staining in the uterine horn intense.

In the first two animals which were killed earlier in oestrus than the last two, a very small number of dye cells is seen. These are more or less confined to the stratum compactum in which they are loosely scattered. The dye granules are coarse, but seldom obscure the nucleus, and some of the cells contain only one or two granules. Dye cells are only occasionally seen in the mucosa of the cervix or vagina, and none are present in the vaginal epithelium.

In the second two animals which show almost identical appearances a considerable number of dye cells is present. They are most numerous in the stratum compactum, where they are loosely arranged and not concentrated under the uterine epithelium as at the 12th day of the cycle (animal No. 28), but they also extend out into the stratum spongiosum. As in the first two animals the dye granules are coarse but seldom obscure the nucleus, and in some cells only few granules of dye are present. Very few examples of cells bursting and shedding the dye are seen. Fine dye granules are present in many of the endometrial cells of the stratum compactum, and also in some of the epithelial cells of the uterine lumen. Around the large blood vessels in the circular muscle zone a considerable number of vitally stained rounded cells and fibrocytes is present. In the vaginal submucosa a few rounded dye cells and a moderate number of vitally stained fibrocytes is seen. No dye cells are seen in the uterine, cervical or vaginal epithelium.

Group II.

The three animals in this group were killed in the early part of a new cycle, but in all three ovulation has failed to occur, and the appearances suggest that the oestrous changes had been prolonged.

The dye appearances resemble those in the last two animals in Group I. A considerable number of dye cells is scattered

through the stratum compactum especially around the antimesometrial side of the uterine lumen, but also extending out into the stratum spongiosum. In practically all the cells the nucleus is visible as in the animals of the former group. In one of the animals, where the differential staining with carbol fuchsin is particularly good, there seems to be ample proof of the origin of the dye cells from the blood. Many cells with dense nuclei are seen in the endometrium, some with dye and others without dye. They vary in size but the largest of them seem definitely to be the type of cell that takes up the dye.

No vitally stained cells are present in the uterine, cervical or vaginal epithelium. A few rounded dye cells and a moderate number of small dye-containing fibrocytes are seen in the vaginal submucosa.

Group III.

The two animals in this group were killed about the sixth or seventh day of the cycle, oestrus and ovulation having occurred normally. Both animals present similar appearances.

A small number of dye cells is seen in the dense stratum compactum, and some of these are close to the uterine epithelium, especially around the antimesometrial side. The dye granules are coarse, and some of the cells seem to be in a state of degeneration and to be shedding the dye. Small masses of "free" dye granules are also seen, presumably the contents of dye-containing cells which have degenerated or shed the dye. Practically no vitally stained cells are seen elsewhere in the uterine horn. No dye is visible in the endometrial cells.

Very few dye cells are seen in the vaginal submucosa, and none in the uterine, cervical or vaginal epithelium.

A number of free vitally stained cells is present in the uterine lumen.

Group IV.

The three animals in this group were killed approximately about the midpoint of the cycle, the previous oestrus and ovulation having occurred normally. All show similar appearances.

A relatively small number of dye cells is seen in the very wide endometrium. The dye cells are scattered singly in the dense stratum compactum, and not concentrated against the uterine epithelium. In smaller numbers they extend out into the stratum spongiosum. They are slightly more numerous in the antimesometrial half of the stratum compactum. The dye granules are coarse in the stratum compactum, but seldom obscure the nucleus, and in the stratum spongiosum fewer and smaller granules are present in the dye cells. Generally speaking the dye granules are coarser the nearer to the uterine lumen. A few fine dye granules are seen in some of the endometrial cells of the stratum compactum. In the vaginal submucosa a moderate number of rounded dye cells and vitally stained fibrocytes is seen. No dye cells are present in the uterine, cervical or vaginal epithelium.

In the lumen of the uterus, cervix and vagina, a small number of rounded vitally stained cells is present. They are larger than the vitally stained cells in the endometrium, and the dye granules are coarser, and they seem to be cells which have wandered from the Fallopian tube and peritoneal cavity.

Group V.

The three animals in this group were killed approximately at the twelfth day of a 16-day cycle, the previous oestrus and ovulation having occurred normally.

All show the pronounced dye appearances described for animal No. 28. The vital staining in the uterine horn is very intense, confined to the stratum compactum, and the dye cells are concentrated especially close to the uterine lumen, where the dye often appears in large masses and many of the cells appear to be bursting and shedding the dye. In the cervical and vaginal submucosa a relatively small

number of vitally stained cells is present.

Group VI.

This group consists of one animal killed approximately at the 15th day of a 17-day cycle, and three animals killed at the time of oestrus, before ovulation should have occurred.

In the first animal in this group only a small number of dye cells is seen in the uterine endometrium, and in these the nucleus is usually easily seen. They are chiefly confined to the stratum compactum and usually near the uterine lumen. Very few dye cells are present in the stratum spongiosum. A small number of vitally stained rounded cells and elongated fibrocytes is present in the vaginal submucosa. No dye cells are seen in the uterine, cervical or vaginal epithelium. A small number of rounded dye cells with coarse granules is present in the lumen of the uterus, cervix and vagina.

The other three animals each show practically identical appearances. A moderate number of dye cells is present, and chiefly confined to the stratum compactum. They are arranged in an incomplete row close to the uterine lumen, where some are intensely stained and a few appear to have degenerated. A few dye cells are present in the stratum spongiosum and contain only a small amount of dye. Some of the endometrial cells contain fine dye granules, and fine granules may also be seen in some of the epithelial cells of the uterine lumen, situated in the supranuclear part of the cells. A small number of dye cells is present in the submucosa of the cervix, and a moderate number in the vaginal submucosa. No large dye cells are seen in the uterine, cervical or vaginal epithelium. In two of these animals great numbers of rounded dye cells of varying size and with very coarse granules are present among the desquamated epithelial cells in the lumen of the cervix and vagina. No such intensely stained cells are present in the mucosa of the cervix and vagina. They are definitely the type of free dye cells found in the ovarial sac and tube whence they have probably migrated, but some of them

may have been added from the uterine endometrium.

Group VII.

The two animals in this group received the dye about the time of the previous oestrus which was missed or suppressed. Both were killed at the time of the next oestrus, one before ovulation should have occurred, and the other after the new ovulation.

Both animals show similar dye appearances. A very small number of dye cells is present in the stratum compactum. They are relatively intensely stained and occur singly. Occasionally a dye cell with a small number of dye granules is seen in the stratum spongiosum. Very few dye cells are present in the vaginal submucosa. No dye cells are seen in the cervical and vaginal epithelium. It should be noted that in the ovaries of these two animals intense vital staining is present in some of the atretic follicles, and this fact, together with the small number of relatively intensely stained cells in the endometrium, shows that dye was available should there have been more cells prepared to take it up.

These two animals have been included in Table II to show that at oestrus practically similar dye appearances are found in animals which were killed soon after the dye was injected, and in animals which received the dye at the time of the previous oestrus.

THE VITAL STAINING APPEARANCES IN THE VAGINAL SMEAR.

In the smears stained with haemalum and eosin the dye was obscured. A few of the smears were, however, stained with carbol fuchsin and in these many of the cells showed granules of dye. The dye granules are usually very fine and scattered through the cell, but sometimes they appear to be collected around vacuoles in the cell, and may be relatively coarse. Many of the cells are larger than the vitally stained cells in the uterus and some appear to contain a polymorph. In some cells no nucleus is visible, but in others the nucleus is very distinct, and is large, oval, rounded or irregular in shape, and very densely stained. At least some of these cells appear

to be similar to those in the endometrium, but especially to those found free in the lumen of the tube and ovarial sac of many of the animals. Owing to the small number of dye cells in the vaginal submucosa and their absence in the vaginal epithelium it seems most probable that the dye cells in the smear have come chiefly from the peritoneal cavity via the tube, though perhaps a few have migrated from the uterine endometrium.

APPENDIX V.

DISTRIBUTION OF FAT IN THE UTERUS DURING THE OESTROUS CYCLE: DESCRIPTION OF APPEARANCES IN THE ANIMALS SHOWN IN TABLE V.

At Heat: Both animals show similar appearances. Practically no fat, or only a very small amount, is seen in the epithelium of the uterine lumen and gland ducts. A small amount is, however, present in the epithelium of the gland fundi.

In the stratum compactum a small number of well defined "masses" of fat is visible.

About the Sixth Day of the Cycle: Both animals show comparable appearances, one containing slightly more fat than the other.

A considerable amount of fat is present in the uterine epithelium in one animal, but only a small amount in the other. The epithelium of the gland ducts shows practically no fat as in the animals killed at oestrus, but slightly more fat, irregularly distributed, is present in the gland fundi. The amount of fat in the connective tissue of the endometrium is, however, definitely increased.

Small particles of fat are clearly seen in some of the large blood vessels.

About the Midpoint of the Cycle: In one animal the appearances are practically similar to those at the sixth day of the cycle, though slightly more intense. In the other animal, however, the uterine and gland duct epithelium are loaded with fat. A lesser amount is present in the gland fundi. In the connective tissue of the

endometrium the amount of fat is practically similar in both animals to the appearances found at the sixth day of the cycle.

A considerable amount of fat irregularly distributed may be seen in the muscular coats and around the large vessels.

About the Thirteenth Day of a 17-Day Cycle: The three animals show comparable appearances. In the uterine epithelium a moderate amount of fat is present, irregularly distributed, in one animal, but only a small amount in the other two animals. A moderate amount is present in the gland fundi and a small amount in the epithelium of the gland ducts. In the connective tissue of the endometrium a slightly greater amount of fat is observed than in any of the previous animals.

At the Fifteenth Day of a 17-Day Cycle: In this animal the appearances are definitely less marked than at the thirteenth day of the cycle, and resemble the appearances in the animals killed at the time of "heat". A very small amount of fat is present irregularly scattered in the uterine epithelium. Practically no fat is seen in the epithelium of the gland ducts, and in the epithelium of the gland fundi a small amount is present only in some glands.

In the connective tissue of the endometrium only a small amount of fat is observed.

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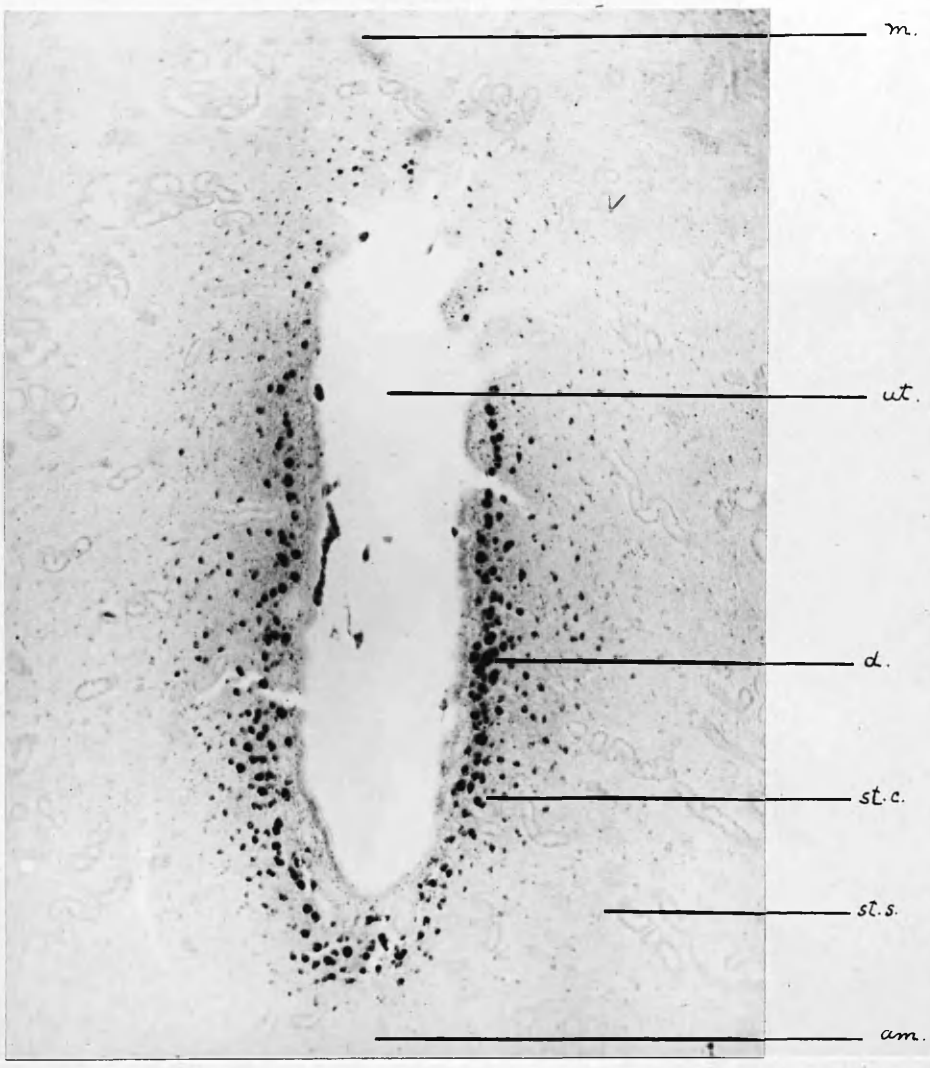


Fig. 1. Transverse section of uterine horn of virgin guinea-pig No. 28 (Table II) killed at 12th day of 16-day cycle. Shows intense vital staining practically confined to stratum compactum, and increased around antimesometrial half of uterine lumen. Intravitally stained with trypan blue.

am. Antimesometrial side of uterus.
d. Dye.
m. Mesometrial side of uterus.
st.c. Stratum compactum.
st.s. Stratum spongiosum.
ut. Uterine lumen.

