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SOME OBSERVATIONS ON THE UTERUS OF THE RAT DURING THE PUERPERIUM.

by

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This thesis deals with some of the changes that occur in the Rat's uterus during the post-partum period. In the first part the behaviour of the endometrium is described with particular attention being paid to that of the epithelium. The area of endometrium left devoid of epithelium by the separation of the placenta was re-epithelialised by cells which spread inwards from the existing marginal epithelium. There was no evidence of the bare area being covered by a new epithelium arising from stromal cells. Re-epithelialisation was rapid and was completed within thirty-six hours. Glycogen was absent from both the normal and the spreading epithelium. Ribonucleic acid was present in the cytoplasm of the normal epithelial cells but was much reduced in the spreading epithelial cells. There was a vacuolar degeneration of the epithelium, similar to that which occurs during oestrus, and which was most marked at thirty-six/

thirty-six hours. There was no evidence of exfoliation of the placental site and the endometrial portion of the placental artery appeared to be absorbed in situ.

In the second part the involution of the metrial gland is considered. The metrial glands of lactating and non-lactating animals involute in the same way and at the same speed. Involution of the gland is rapid in the early stages, is nearly complete by fifteen days, and is finished by the twentieth day of the puerperium. Typical granulated metrial gland cells form only a small proportion of the total cell population of the gland. They disappear by the fifth day, presumably disintegrating. They do not become lipoid containing or phagocytose pigment. The specific granules of the metrial gland cells are probably formed by a mucopolysaccharide joined to an alkaline protein. "Encapsulated" giant cells derived from the placental artery were present in the glands and the adjacent endometrium. These cells were multinucleate, basophilic and surrounded by a "capsule" of neutral mucopolysaccharide. They persist until the fourteenth day. The placental artery within the metrial gland was absorbed by the/

the fifteenth day.

The third section is devoted to a consideration of the characteristics of a pigment which is to be found in the metrial glands and the adjoining part of the endometrium from the second day of the puerperium onwards. The pigment was yellowish-brown in colour and was intra-cellular. It contained ferric iron and can therefore be regarded as a haemosiderin. However, it had other properties, which suggest that there is also a lipid component present. This lipid component behaved in many ways like the lipogenic pigments. Lipid is not usually associated with haemosiderin.

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by

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A Thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy.

PREFACE

Some of the changes that occur in the rat's uterus during the post-partum period are described in this thesis. Particular attention has been given to the repair of the uterine epithelium; to the involution of the metrial gland; and to a pigment which appears on the second day of the puerperium.

Part of the substance of the thesis has been published as follows:-

1. "Post-Partum Changes in the Uterus of the Rat". Journal of Embryology and Experimental Morphology, 1955, 3, 256.

2. "A Pigment in the Rat's Uterus". Quarterly Journal of Microscopical Science, 1956, 97, 11.

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CONTENTS

PART I.

The repair of the endometrium with special reference to the uterine epithelium.

INTRODUCTION

Relatively little attention, especially in recent years, has been given to the repair of the endometrium of the post-parturient uterus. Perhaps rather surprisingly, in view of the great difficulty in obtaining suitable specimens, most work has been carried out on the human uterus. Because of this, although the present investigation deals with the rat, the findings in man will be described first and those in other mammals second. Later, in the discussion, a comparison will be made between some aspects of repair in the human and in the rat.

The scarcity of human material, its autolysis after death, and its possible involvement by pathological processes during life, make it easy to understand how differences of opinion as to the course of endometrial regeneration, in this species, have arisen.

Before the reparative processes themselves could be understood it was necessary that the nature of the decidual lining of the uterus should be recognised and that the thickness of decidua remaining after the separation of the placenta be known. An early statement on this topic is that/

/that of William Hunter (1774) who wrote that the decidua "is an efflorescence of the internal coat of the uterus itself, and is, therefore, shed as often as a woman bears a child or suffers a miscarriage. It is of considerable thickness and one stratum of it is always left upon the uterus after delivery, most of which dissolves and comes away with the lochia. Frequently a thicker stratum separates from the uterus in one part and a thinner in another". On the other hand, Cruveilhier (1834) was of the opinion that the whole of the decidua was shed during labour and that the muscular coat was left naked to the uterine lumen. Others, particularly Robin (1848), held the view that the new mucous membrane started to form in the fourth month of pregnancy and replaced the original one after labour.

A study of the involution of the uterus was made by Heschl (1852) but he paid little attention to the repair of the mucous membrane. He did, however, believe that it was not until the uterus had regained its normal size that the repair of the non-placental mucosa was completed, while that of the placental site took an even greater length of time. He noted/

/noted that the vessels at the placental site were thrombosed and he regarded this as typical of that region.

Farre (1839) writing about the decidua said "one portion remains covering the muscular structure of the uterus, but is in parts so thin that the latter appears to be nearly bare". He also described the development of hypertrophic masses of new material in the area of the placental site and their subsequent separation and shedding into the uterine lumen some months after the end of pregnancy.

A major contribution was made by Friedländer (1870, 1876) who was responsible for the first clear account of the division of the decidua into compact and spongy layers. He was of the opinion that separation of the placenta took place in the deeper part of the compact layer and that thus the whole of the spongy layer, which contained the fundic parts of the uterine glands, remained in situ. He thought that the fundi of the glands played a major role in the regeneration of the mucosa. He showed that the non-placental and placental regions of the mucosa were restored at different times. Repair of the former was completed by the end of four weeks but the latter required several weeks more. The most distinctive/

/distinctive characteristic of the placental region was the thrombosed vessels which were being organised. He thought that this process began in the final week of pregnancy and that its onset was marked by hyaline degeneration of the vessel walls.

Kundrat and Engelmann (1873) and Wheeler (1875) agreed with Friedländer that placental separation occurred in the deeper part of the compact layer. The former also confirmed his observation that the non-placental mucosa was repaired by the fourth week post-partum, at which time the area of the placental site was still devoid of an epithelial covering. Incidentally, it was these authors who first recognised cyclical changes in the uterine mucosa although they were unaware of their significance.

The next advance was due to Langhans (1875) who showed that the decidua consists, not of two layers as maintained by Friedländer, but of three layers. The extra layer described by Langhans was the basal layer which lies next to the muscular coat. The stroma and the glands of this layer retain their non-pregnant appearance during pregnancy. He thought that separation took place through the spongy layer and not through the compact layer as described by Friedländer. With separation through/

/through the spongy layer the deepest part of this layer and the whole of the basal layer are retained. He believed that regeneration of the mucosa occurred in the same way throughout the whole of the uterus (e.g. placental and non-placental site areas) and that the greatest contribution to the restoration of the mucosa was made by the basal layer.

De Sinety (1878) maintained that the decidua was devoid of epithelial structures - including glands - and was thus forced to the view that during the repair of the mucosa new epithelial cells arose from embryonic cells present in the decidua.

Leopold (1878) devoted much attention to the behaviour of the placental vessels. Like Friedländer he found that the vessels at the placental site began to show hyalinisation during the eighth month of pregnancy, that their walls were infiltrated by foetal giant cells and that they become thrombosed, either wholly or in part. During the puerperium the thrombi at the placental site were made up partly from these vessels and partly by thrombi which arose during the first few days post-partum. He believed that the hyalinised and thrombosed vessels underwent organisation, vascularisation, and/

/and eventually disappeared. In the non-placental areas the decidua, which is retained after labour, degenerated and was shed into the uterine lumen. It was replaced by a new endometrium which arose from the residual stroma and from glandular epitholium. This process was completed by the end of the third week. On the other hand, repair of the mucosa at the placental site took until the sixth week. As only a very small number of glands was to be found in the decidua basalis of this region, he maintained that new epithelium and glands arose from cells which he termed serotinal cells. These were to be found between bundles of muscle fibres.

A histological study by Mayor (1887) was mainly devoted to the involution of the uterine muscle but he does say that repair of the mucosa was not finished until the second month and that he was unable to detect glands in the region of the placental site even at twenty-four days post-partum.

It was Klein's (1891) opinion that small pockets of decidua, which persisted in the intervals between bundles of muscle fibres, gave rise to the new mucous membrane. He thought that the regenerative process might occur as early as the third day of the puerperium.

In/

/In 1892 a valuable contribution was made by Emil Rice. He examined the placental site manually and removed fragments of tissue with his finger for histological study. He described the fate of the thrombosed vessels. These he believed separated from the uterine wall and formed clots of varying size in the uterine lumen from which they were eventually extruded. He thought that the separation of the thrombosed vessels was brought about "by the epithelium from the glands gradually spreading over the surface and beginning to secrete again, so that eventually a layer forms between the mucosa and the clot, detaching it from its base and causing its exfoliation". Unfortunately, although his observations were based upon thirty-six patients, they did not extend beyond the twelfth day of the puerperium and thus he could only deal with the early stages of mucosal repair. However, the material used was obtained from normal women and the picture was not complicated by infection or other pathological conditions as in so many of the earlier investigations.