Photomicrograph. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. X 75

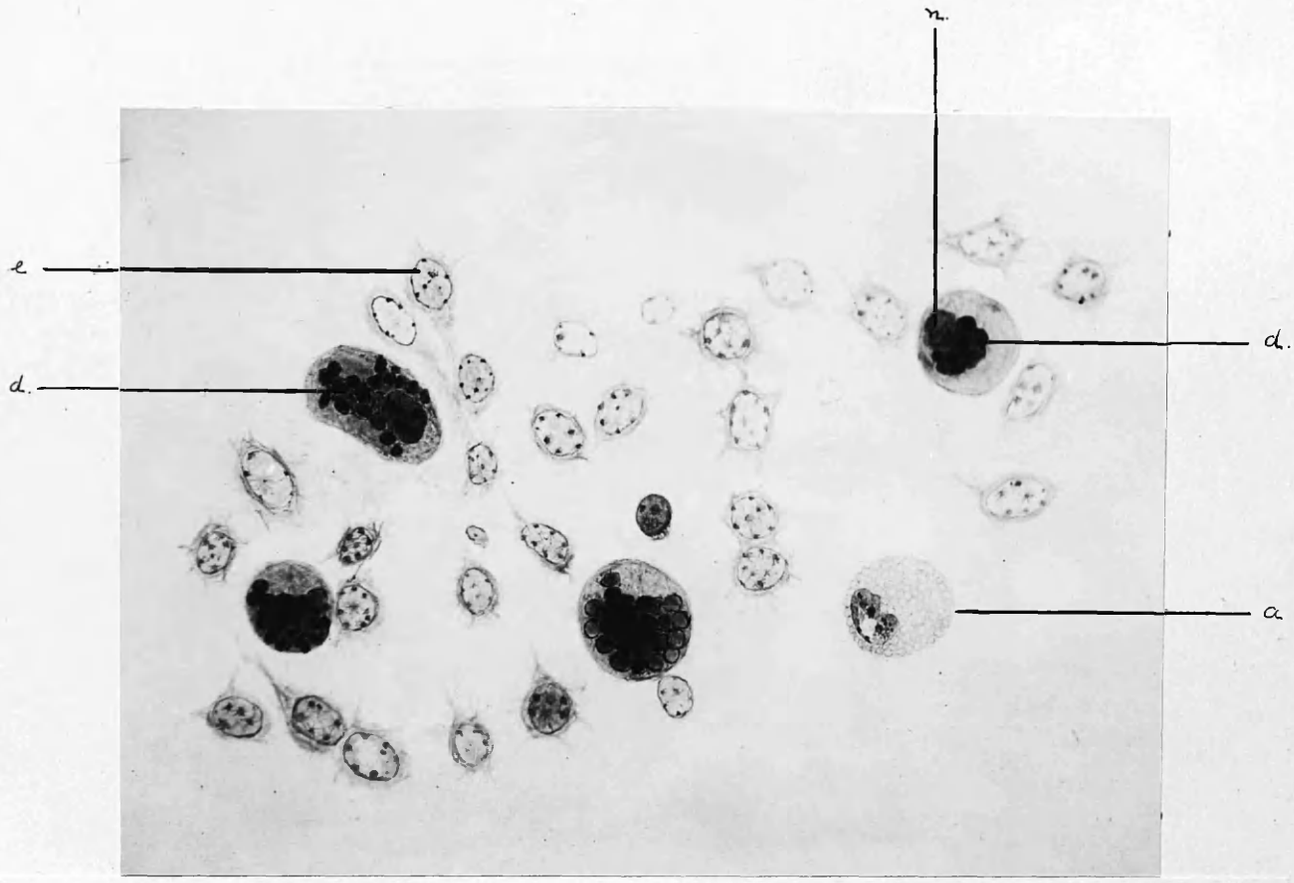


Fig. 2. A group of rounded vitally stained cells at the junction of stratum compactum and stratum spongiosum of uterine horn. From same guinea-pig (No. 28) as Fig.1 - killed at 12th day of 16-day cycle. Intravitally stained with trypan blue.

a. Rounded cell without dye, but which appears to correspond to the cells which take up the dye.
d. Dye granules.
e. Nucleus of endometrial cell.
n. Nucleus.

Drawing by Professor Bryce. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x 1200.

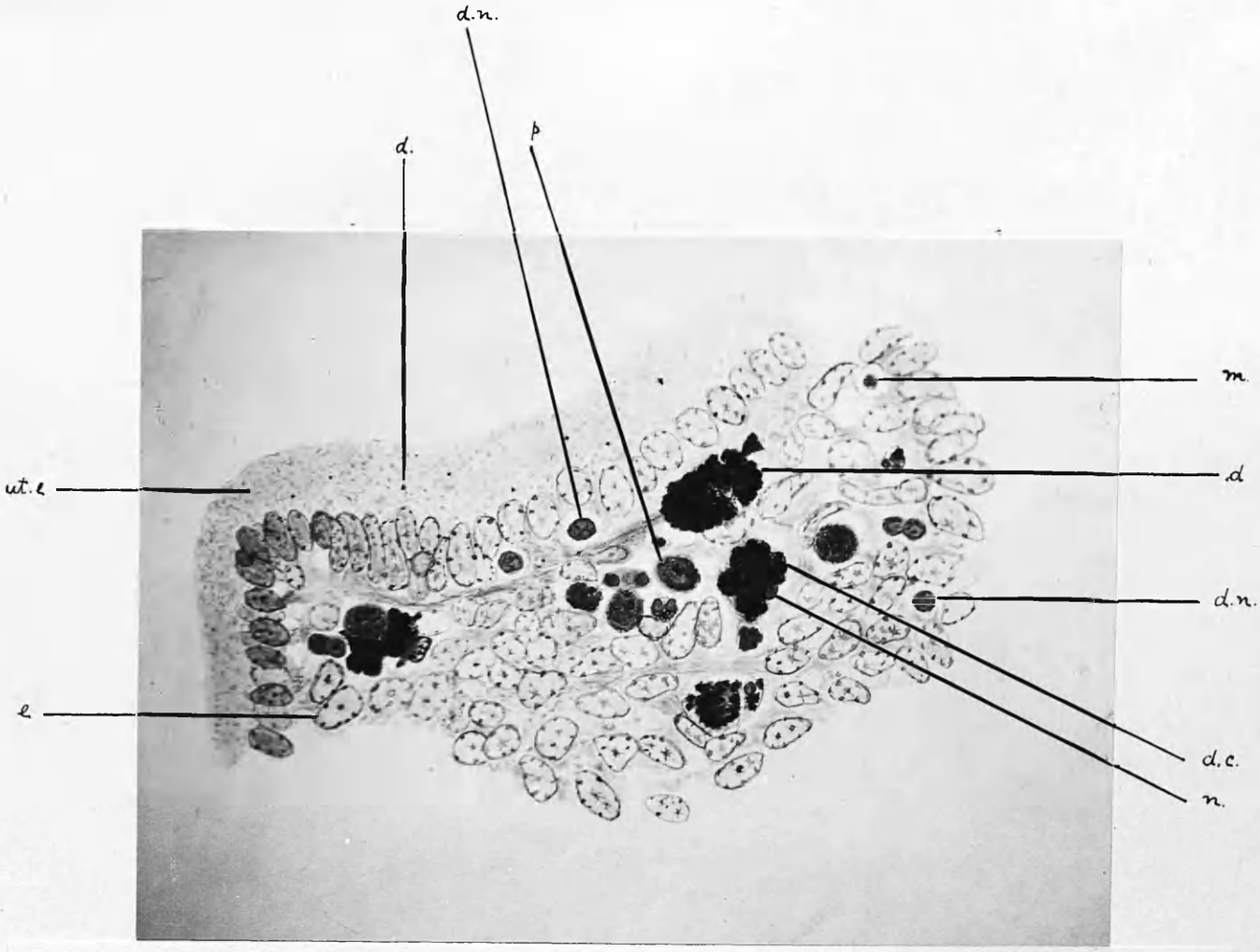


Fig. 3. Part of stratum compactum of uterine horn from same guinea-pig as Figs. 1 and 2 - killed at 12th day of 16-day cycle. Illustrates the large dye-containing cells close to the uterine epithelium. The dye granules form large "dye masses" in which the nucleus is practically obscured. The part illustrated shows moderately intense vital staining compared with other parts of the stratum compactum, but was chosen because of the variety of cells seen.

d. Dye.
d.c. Large dye-containing cell.
d.n. Densely stained nucleus without related dye, and apparently similar to nuclei of dye-containing cells.
e. Nucleus of endometrial cell.
m. Very small mononuclear.
n. Nucleus.
p. Vitally stained polymorph.
ut.e. Epithelium of uterine lumen.

Drawing by Professor Bryce. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x 1200.

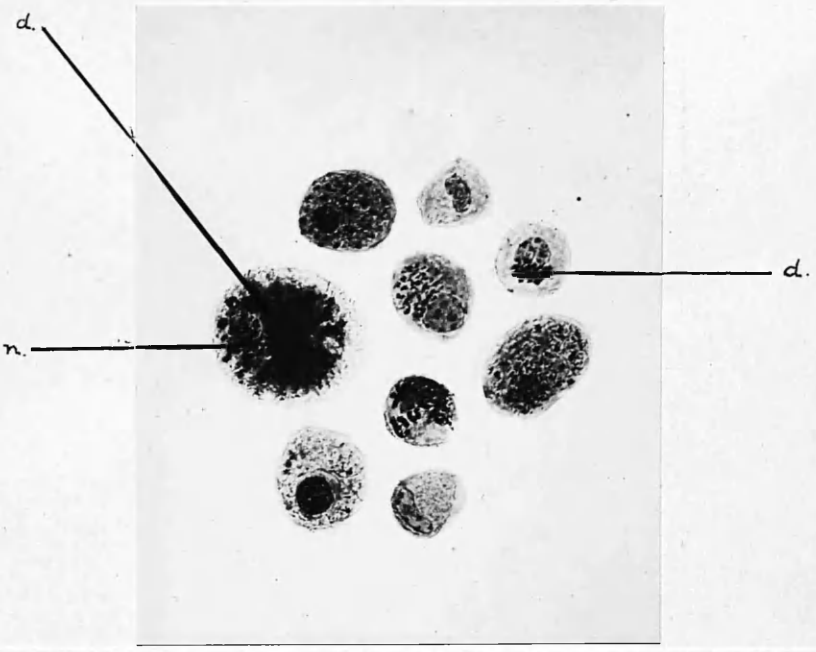


Fig. 4. A group of dye-containing cells free in the lumen of the Fallopian tube. The cells vary in size, and the dye granules may be scattered throughout the cytoplasm, or concentrated in one part of it. Two smaller cells without dye are also shown. From same animal as previous Figs.

d. Dye.
n. Nucleus.

Drawing by Professor Bryce. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x 1200.

FIG. 5.

Series of diagrams made with the projection apparatus illustrating the distribution and intensity of the vital staining in the endometrium of the uterine horns during the oestrous cycle. The diagrams demonstrate the appearances in the groups of animals shown in Table II. The sizes of the vitally stained cells are slightly exaggerated. x circa 40.

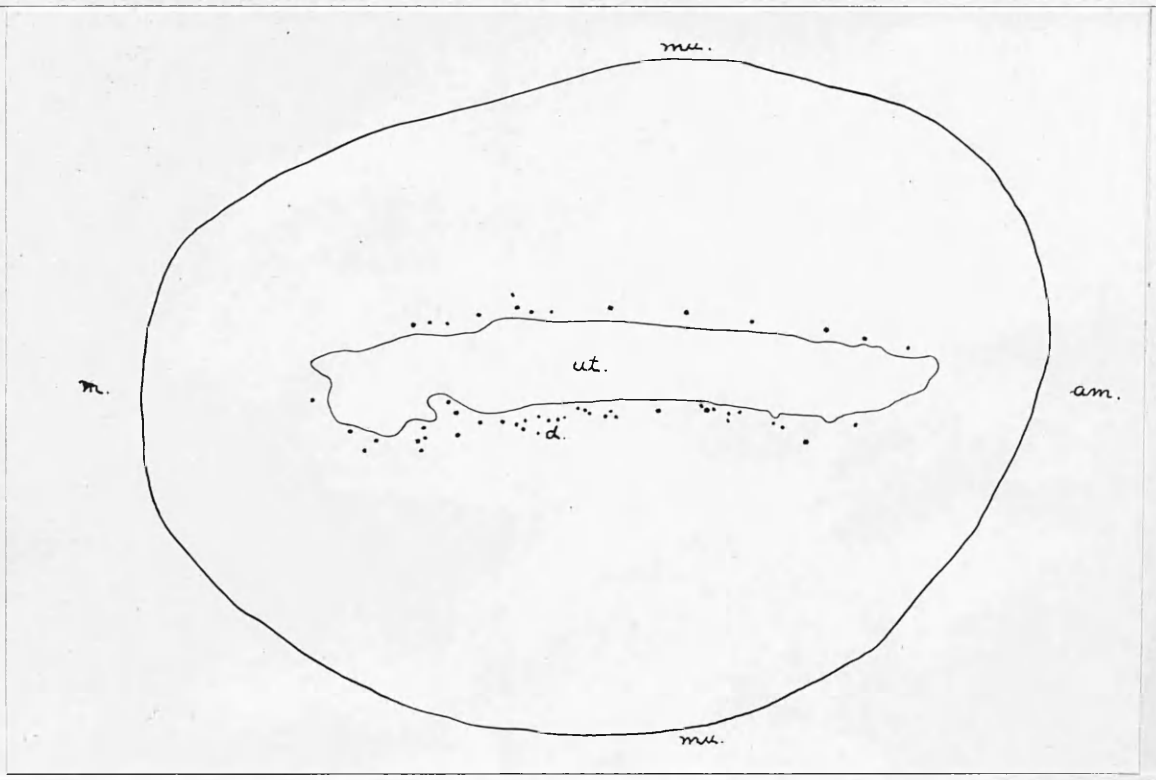


Fig. 5.a. Transverse section of uterine horn of animal No. 32 (Group I, Table II) killed at oestrus. A relatively small number of dye-containing cells is present. The vital staining is not intense, and the dye-containing cells are confined to the stratum compactum.

am. Antimesometrial side of uterus. mu. Muscularis mucosae.
m. Mesometrial side of uterus. ut. Uterine lumen. d. Dye.

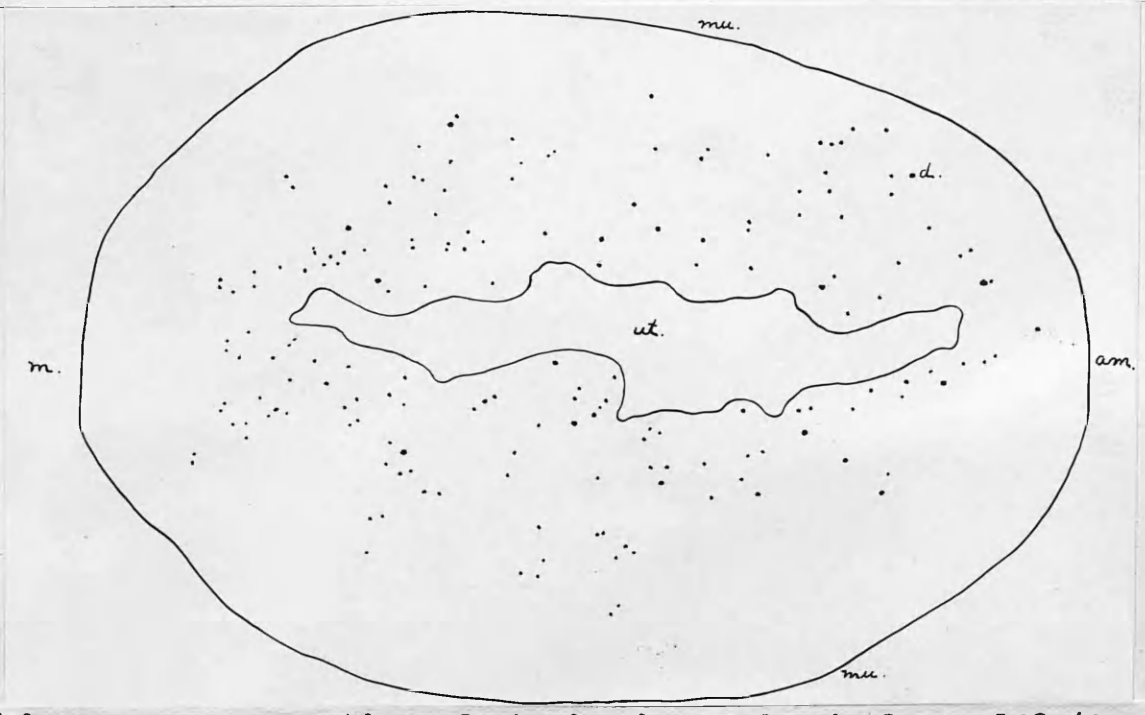


Fig. 5.b. Transverse section of uterine horn of animal No. 109 (Group I, Table II) killed at oestrus. The dye-containing cells are more numerous than in previous animal No. 32 (Fig. 5.a), and are scattered loosely through both stratum compactum and stratum spongiosum. The vital staining is slightly more intense.

For abbreviations see Fig. 5.a.

FIG. 5.

Series of diagrams made with the projection apparatus illustrating the distribution and intensity of the vital staining in the endometrium of the uterine horns during the oestrous cycle. The diagrams demonstrate the appearances in the groups of animals shown in Table II. The sizes of the vitally stained cells are slightly exaggerated. x circa 40.

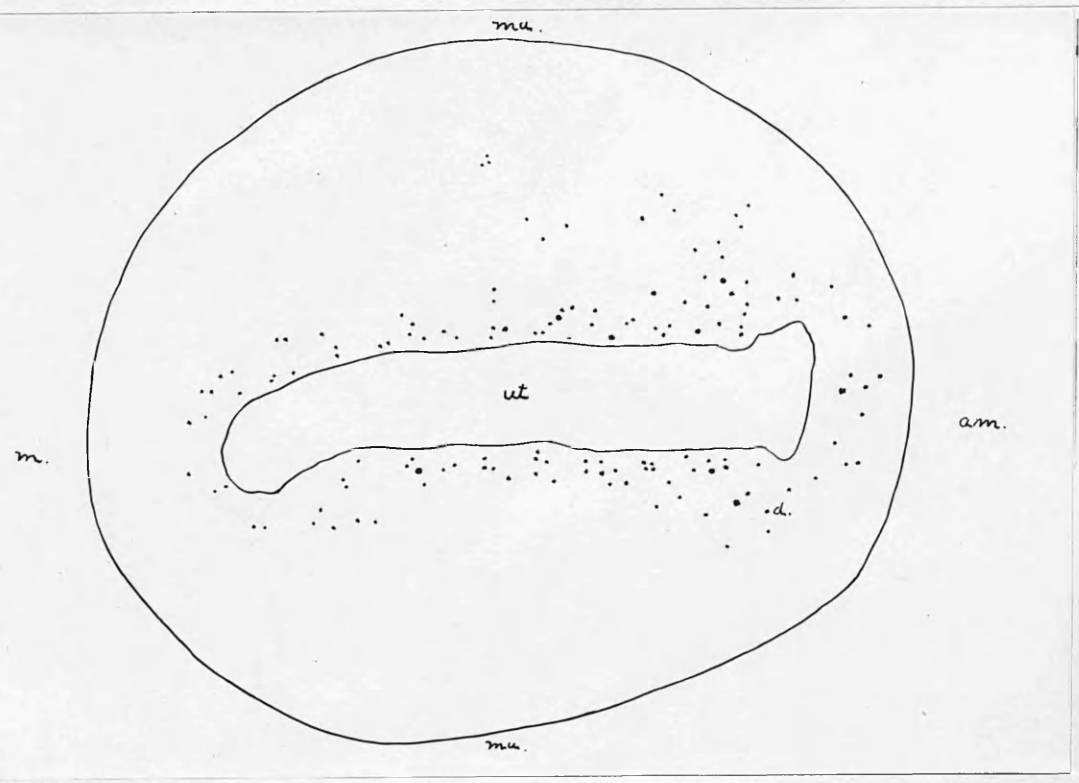


Fig. 5.c. Transverse section of uterine horn of animal No. 42 (Group II Table II) killed at 3rd day of cycle. The new ovulation has failed to occur. The dye-containing cells are chiefly confined to the stratum compactum, but the staining is not intense. (The uterine and vaginal appearances suggest that the oestrous changes had been prolonged).

am. Antimesometrial side of uterus. mu. Muscularis mucosae.
m. Mesometrial side of uterus. ut. Uterine lumen. d. Dye.

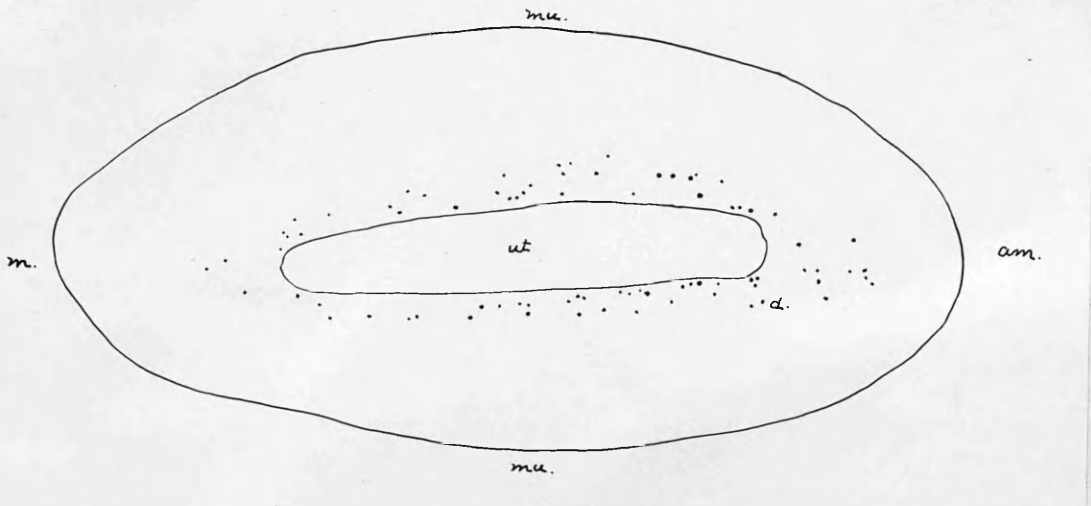


Fig. 5.d. Transverse section of uterine horn of animal No. 22 (Group III, Table II) killed at 7th day of cycle. A relatively small number of vitally stained cells is present, practically confined to the stratum compactum. The vital staining is small in amount.