Werth (1895) made an experimental study of the repair of the uterine mucosa. He removed uteri at varying periods after he had curetted them, and examined his material by histological methods. He found that a new mucosa was formed very rapidly and that/

/that this was brought about by the tissue which remained in the intervals between bundles of muscle fibres.

Leusden (1897) was impressed by the scarcity of glands in the decidua basalis. He noted the presence of giant cells - the so-called serotinal giant cells - in the decidua basalis in the early stages of the puerperium and thought that these played a major role in the subsequent formation of new glandular structures. This concept was, however, vigorously challenged by D'Erchia (1897). Likewise Aschoff (1899) was opposed to this idea. He held that the giant cells were of foetal origin rather than maternal and that they were not responsible for the regeneration of the uterine glands. He maintained that the decidua basalis retained a sufficiently large number of glands to effect subsequent glandular regeneration and that no other explanation was required for this process.

An account of the changes in the uterine mucous membrane during the early post-partum period based upon normal material was made by Krönig (1901) who pointed out that, although the line of cleavage of the placenta and membranes is generally to be found in the spongy layer of the decidua, it is not always so. He demonstrated that in some parts of the uterus a layer only a

few/

/few cells thick remains while in others most of the spongy layer persists - this being particularly true of the cervix, the mucous membrane of which is retained almost in its entirety. In all parts of the uterus, including the placental site, sufficient decidua is left to ensure that a new mucous membrane can be formed from it. He was of the opinion that the non-placental mucous membrane was repaired by the eighteenth day while that of the placental site took somewhat longer. Infection appeared to have no effect upon the rate of mucosal repair.

Wormser (1903) published a paper which for long remained the standard work on the subject of endometrial repair in man. It was based upon a considerable quantity of material. Apart from eight whole uteri which could be regarded as showing little or no pathological change, he obtained specimens by curettage from thirty-six women and he also removed material manually from the placental site in four others. A considerable portion of the material obtained, both by curettage and manually, came from patients who showed symptoms and signs of infection or of haemorrhage. The majority of his specimens cover the first eleven days of the puerperium and the material practically/

/practically ends at the twenty-third day.

He was of the view that a line of demarcation developed between the superficially placed decidua, which was to become necrotic and be shed into the uterine lumen, and the deeper decidua which was to survive and form the basis from which a new uterine mucosa arose. This process, with the formation of the line of demarcation, can be detected by the second day of the puerperium and is easily recognisable by the sixth day. In the zone of the decidua vera the degenerative changes are well marked by the tenth day when the proliferation of the deeper layer giving rise to a new mucosa is clearly apparent, although this mucosa is still basically, at this time, formed by the remnants of the decidua. In places the surface of the new mucous membrane is still not covered by epithelium and these areas resemble granulating surfaces. Some of them are clean while others have pieces of necrotic matter adhering to them. Elsewhere the surface of the membrane is covered by an irregular and thin epithelium. He called this a "provisional wound cover".

In general, by the fourteenth day the remaining decidua has been replaced by the new mucous membrane. Some decidual cells do/

/do remain in the superficial parts of the mucosa but these show evidence of degeneration, while the stroma of the mucous membrane has for the most part regained its endometrial characteristics. The newly formed endometrium is exceedingly thin - indeed in places it may be thinner than the decidua from which it was formed. The surface of the endometrium is irregular and is not yet entirely covered by epithelium, although most of it has been epithelialised by a spread of cells from the mouths of the uterine glands. Some of this epithelium is normal or almost normal in appearance. Only a few uterine glands are present and these are of irregular outline and make a great variety of angles with the surface. By the end of the third week the endometrial characters of the membrane are even more pronounced but it is still thinner than usual. However, Wormser regarded the repair of the non-placental regions, in all its essentials, as having been completed by this time.

Similar degenerative and reparative processes occur in the region of the placental site as in the decidua vera (non-placental areas). However, in the region of the placental site there is a greater abundance of necrotic material, some of which is very resistant to the cleansing which takes place. As

a/

/a result of this, regeneration at the site takes longer and even under optimal conditions the site may still be recognised two months after labour by the presence of remnants of the larger blood vessels and of pigment containing phagocytes. Soon after delivery the uterine lumen is filled by a large blood clot which is readily separated from the decidua vera but which, at the placental site, is continuous through the open ends of the maternal placental vessels with the thrombi that fill them. This results in the clot being more adherent in the placental region. Although the line of separation forms here, as elsewhere, the great vessels make it irregular and, after the shedding of the surface layers, the muscle coat may be laid bare in places. The pieces of clot and the necrotic matter, which are shed, appear to be separated from the underlying decidua by a process which seems to involve digestion and solution. This may be assisted by leucocytes which are always present even in the absence of any infection. Dead material which is not shed is enveloped by newly formed endometrium and after a time it is removed. In particular the terminal portions of the great vessels behave in this way. The other necrotic material, which is shed into the lumen, may be in the form of large sloughs or it may seem to dissolve away. Likewise blood clot/

/clot may be shed as large pieces or may soften and break down.