For abbreviations see Fig. 5.c.

FIG. 5.

Series of diagrams made with the projection apparatus illustrating the distribution and intensity of the vital staining in the endometrium of the uterine horns during the oestrous cycle. The diagrams demonstrate the appearances in the groups of animals shown in Table II. The sizes of the vitally stained cells are slightly exaggerated. x circa 40.

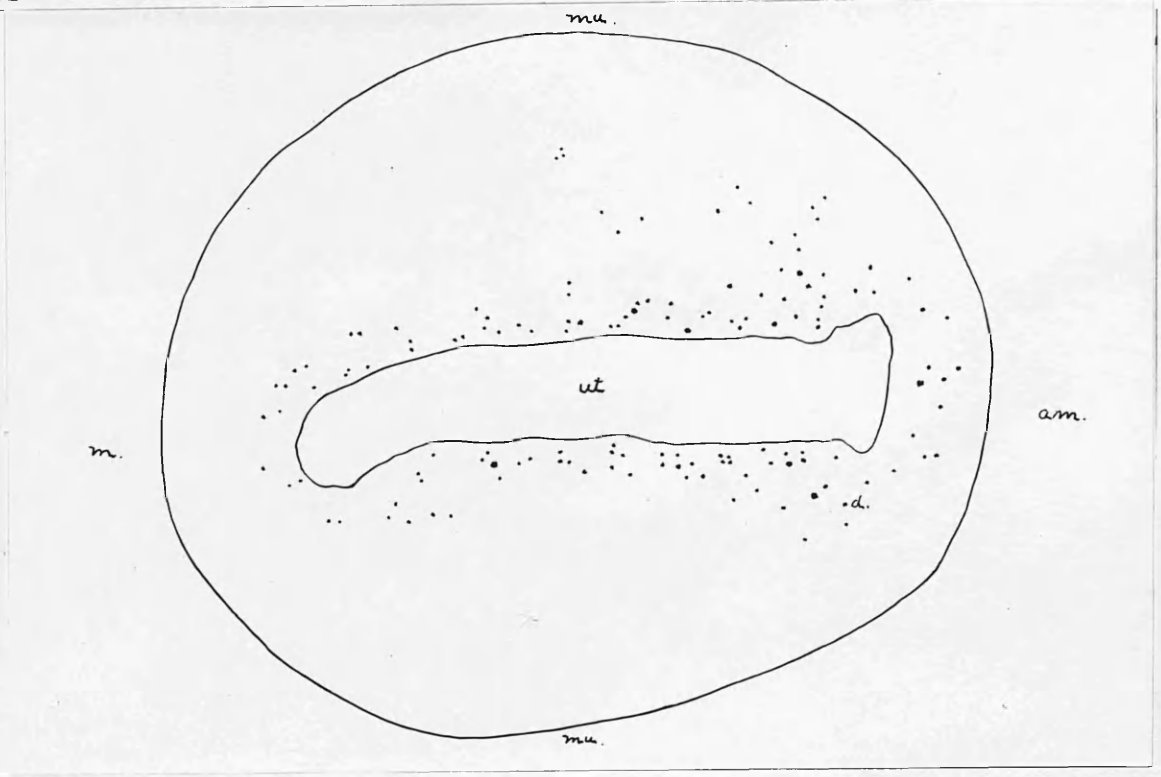


Fig. 5.c. Transverse section of uterine horn of animal No. 42 (Group II Table II) killed at 3rd day of cycle. The new ovulation has failed to occur. The dye-containing cells are chiefly confined to the stratum compactum, but the staining is not intense. (The uterine and vaginal appearances suggest that the oestrous changes had been prolonged).

am. Antimesometrial side of uterus. mu. Muscularis mucosae.
m. Mesometrial side of uterus. ut. Uterine lumen. d. Dye.

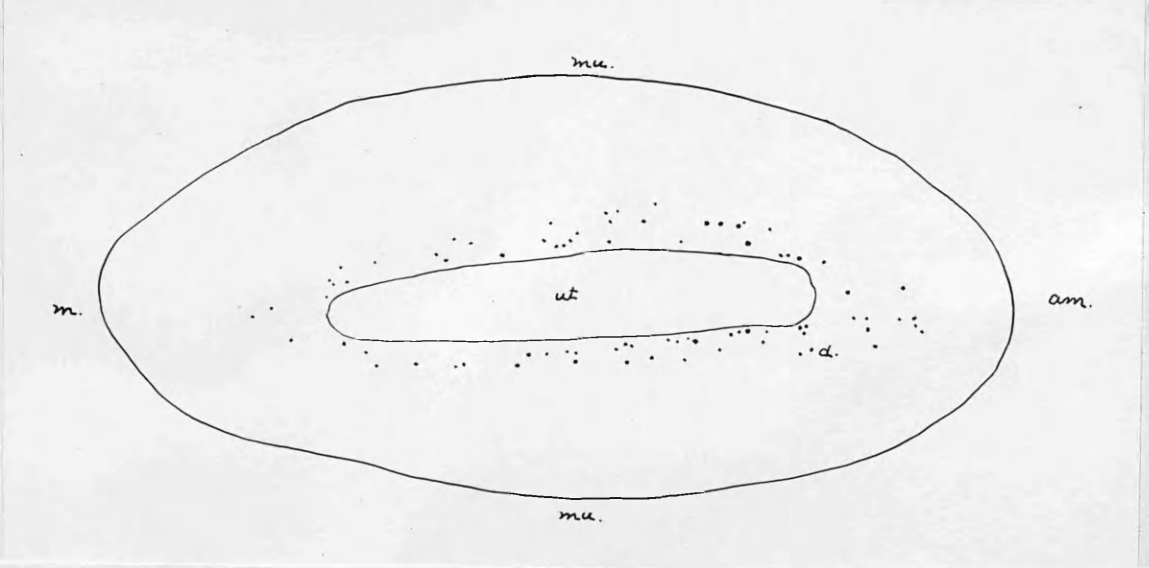


Fig. 5.d. Transverse section of uterine horn of animal No. 22 (Group III, Table II) killed at 7th day of cycle. A relatively small number of vitally stained cells is present, practically confined to the stratum compactum. The vital staining is small in amount.

For abbreviations see Fig. 5.c.

FIG. 5.

Series of diagrams made with the projection apparatus illustrating the distribution and intensity of the vital staining in the endometrium of the uterine horns during the oestrous cycle. The diagrams demonstrate the appearances in the groups of animals shown in Table II. The sizes of the vitally stained cells are slightly exaggerated. x circa 40.

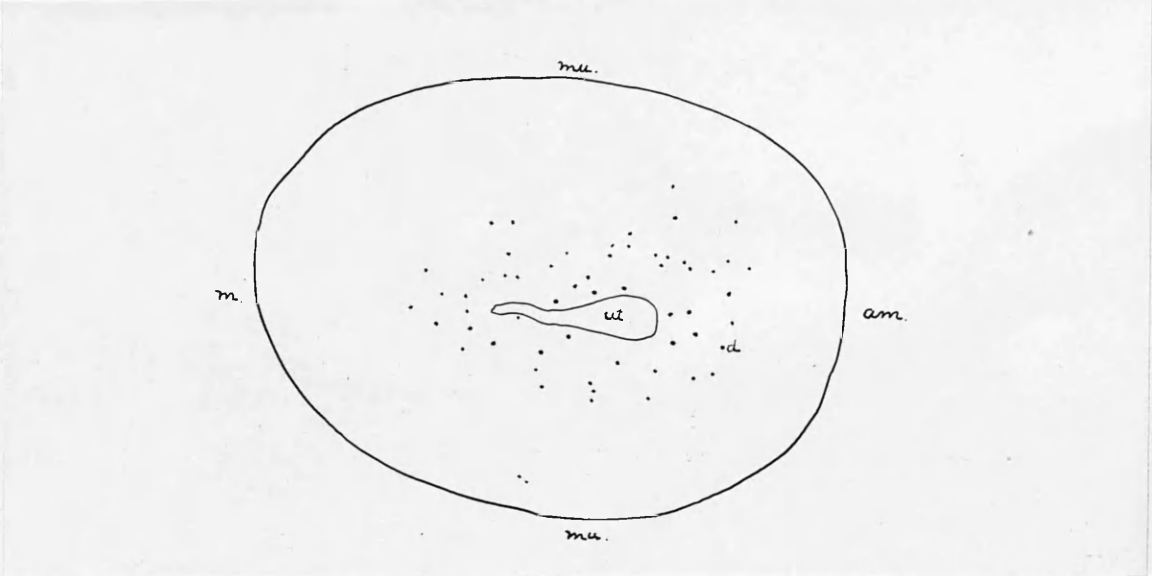


Fig. 5.e. Transverse section of uterine horn of animal No. 43 (Group IV, Table II) killed about midway in the cycle. A relatively small number of dye-containing cells is present. The cells are scattered loosely in the stratum compactum and extend into stratum spongiosum. The vital staining is small in amount.

am. Antimesometrial side of uterus. mu. Muscularis mucosae.
m. Mesometrial side of uterus. ut. Uterine lumen. d. Dye.

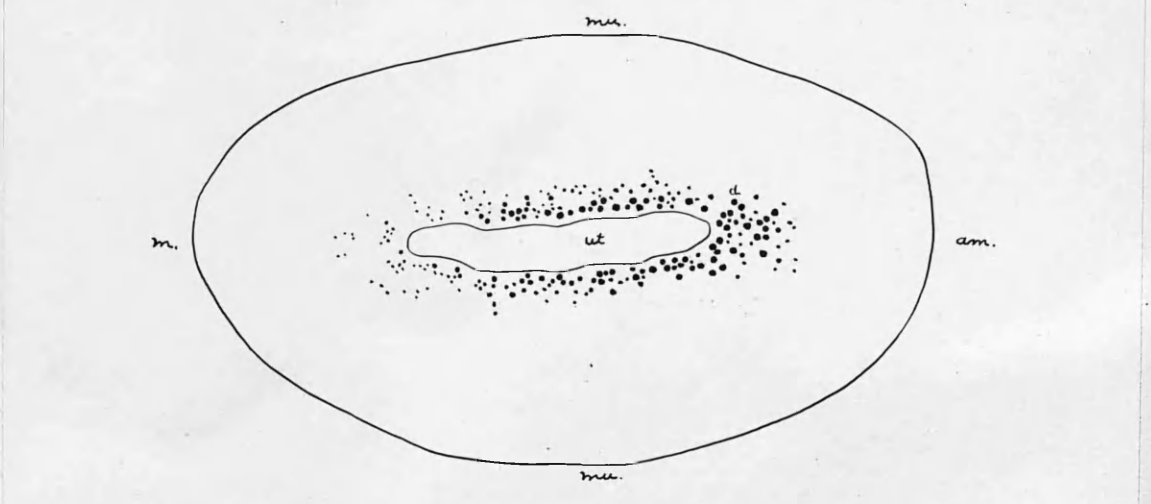


Fig. 5.f. Transverse section of uterine horn of animal No. 28 (Group V, Table II) killed at 12th day of 16-day cycle. A large number of intensely stained cells is present, especially concentrated close to the uterine lumen, and practically confined to the stratum compactum. The vital staining is more pronounced around the antimesometrial half of the uterine lumen.

For abbreviations see Fig. 5.e.

FIG. 5.

Series of diagrams made with the projection apparatus illustrating the distribution and intensity of the vital staining in the endometrium of the uterine horns during the oestrous cycle. The diagrams demonstrate the appearances in the groups of animals shown in Table II. The sizes of the vitally stained cells are slightly exaggerated. x circa 40.