As already pointed out, some of Wormser's material came from patients who had post-partum bleeding or showed evidence of infection. He found that uterine repair is unaffected by slight degrees of infection and may occur even when infection is severe. Against this, the retention of some of the products of conception within the uterus could cause considerable variation in the time taken for repair to occur and even great delay could result. He found no evidence of the very rapid repair of the uterine mucosa as postulated by Klein (1891) who claimed that a new mucous membrane was formed in the first few days post-partum.

In 1910 a long monograph by Goodall appeared. This dealt, for the most part, with the behaviour of the uterine vessels and was based upon material stained by Van Gieson's method and Weigert's elastic technique. Goodall was of the view that the post-partum uterus required less blood than during pregnancy and that this could be correlated with changes in all the uterine vessels. These changes affected, especially, the vessels in the inner two-thirds of muscle coat and were well marked in those lying deep to the placental site. He thought that during the/

/the puerperium all the arteries were replaced by the growth of a new, small vessel inside the lumen of the original one. After this the wall of the original vessel broke down by hyaline and elastoid degeneration and was absorbed. This absorption was accomplished quickly and was complete in young individuals but was slower and less complete in older women.

Schickele (1910) was also interested in the behaviour of the vessels but he limited himself to studying those at or near to the placental site. He thought that the vessels were finally absorbed and that their walls were prepared for this by an infiltration of trophoblastic cells which caused a hyaline degeneration to develop. He also believed that this made their walls more easily compressible by the contracting and retracting muscularis after labour and that this resulted in haemostasis occurring.

Hinselmann (1913) described the thrombosis of vessels at the placental site. He did not find any evidence that thrombosis occurred early in pregnancy. He noted that thrombi were present in both arteries and veins so that these were often difficult to distinguish.

The next work of importance is that of Teacher (1927) who based /

/based his conclusions upon a study of seven uteri obtained at post-mortem and one which was removed at operation. These covered the period from the fourteenth to the forty-second day of the puerperium. He also obtained a few curettings during the first fourteen days post-partum but these came from diseased uteri so that he placed little reliance upon them and based his account of this early period on the work of Wormser.

He agreed with Wormser that in the early phase the superficial layer of the decidua is demarcated from the rest and, after undergoing necrotic changes, is shed into the uterine lumen to form part of the lochia. He differed from Wormser in thinking that the restoration of the non-placental mucosa took somewhat longer than fourteen days. Repair at the placental site is slower than elsewhere and takes six weeks as against about three weeks. The placental site is characterised by thrombosed vessels which give it a nodular appearance. He claimed that the thrombi become organised and that the vessels are absorbed in situ. The site can also be identified by the many pigment bearing phagocytes which are present at it along with giant cells of chorionic origin.

Like Wormser he found that slight degrees of infection had/

/had no effect upon the rate of repair. This he thought was an important observation from a medico-legal viewpoint.

Schroeder (1930), also, was of the opinion that repair at the placental site took longer than in the extra-placental region. He found that in the latter region it was completed in three weeks while the former took six. He attributed the delay in the placental region to the presence there of hyalinised and thrombosed placental vessels, which seemed to interfere with the formation of a new epithelium. The vessels themselves disappear very slowly. Hyaline remnants could be found at the site up to a year after delivery. It appears that the vessels are absorbed in situ and that they are not shed into the uterine lumen.

A major contribution was made by Williams (1931) who was particularly concerned with the fate of the placental site, and especially with that of the vessels in this region. He obtained a large number of specimens which can be divided into three groups. The uteri in the first group were obtained by hysterectomy performed immediately after Caesarean Section. Some of these uteri were removed before and others after the expulsion of the placenta, so that the conditions just before, and/

/and just after, placental separation could be studied. In the next group the specimens were obtained at post-mortem. These specimens covered the first three days of the puerperium and were removed from women who had died of pneumonia, heart disease, or of a general toxaemia. No specimens from women dying of uterine infection were placed in this group, so that this material was not complicated by the presence of uteri showing pathological changes. The third group was made up of uteri which were removed by hysterectomy for the purpose of sterilising the individual. The indications for sterilisation included chronic nephritis, serious heart disease, feeble-mindedness, and other psychological disorders. In all there were eighteen specimens in this group which covers the period of from one week to four months. The earlier part of this period is better represented than the later - nine specimens were obtained from between the seventh and twentieth day, seven between the twenty-first and fifty-first day, one at three months, and one at four months. The specimens in this group, like those in the first, were fixed immediately after removal in ten per cent formalin. After twenty-four hours they were cut open by either a vertical or transverse incision to allow greater penetration of the fixative.