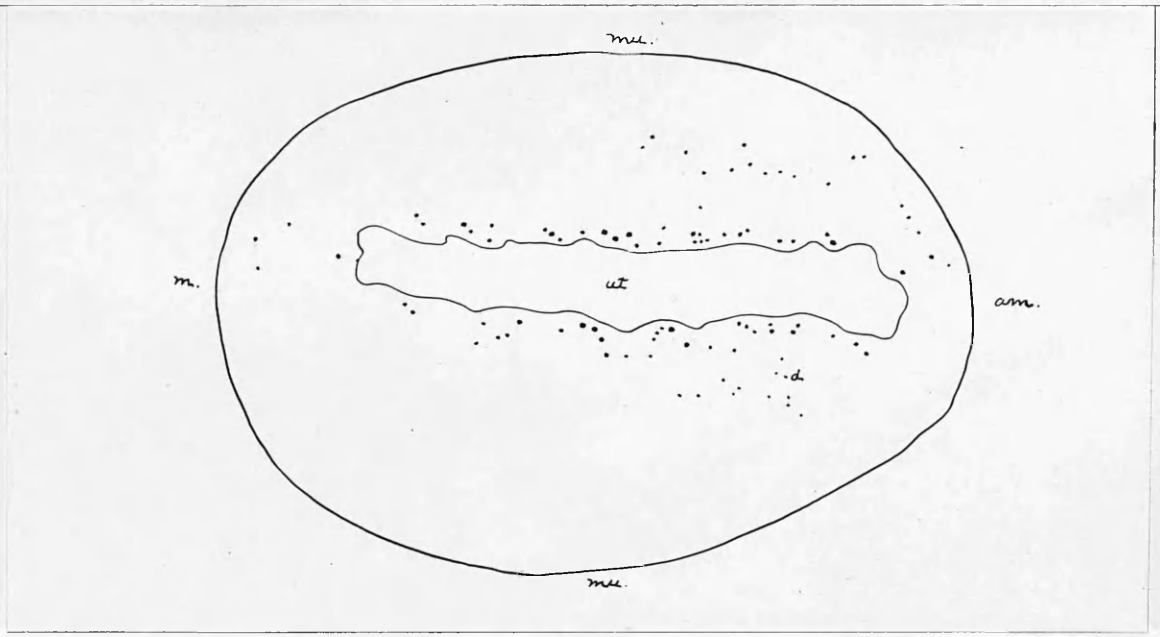


Fig. 5.g. Transverse section of uterine horn of animal No. 24 (Group VI, Table II) killed at oestrus. The dye-containing cells are fewer in number than at 12th day of cycle (Fig. 5.f), and are more loosely arranged. The vital staining is also less intense.

am. Antimesometrial side of uterus. mu. Muscularis mucosae.
m. Mesometrial side of uterus. ut. Uterine lumen. d. Dye.

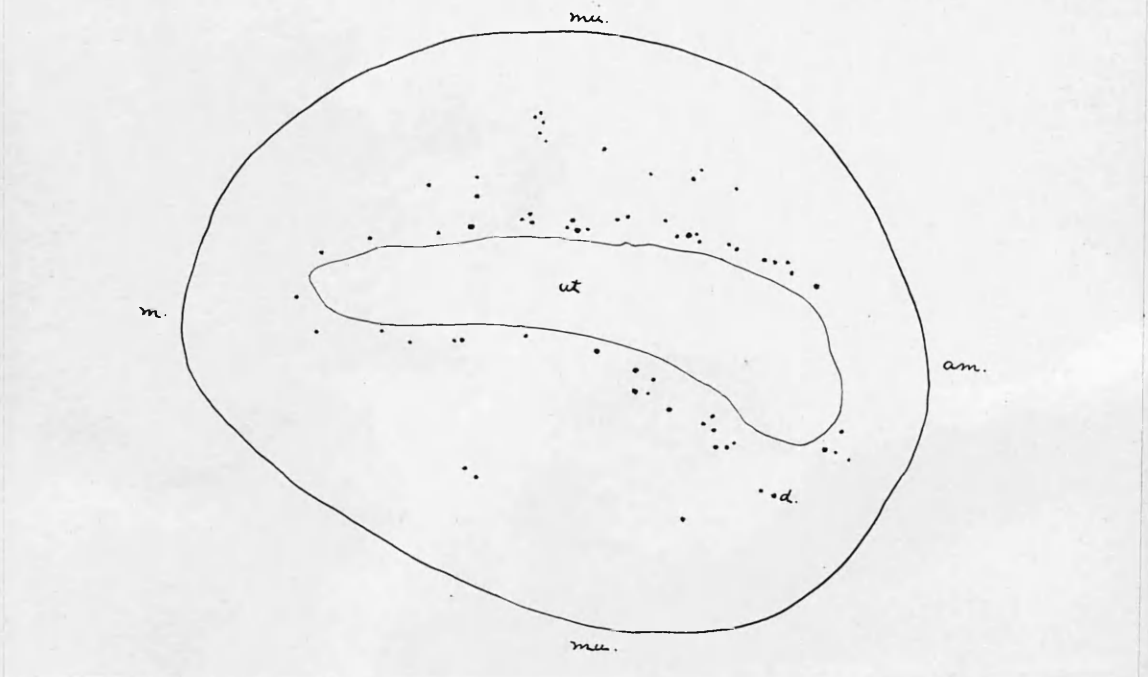


Fig. 5.h. Transverse section of uterine horn of animal No. 51 (Group VII, Table II) killed at oestrus after new ovulation has occurred. The dye was injected at the time of the previous oestrus, which was suppressed. A relatively small number of dye-containing cells is present, and the appearances compare with those of the other animals killed at oestrus.
For abbreviations see Fig. 5.g.

FIG. 6.

Series of diagrams made with the projection apparatus illustrating the distribution and amount of fat in the connective tissue of the endometrium of the uterine horns during the oestrous cycle. The diagrams demonstrate the appearances in the animals shown in Table V. The sizes of the "fat masses" are slightly exaggerated. x circa 40.

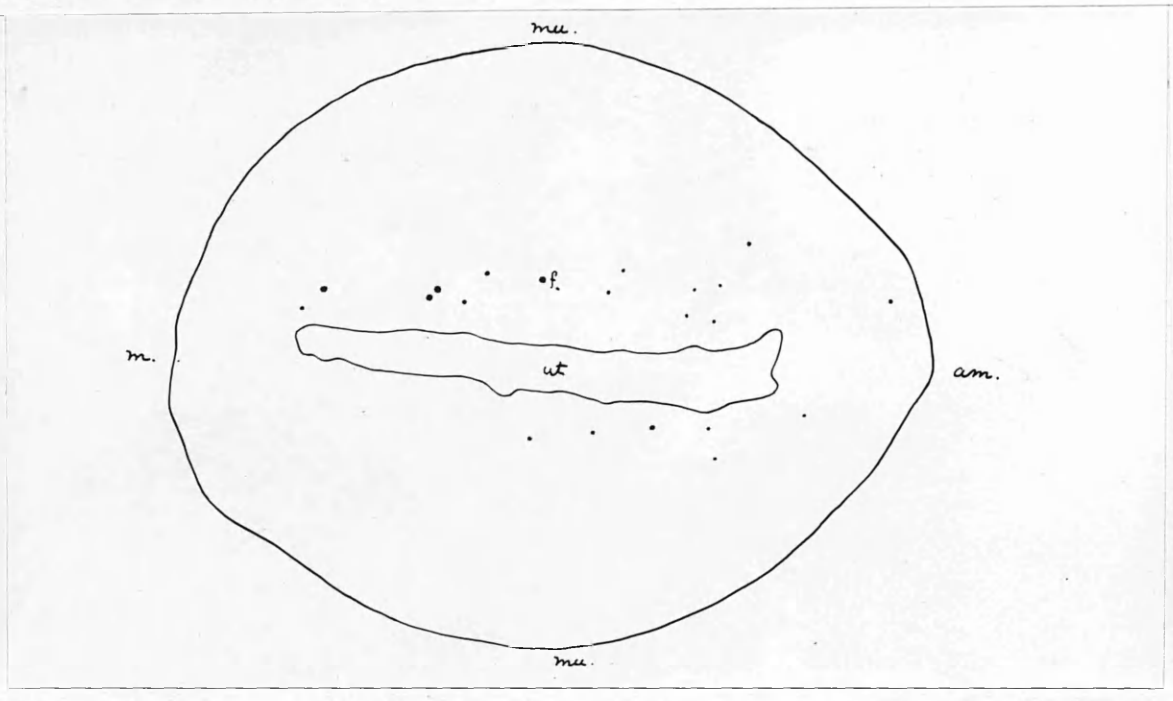


Fig. 6.a. Transverse section of uterine horn of animal No. 67 (Table V) killed at oestrus. A relatively small amount of fat is present in the connective tissue of the endometrium.

am. Antimesometrial side of uterus. mu. Muscularis mucosae.
m. Mesometrial side of uterus. ut. Uterine lumen. f. Fat.

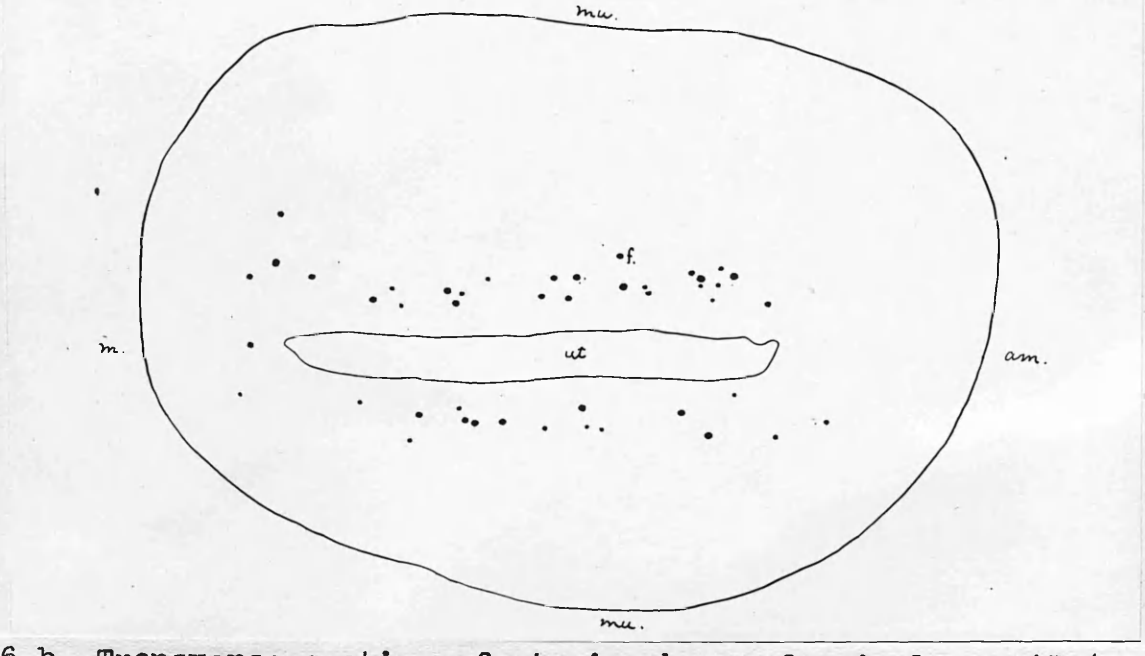


Fig. 6.b. Transverse section of uterine horn of animal No. 45 (Table V) killed at 6th day of 17-day cycle. A greater amount of fat than at oestrus (Fig. 6.a) is now present in the connective tissue of the endometrium.

For abbreviations see Fig. 6.a.

FIG. 6.

Series of diagrams made with the projection apparatus illustrating the distribution and amount of fat in the connective tissue of the endometrium of the uterine horns during the oestrous cycle..The diagrams demonstrate the appearances in the animals shown in Table V. The sizes of the "fat masses" are slightly exaggerated. x circa 40.

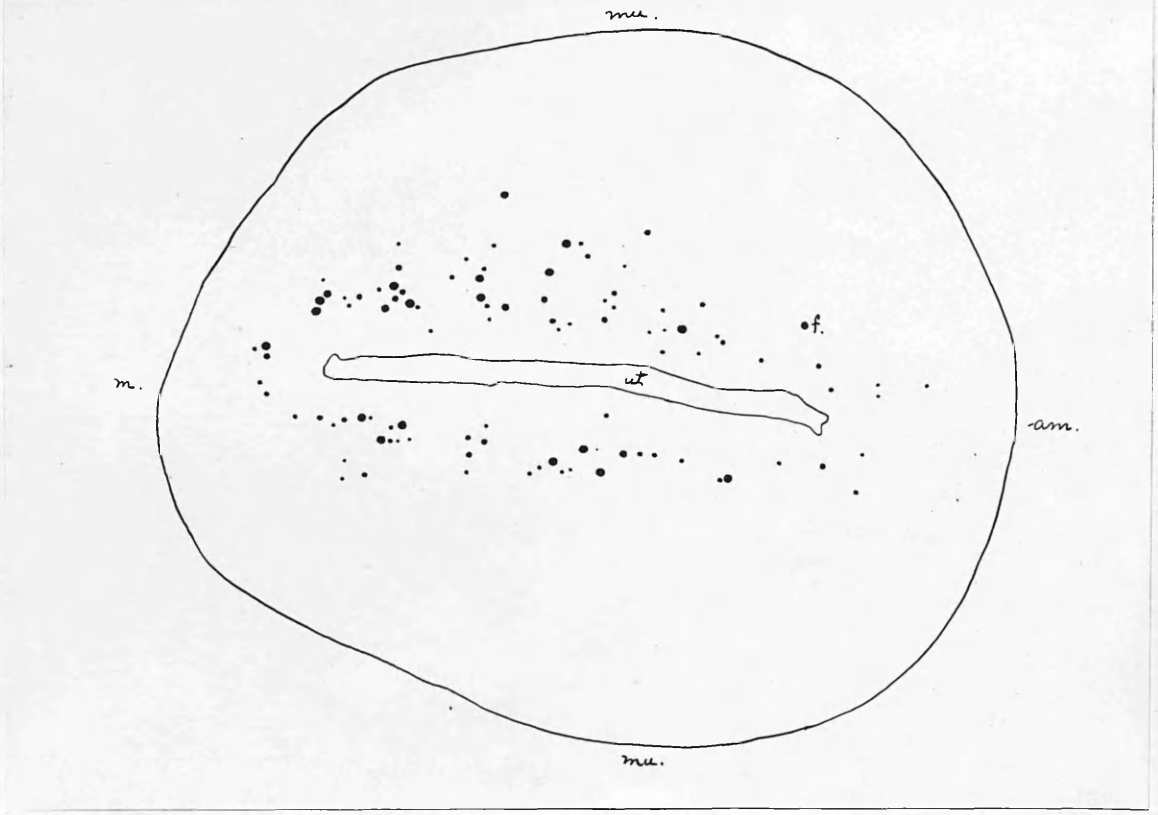


Fig. 6.c. Transverse section of uterine horn of animal No. 54 (Table V) killed at 6th day of 17-day cycle. The increase in the amount of fat in the connective tissue of the endometrium is still more apparent (cf. Fig. 6.a.).

am. Antimesometrial side of uterus. mu. Muscularis mucosae.
m. Mesometrial side of uterus. ut. Uterine lumen. f. Fat.

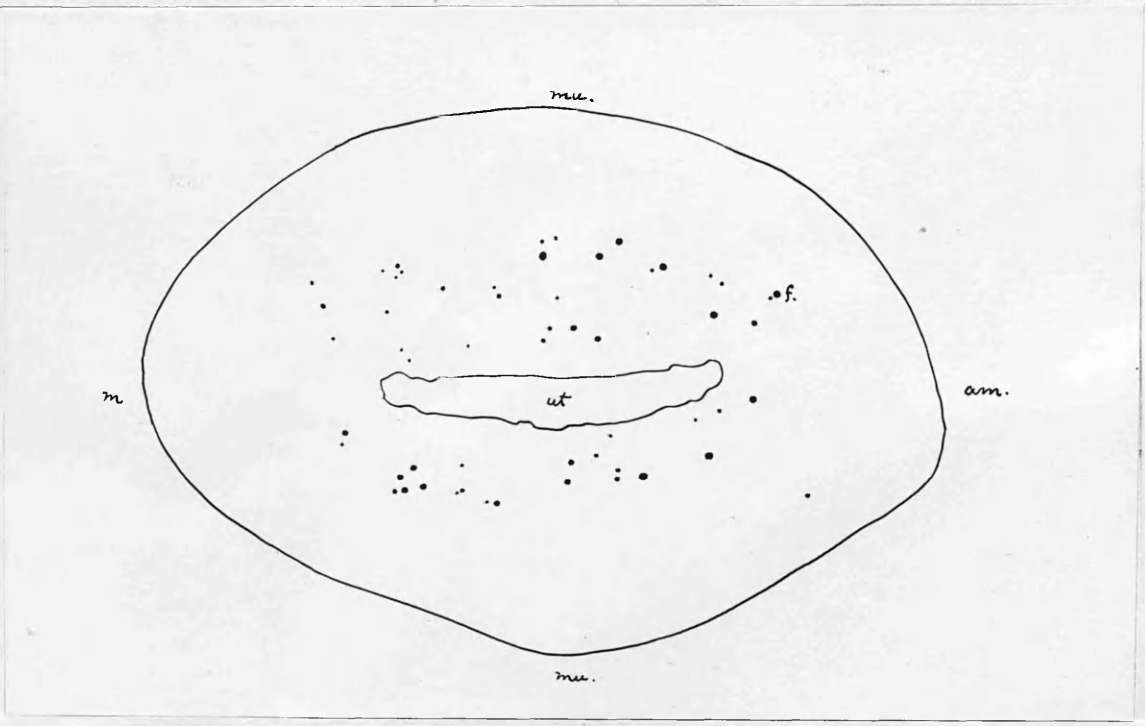


Fig. 6.d. Transverse section of uterine horn of animal No. 53 (Table V) killed at 9th day of 17-day cycle. The increase in the amount of fat in the connective tissue of the endometrium is more or less maintained.

For abbreviations see Fig. 6.c.

FIG. 6.

Series of diagrams made with the projection apparatus illustrating the distribution and amount of fat in the connective tissue of the endometrium of the uterine horns during the oestrous cycle. The diagrams demonstrate the appearances in the animals shown in Table V. The sizes of the "fat masses" are slightly exaggerated. x circa 40.

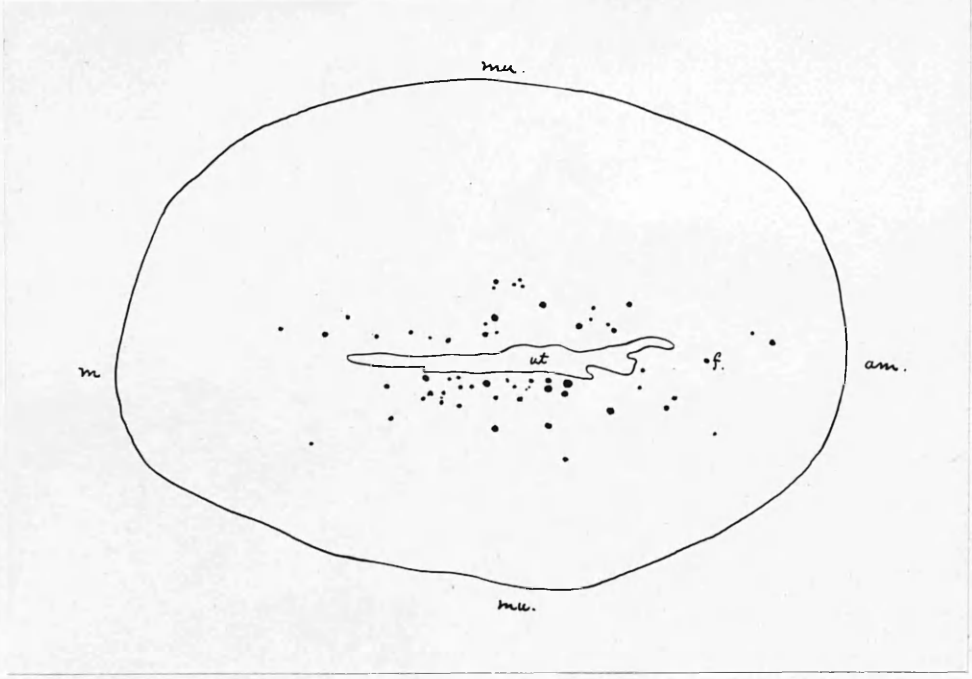


Fig. 6.e. Transverse section of uterine horn of animal No. 65 (Table V) killed at 9th day of 17-day cycle. A considerable amount of fat is still present in the connective tissue of the endometrium.

am. Antimesometrial side of uterus. mu. Muscularis mucosae.
m. Mesometrial side of uterus. ut. Uterine lumen. f. Fat

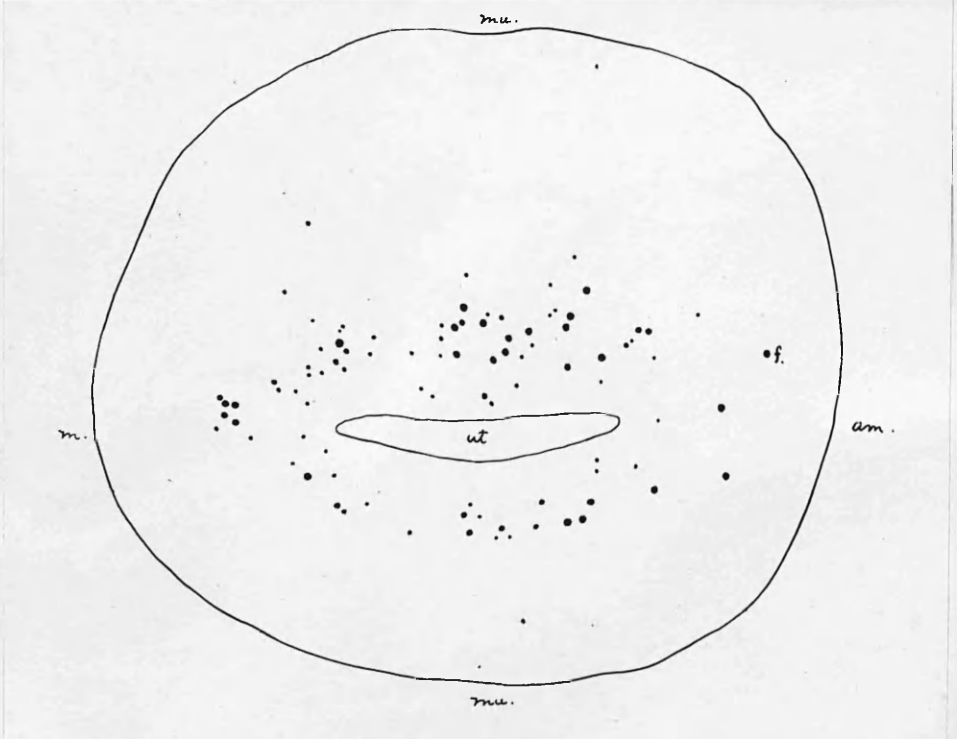


Fig. 6.f. Transverse section of uterine horn of animal No. 55 (Table V) killed at 13th day of 17-day cycle. A great amount of fat is present in the connective tissue of the endometrium. For abbreviations see Fig. 6.e.

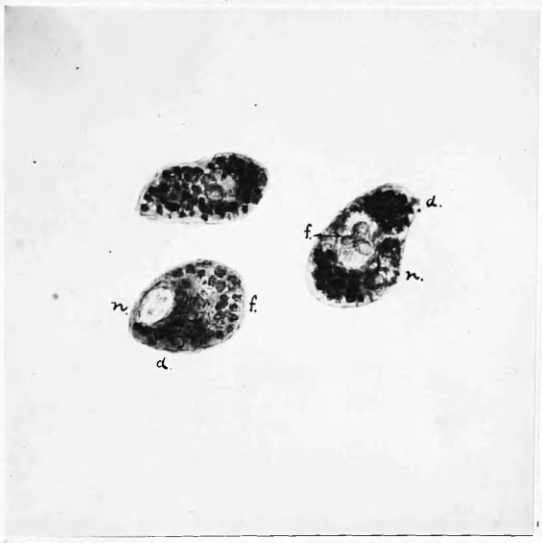


Fig. 7. A group of vitally stained cells containing particles of fat. From animal No. 47 (Table V). Killed at 13th day of 17-day cycle. The cells were situated in the stratum compactum of the uterine horn, near the antimesometrial side of the uterine lumen.

d. Dye. f. Fat. n. Nucleus.

Drawing by Professor Bryce. Fixation Formalin and Corrosive sublimate. Stained Grubler's Sudan III. x 1200.