Williams/

/Williams was in agreement with many of the previous workers that the repair of the non-placental mucosa is rapid. He found that the decidua vera is from 1 to 3 mm. thick during the first few days. By the seventh day the surface layer is infiltrated with blood, although the decidual cells show no signs of degeneration. Immediately below the surface layer the mucosa is infiltrated with leucocytes while deeper still there are many glands which are embedded in a stroma that gives no indication of a decidual reaction. By nine days the glandular epithelium is showing changes - the epithelial cells are irregular in size and shape with enlarged and poorly staining nuclei. No mitotic figures are present. The surface epithelium is replaced by cells from the fundi of the glands. Re-epithelialisation is rapid and is completed by about the fourteenth day post-partum, but at this time the stroma is not entirely normal and does not usually become so until about the twenty-first day.

On the other hand the placental site is not repaired until the sixth or seventh week. Soon after labour the placental site is characterised by numerous clot or fluid filled blood vessels which are tightly packed together. The site is about 10 cm. in diameter and is slightly elevated. Its surface is irregular/

/irregular with raised nodules and there is much extruded blood. Williams believed that the site and particularly the blood vessels are shed into the uterine lumen by a process of exfoliation. This is brought about by the undermining of the placental site by the ingrowth of endometrium from round its edges - this endometrium gradually spreading inwards deep to the site and thus separating it from the still deeper structures. He thought that the cellular remnants of the decidua basalis played little or no part in the process of exfoliation, which was brought about by the migration of the new non-placental endometrium. He believed that the shedding of the degenerating and thrombosed vessels by the process of exfoliation is highly beneficial in that it results in the formation of a new mucosa at the placental site much more rapidly than could occur if the vessels and thrombi were absorbed in situ. Further, if the vessels were absorbed in situ considerable scarring of the mucosa would result, so that after a few pregnancies the scarring might be so great as to interfere with the menstrual changes in the endometrium and with the embedding of a fertilised ovum so that "the reproductive career would come to an untimely end."

He noted that a moderate degree of infection or the retention/

/retention of fragments of membrane did not markedly alter the time taken for repair of the non-placental site endometrium. However, he felt that repair at the site itself was delayed.

Rutherford and Mezer (1942) studied the regeneration of the uterine mucosa in normal women. In all twenty five women were investigated and, from each, one sample of the uterine lining was obtained by suction curettage. One specimen for each of the first fourteen days of the puerperium was obtained while the remaining eleven specimens covered from the third to the fourteenth week. They are in general agreement with earlier writers. They described the regeneration of both the surface and the glandular epithelium and state that evidence of this can be detected as early as the first day post-partum. Regeneration occurs initially without cell division but a few mitoses are present by the fourth day. In a comparison of lactating and non-lactating females they found no difference in the rate of endometrial repair.

A study by Sharman (1953) was based upon an extensive collection of material. He employed ten uteri which were removed at post-mortem and which ranged from the day of delivery to the twelfth day of the puerperium. He also obtained a fifty-fourth day specimen by hysterectomy. Apart from these whole/

/whole uteri he had 636 biopsy specimens of the endometrium which were removed by curettage from 285 women. These specimens cover every day from the fifth to the eighty-first. After this each week is represented by several specimens up to the thirty-ninth week post-partum. All the biopsy material was taken from women who showed no evidence of uterine infection.

He was particularly concerned with the repair of the endometrium outside the placental site but he includes observations on the site when portions of this region happened to be present in his sections. Repair is rapid. By the eighth day a proliferative mucosa can be identified although the surface epithelium is not quite completely restored at the fourteenth day. However, the mucosa is entirely repaired by the end of the sixteenth day. Cell division in the glandular epithelium can be detected on the eighth day and mitosis continues at a high rate while restoration is going on. He noted that giant cells could be found up to the seventh day.

Sharman draws attention to decidual cells persisting in the new endometrium. Some of these cells divide and he believes that they may change into typical endometrial cells of a connective tissue type thus playing a part in the reconstruction of/

/of the new mucosa.