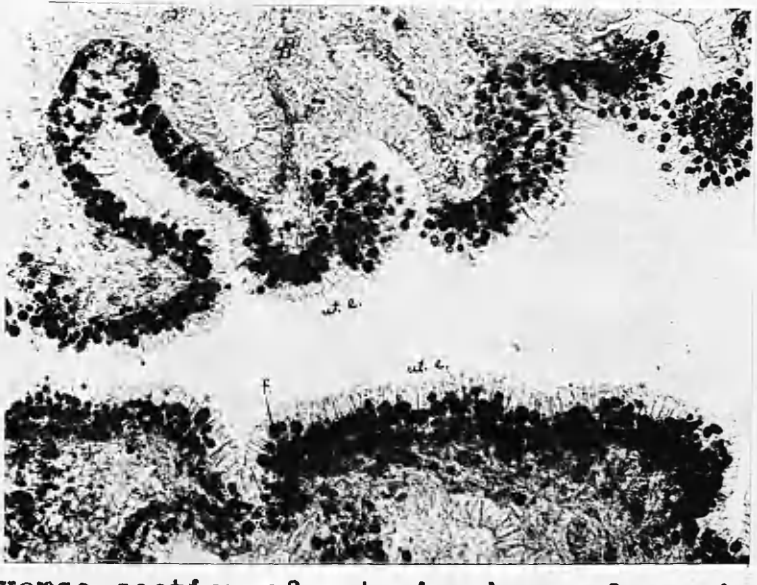


Fig. 8. Transverse section of uterine horn of ovariectomised animal No. 57 (Table IX). Animal received corpus luteum extract - uterus previously sensitised by small doses of oestrin. Demonstrates abundant coarse fat in epithelium of uterine lumen.

f. Fat. ut.e. Epithelium of uterine lumen.

Photomicrograph. Fixed in 10% Formalin. Stained Grübler's Sudan III. x 240.

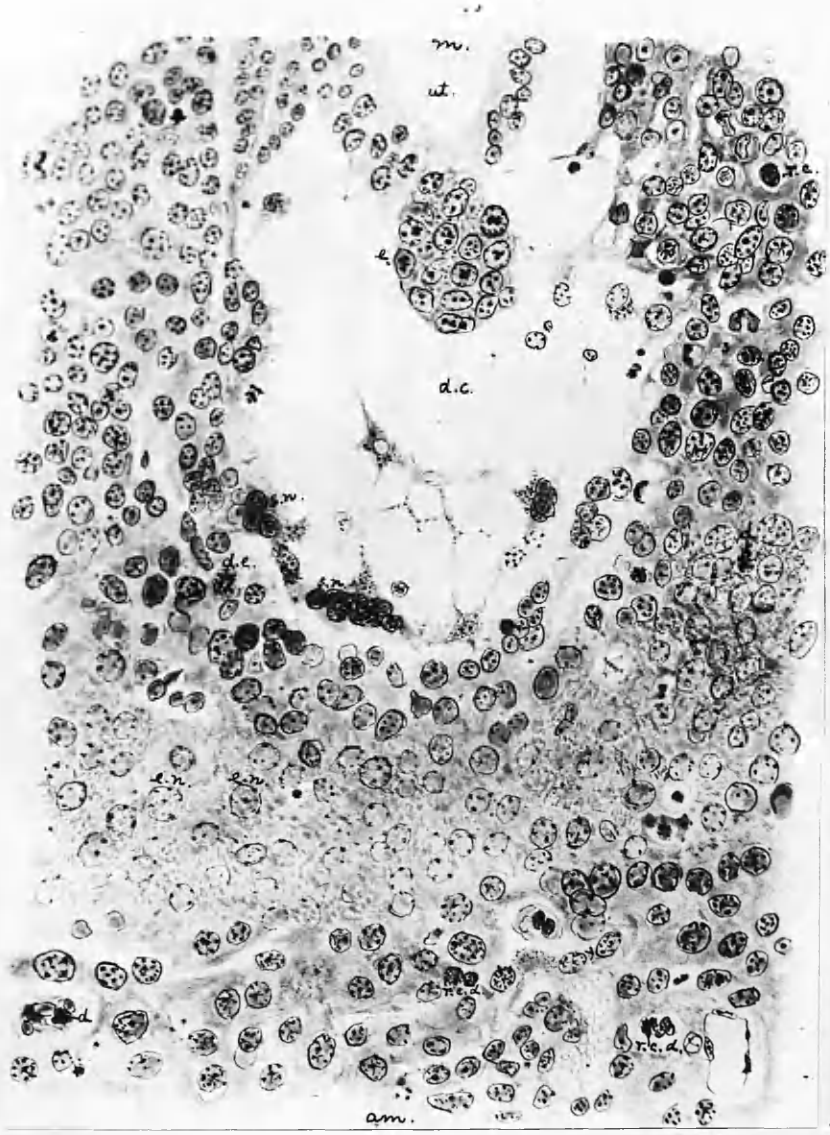


Fig. 9. Transverse section of uterine horn of pregnant animal No. 261. Intravitally stained with trypan blue. Shows embryo aged 6 days 9 hours just implanted.

am.	Antimesometrial side of uterus.
d.	Dye.
d.c.	Decidual cavity.
d.e.	Dye granules in large endometrial cell.
e.	Embryo.
e.n.	Large pale endometrial nuclei of compact zone.
m.	Mesometrial side of uterus.
r.c.	Rounded cell with dense nucleus and no dye - as in the virgin uterus.
r.c.d.	Rounded dye-containing cell with dense nucleus similar to vitally stained cells in virgin uterus.
s.n.	Intensely stained smaller nuclei on inner wall of decidual cavity.
ut.	Uterine lumen.

Drawing by Professor Bryce. Fixation Zenker . Stained Carbol-Fuchsin. x 580.

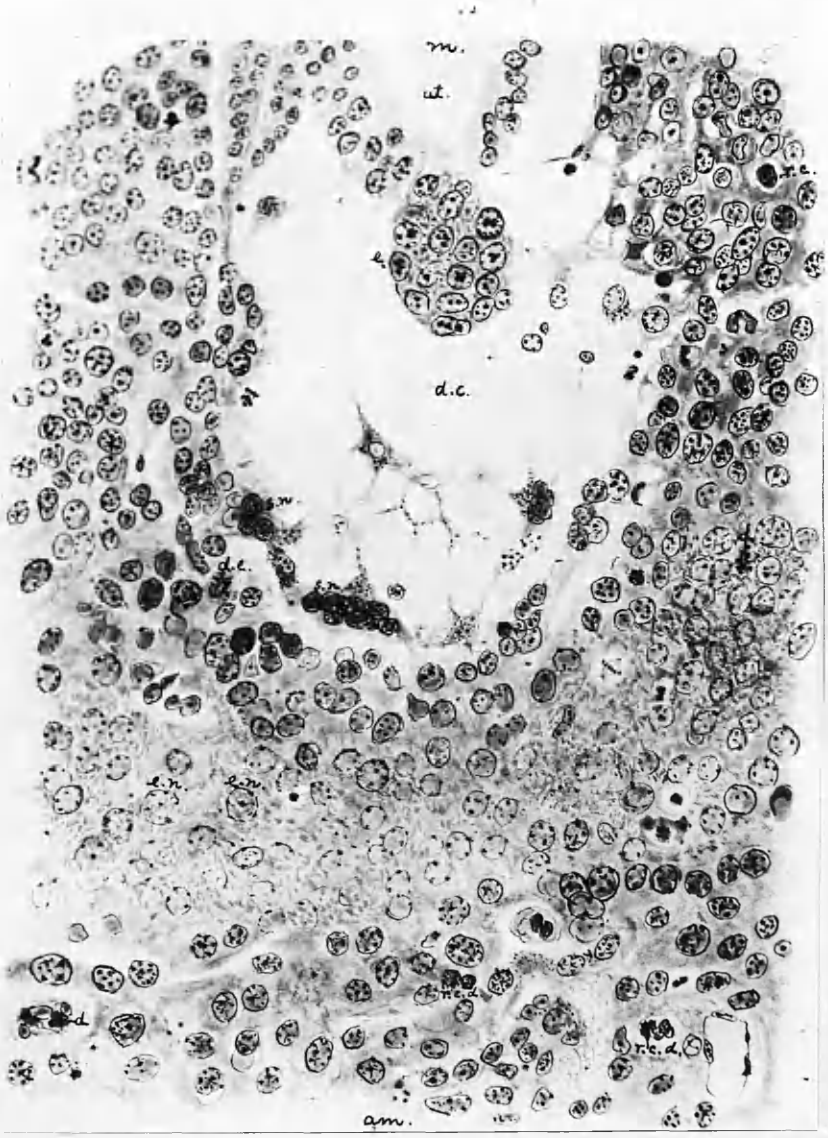


Fig. 9. Transverse section of uterine horn of pregnant animal No. 261. Intravitally stained with trypan blue. Shows embryo aged 6 days 9 hours just implanted.

am.	Antimesometrial side of uterus.
d.	Dye.
d.c.	Decidual cavity.
d.e.	Dye granules in large endometrial cell.
e.	Embryo.
e.n.	Large pale endometrial nuclei of compact zone.
m.	Mesometrial side of uterus.
r.c.	Rounded cell with dense nucleus and no dye - as in the virgin uterus.
r.c.d.	Rounded dye-containing cell with dense nucleus similar to vitally stained cells in virgin uterus.
s.n.	Intensely stained smaller nuclei on inner wall of decidual cavity.
ut.	Uterine lumen.

Drawing by Professor Bryce. Fixation Zenker . Stained Carbol-Fuchsin. x 580.

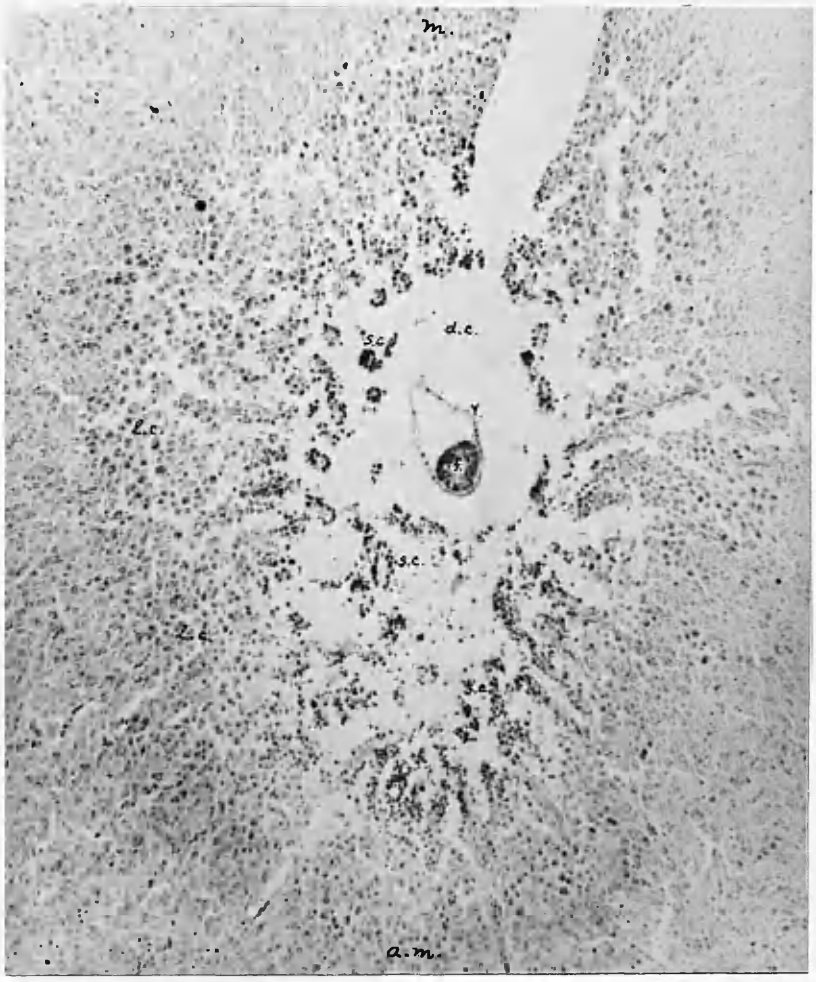
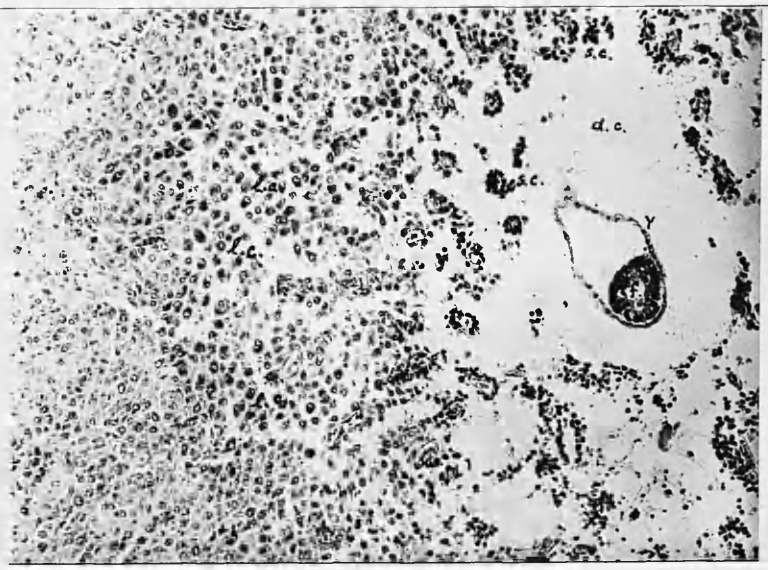


Fig. 10. Transverse section of uterine horn of pregnant animal No. 158. Intravitally stained with trypan blue. Shows embryo aged $9\frac{1}{2}$ days.

a.m. Antimesometrial side of uterus.
d.c. Decidual cavity.
f. Formative knob.
l.c. Large endometrial cells forming main part of wall of decidual cavity - and showing intense vital staining.
m. Mesometrial side of uterus.
s.c. Intensely stained small cells in decidual cavity.
y. Yolk sac endoderm.

Photomicrograph. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x 70



Higher power view of above figure. x 90.

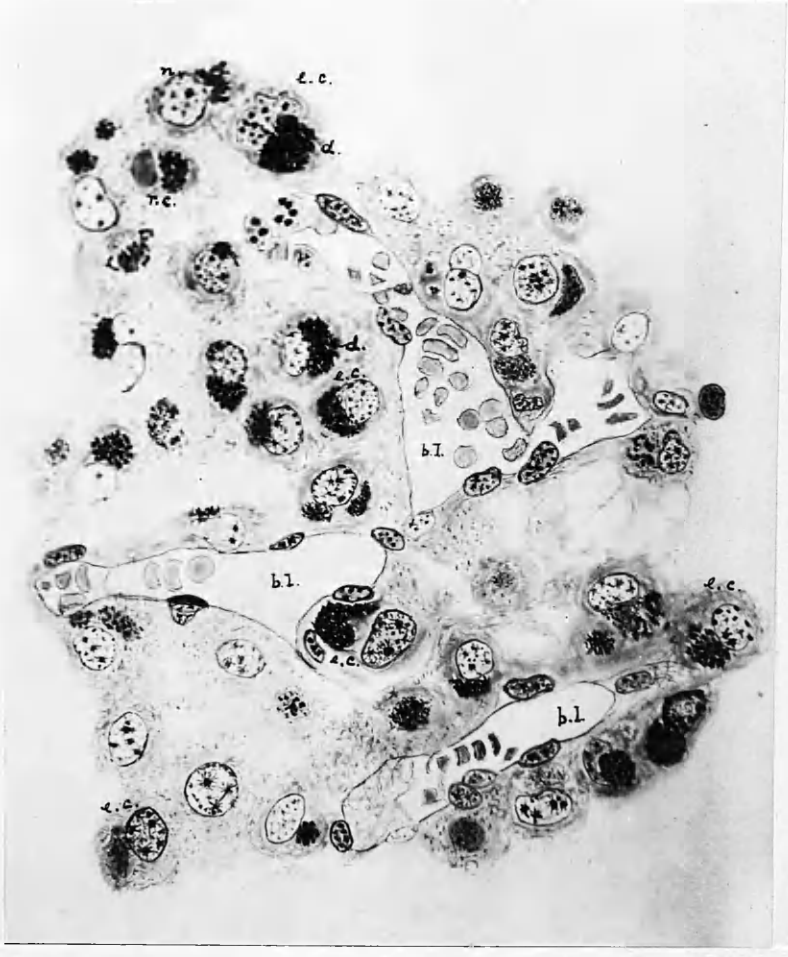
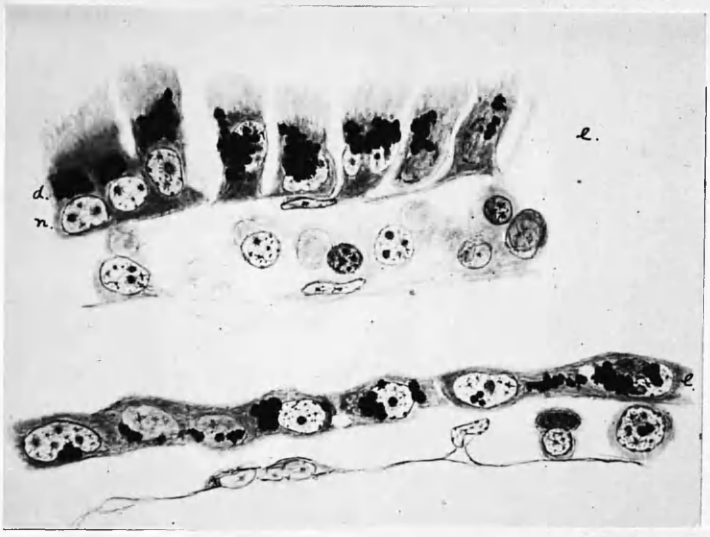


Fig. 11. Part of vitally stained wall of decidual cavity. From embryo aged 9½ days (animal No. 158 - compare Fig. 10). Illustrates the intense vital staining in the endometrial cells.

b.l. Blood vessel.
d. Dye.
e.c. Large vitally stained endometrial cell.
n. Nucleus.
r.c. Vitally stained rounded cell with dense nucleus - similar to rounded vitally stained cells in virgin uterus.

Drawing by Professor Bryce. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x circa 800.



FIGS. 12 and 13.

Parts of vitally stained yolk sac endoderm from embryo aged 18 days (animal No. 83). Intravitally stained with trypan blue.

Fig. 12. Part of dorsal third of yolk sac endoderm where the cells are columnar.

Fig. 13. Illustrating the vital staining in the yolk sac endoderm where the cells are flattened. The dye granules are not so numerous as in Fig. 12, but are still large and coarse.

d. Dye granules.
e. Yolk sac endoderm.
n. Nucleus.

Drawings by Professor Bryce. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x 1200

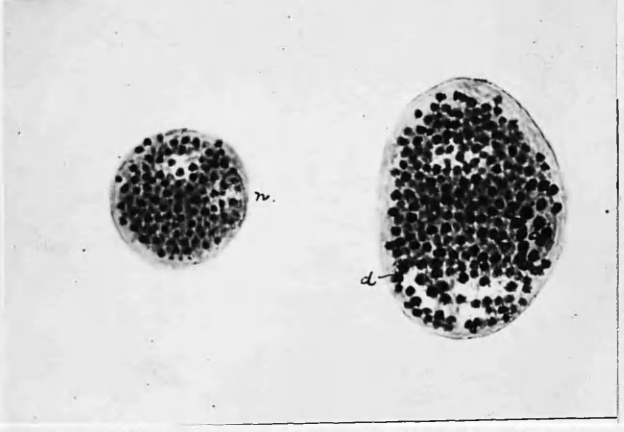


Fig. 14. Illustrating two vitally stained cells from follicular cavity of atretic follicle. From animal No. 28 (Table II) killed at 12th day of 16-day cycle. Intravitally stained with trypan blue.

d. Dye granules.
n. Nucleus.

Drawing by Professor Bryce. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x 1200.

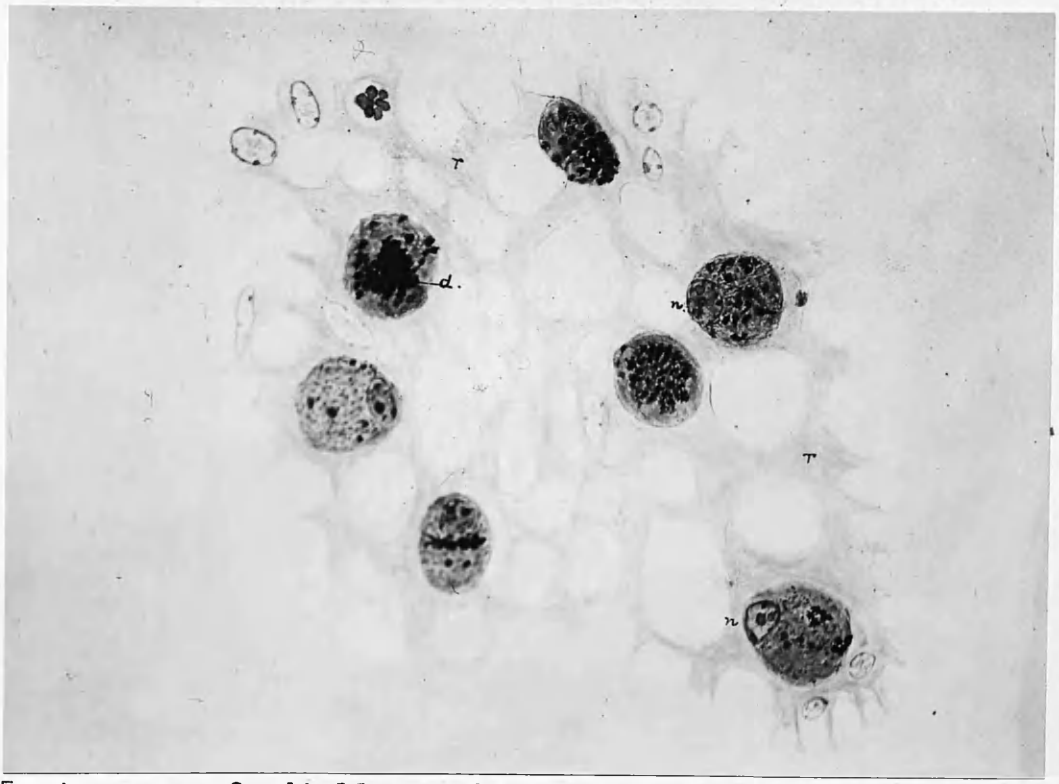


Fig. 15. A group of vitally stained rounded cells from the centre of a young corpus luteum. From animal No. 22 (Table II) killed at 7th day of 17-day cycle. Intravitally stained with trypan blue.

d. Dye granules.
n. Nucleus.
r. Loose reticulum.

Drawing by Professor Bryce. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x 1200.

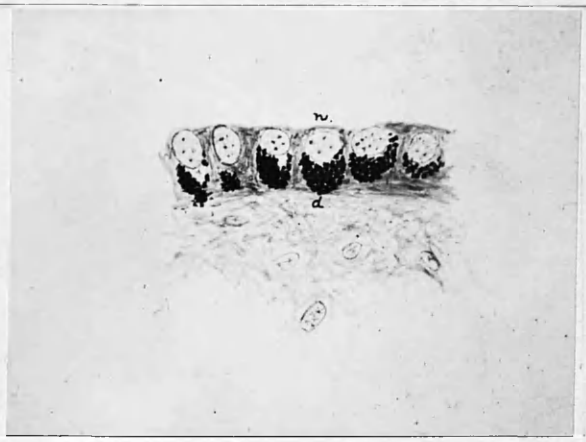


Fig. 16. Illustrating intense vital staining in germinal epithelium over projecting part of corpus luteum. From animal No. 28 (Table II) killed at 12th day of 16-day cycle. Intravitally stained with trypan blue.

d. Dye granules.
n. Nucleus.

Drawing by Professor Bryce. Fixation Formalin and Corrosive Sublimate. Stained Carbol-Fuchsin. x 1200