He notes that the stroma immediately after labour contains large numbers of polymorphonuclear leucocytes and of lymphocytes. During the first ten days of the puerperium the polymorphs decline in number and after this time are rarely found. On the other hand, after the tenth day plasma cells may be found in the stroma and these, along with lymphocytes may persist until the third or fourth month. Indeed they do so in 37% of the biopsies obtained during this period. As this infiltration of plasma cells and lymphocytes occurs in normal women it should not, in women who have had a recent pregnancy, be taken to indicate the presence of infection, as has been the case.

Sharman makes no mention of the fate of the blood vessels at the placental site and he does not describe the process of exfoliation which Whitridge Williams noted in this area.

Attention will now be turned from man to the mammals as a whole. Surprisingly little work has been done in this field. Of the earlier writers on the comparative side, Duval (1891) and more particularly Strahl (1895, 1903, 1906a, 1906b) and Strahl and Henneberg (1901, 1902) are prominent. Strahl, on the results of his work, divided the Deciduata into three groups/

/groups on the basis of the way in which uterine involution occurs, more particularly that of the epithelium. In one group, in which is to be found man and monkeys, there is no uterine epithelium left covering the decidua after labour so that during the puerperium a completely new epithelium is regenerated. In the next group, to which the Carnivora belong, the non-placental regions of the mucosa are covered by epithelium while this is absent deep to the placenta itself. There is thus a smaller area than in the previous group to be re-epithelialised. The third group is represented by the Rodentia. In it not only is the non-placental mucosa covered by epithelium but epithelium spreads inwards from the placental margin to lie deep to the placenta itself so that ultimately the placenta remains attached to the uterus by little more than a stalk formed by the uterine vessels. This arrangement, of course, leads to rapid epithelial and endometrial repair during the puerperium and to the establishment of conditions which could support an early further pregnancy. In this regard the immediate post-partum oestrus of the rat will be recalled.

Apart from Duval and Strahl, studies on rodents have been made by Chipman (1903) who carried out a general survey of the group, by Stolper and Herrmann (1904), Hamilton (1933) and Nicol/

/Nicol (1933) on the guinea pig and Greenwald (1958) on the mouse, while the gundi was investigated by de Lange (1934). The closely related rabbit was studied in detail by Bull (1949). In other orders investigations have been made on the cat by Turner (1876), the mole by Moll (1906), and the cow by Ellenbogen (1930).

The work reported in this thesis has been carried out on the rat. Repair of the uterine epithelium in this animal was described by Duval (1891). As previously noted in this species, the area of endometrium left denuded of epithelium, after the separation of the placenta, is small, this being due at least in part to the undermining of the placenta by the uterine epithelium. Duval believed that the denuded area was re-epithelialised by the transformation of endometrial stromal cells into epithelial cells and he claimed to have observed cells in all the various stages of this process. On the otherhand, Strahl (1906b), who worked with the mouse, was of the opinion that the bare area was recovered by a spread of cells from the epithelium at its margin. He considered that this also occurred in the rat and in other mammalian species. Bull (1949), studying the rabbit, was unable on the material available to him to confirm either of these views. Hamilton (1933), who investigated/

/investigated the guinea pig, was of the opinion that the epithelium is restored from pre-existing epithelium; although he states that at the lower end of the uterus, where the horns are in contact but the lining separate, the possibility of epithelium arising from stromal cells does exist as there are few or no glands in this region to provide a source of epithelial cells. Sharman (1952) investigated the effect of ovariectomy coupled with the administration of either oestradiol monobenzoate or progesterone on the uterus of the post-partum rat and guinea-pig but he makes no comment on the method of regeneration of the epithelium at the placental site.

The investigation reported in this section was undertaken in order to study:-

(1) The mode of regeneration of the epithelium of the placental site in the rat, particularly to determine whether or not epithelial cells arise from cells of the endometrial stroma.

(2) The time at which regeneration is completed and to correlate this with the early occurrence of a new pregnancy.

(3)/

(3) The distribution of glycogen and ribonucleic acid in the regenerating epithelium as no reports of the presence or absence of these substances in the epithelium of the post-partum uterus have been made.

(4) The behaviour of the placental vessels in the endometrium and to determine whether or not exfoliation of the placental site occurs in the rat as described by Williams in man.

(5) In addition certain unexpected observations were made on some other changes that take place in the uterine epithelium and on the behaviour of the endometrial stroma. These will also be reported and commented upon.

MATERIALS AND METHODS.

To ensure standard conditions - as far as possible - particularly with regard to the hormonal state, this work was carried out on lactating animals with six or more offspring and with the possibility of a further pregnancy excluded.

Virgin rats were mated and used for observation provided they had a litter of six or more and were lactating. Either just before or immediately after littering the females were segregated from the males thus avoiding the chance of a new pregnancy/

/pregnancy. Seven animals were killed as soon as parturition was complete (referred to here as 0 hours). Five animals were sacrificed six hours after parturition, three at twelve hours, three at eighteen hours, two at twenty-four hours, two at thirty hours, and three at thirty-six hours. Then five animals were killed on the second day, three on the third day, two on the fourth day, and four on the fifth day. Portions of the uterine horns containing placental sites were removed and fixed in either Bouin's fluid or ice cold Rossman's fluid (nine parts of absolute alcohol saturated with picric acid and one part of neutral formalin). It was possible to recognise the position of the placental sites without opening the uterine horns, partly because the horns are thicker in these regions and partly by means of the metrial gland which bulges into the mesometrium at each site. After embedding in paraffin wax, serial sections ten microns thick were cut and mounted. Those that had been fixed in Bouin's fluid were stained with haematoxylin and eosin. The material fixed in Rossman's fluid was treated to show the presence of either glycogen or of ribonucleic acid. For the demonstration of glycogen the periodic acid - Schiff (P.A.S.) reaction was used. Control sections from which glycogen had been eliminated by digestion/

/digestion with diastase (Gomori, 1952) prior to staining were also prepared. These enabled glycogen to be distinguished from other P.A.S. positive substances present in the sections.

To show cytoplasmic basophilia sections were stained for fifteen minutes in a 0.2 per cent aqueous solution of toluidine blue and then dehydrated in butyl alcohol for ten minutes before clearing and mounting. Dehydration can be carried out in butyl alcohol without appreciable loss of toluidine blue from the sections, as occurs when ethyl alcohol is used for this purpose. Cytoplasmic basophilia due to ribonucleic acid was recognised by incubating control sections in ten per cent perchloric acid for twelve hours at four degrees centigrade before staining. This procedure extracts ribonucleic acid from the sections and a comparison can then be made between treated and untreated sections.

In a number of animals one uterine horn was opened and the placental sites were examined with the naked eye.

OBSERVATIONS

The uterus at 0 hours.

Although the uterus collapses immediately after littering and is reduced in size by contraction of its muscle coat, it is/

/is still much larger than before pregnancy. Measurements made on transverse sections taken through the placental site showed the uterine horn to be about four and a quarter millimetres in width. On opening a uterine horn, the placental sites can be recognised along the mesometrial border of the mucosa. They are about one to two millimetres in diameter, appear as deep pits, and are usually filled with blood clot.

The uterine mucosa is thrown into folds which vary in size and shape and which run in the long axis of the horn. In the region of the placental sites one or more large folds project into the uterine lumen incompletely dividing it into a larger anti-mesometrial portion and a smaller mesometrial one (Fig.1). Duval (1891) referred to these mucosal projections as the utero-placental folds.

The uterine mucosa, except at the placental sites, is lined by a single layer of tall, non-ciliated, columnar epithelial cells (Fig. 2) which tend to become cuboidal in shape at the edge of the bare area of the placental site. The cell outlines are distinct. The cytoplasm contains fine granules, especially in the supranuclear region. At the free surface of the cell there is a clear eosinophilic layer which was referred to by

Pritchard/

/Pritchard (1949) as the distal band. The nuclei, which are situated towards the base of the cells, are round to oval in shape, vesicular in appearance, and contain one or two nucleoli. When stained with toluidine blue the cells show marked cytoplasmic basophilia, particularly in the supranuclear region (Fig. 2). In the control sections, treated with perchloric acid before staining, there is a marked reduction in the amount of cytoplasmic basophilia (Fig. 3) and it is, therefore, concluded that the basophilia lost during perchloric acid incubation is due to ribonucleic acid. With the P.A.S. technique a number of small granules, mainly in the supranuclear region, are stained. There is also a surface layer of P.A.S. positive material and a distinct basement membrane can be made out (Fig. 4). The appearance is similar in the control sections which were incubated in diastase before staining. From this it may be concluded that none of the P.A.S. positive material described above is glycogen. The distribution of the P.A.S. positive material corresponds to that described by Leblond (1950) in the epithelium of the non-pregnant rat uterus.

Mitotic figures are numerous throughout the epithelium except in the regions adjacent to the placental sites where there are only/

/only a few to be found. In the non-placental regions as many as forty mitoses can be seen in a single transverse section.

At intervals between the typical columnar cells a second type of cell can be distinguished. These are narrow and elongated with deeply staining cytoplasm and spindle shaped nuclei (Fig. 5). They resemble the "rod" cells described by Horning (1942) in the uterine epithelium of the non-pregnant rat and by Aykroyd and Gatenby (1941) in man. Mitotic figures can occasionally be seen in these cells.

The epithelium terminates abruptly at the site of insertion of the placental pedicle. At this stage the epithelial cells at the margin of the bare area are cuboidal in shape. The epithelium near the site is infiltrated by a large number of polymorphonuclear leucocytes.

Glands, in small numbers, can be seen in the endometrium of the side walls of the uterine horn and to a much lesser extent along the antimesometrial border, but they are absent from the mesometrial border and thus from the placental site. Because of this distribution they cannot be expected to play any direct part in the re-epithelialisation of the placental site.

The endometrial stroma is most abundant in the mucosal folds, while/

/while between the folds the epithelium is separated from the myometrium by only a thin layer of stroma. The cell density of the stroma varies. In the larger folds there are a few cells scattered in an abundant ground substance giving the endometrium here an oedematous appearance. Elsewhere, especially at the bases of the folds, the cells are more numerous and the ground substance reduced in amount. The stromal cells, which resemble fibroblasts, show no evidence of a decidual reaction except round the placental site where there are a few cells that contain glycogen. In the subepithelial zone the stromal cells are orientated parallel to the surface but otherwise no regular arrangement is apparent. The blood vessels passing through the stroma are dilated and there is a well marked subepithelial plexus.

At the placental site there is an absence of epithelium. Here the stromal cells are more numerous than elsewhere and are fairly closely packed together so that there is no indication of the oedema which is so obvious in the mucosal folds. There is usually one, but there may be two placental arteries at each site (Young, 1956). A placental artery enters the stroma through a gap in the circular layer of the myometrium, the fibres of /

/of which, in this region, are widely separated by cells, some of which resemble those of the adjacent endometrial stroma. The arteries which pass through the condensed stroma towards the bare surface of the placental site contain a mass of thrombus. The wall of a placental artery, as it traverses the stroma, is made up for the most part of a homogeneous eosinophilic staining material which also colours readily with the P.A.S. technique and stains blue with toluidine blue. The presence of this material is taken to indicate a hyaline degeneration of the vessel wall. Within the vessel wall there are a large number of multinucleate giant cells (Fig. 6). These have a basophilic cytoplasm and each cell is surrounded by a thick covering of P.A.S. positive material which has the appearance of forming a capsule for the cell. This "capsule" is eosinophilic and non-metachromatic with toluidine blue. In some cases what seem to be two or three separate smaller cells are present inside one "capsule" and these give the impression of fusing together to produce one large giant cell. On the surface of the bare stroma there is usually a mass of blood clot which may extend into the stroma on either side of the placental artery. Blood clot may also be present within the stroma itself, or between the stroma and the uterine epithelium. Polymorphonuclear leucocytes are already/

/already present in the blood clot and in the adjacent endometrium.

The uterus from 6 to 12 hours post-partum.

By twelve hours post-partum the uterine horns have decreased slightly in size being now about four millimetres wide in the placental regions. The lumen remains large and there is still much folding of the endometrium.

The re-epithelialisation of the bare area of the placental site commences soon after littering. By six hours the epithelial cells at the margin of the bare area have become variable in shape (Fig. 7). In section some appear round, others oval, pear shaped, or flat. Their nuclei are enlarged with prominent nucleoli and the cytoplasm is less basophilic than that of the rest of the uterine epithelium. There is no glycogen within these cells. The intercellular boundaries are very indistinct, and no basement membrane is revealed by any of the staining techniques used. These hypertrophic cells have a thin layer of P.A.S. positive material on their free surface. They form a layer which is one cell thick and which is directly continuous with the adjacent columnar epithelial cells lining the uterine lumen./

/lumen. In some places they lie on the endometrial stroma but in others they are separated from the stroma by blood clot or by fibrin clot with which they are in contact. There was no evidence of a transformation of stromal cells into epithelial cells. Cell types intermediate between the two could not be identified. Similar appearances are present at twelve hours although now the hypertrophic cells are more numerous.

The remainder of the epithelium shows little change. The cells remain tall and columnar but at twelve hours the bases of the cells have become more eosinophilic and in the same region the intercellular boundaries are less distinct. No mitoses were found anywhere in the epithelium although a large number of sections were examined. The polymorphonuclear leucocytic infiltration now extends throughout the whole of the epithelium. The rod-like cells could no longer be discerned (Fig. 8).

The portion of the uterine artery that crossed the endometrial stroma is disintegrating, presumably by a continuation of the hyaline degeneration that was found at the time of littering. By twelve hours the wall may be extensively broken down. In these cases the P.A.S. positive material forms a collection of separate masses which lie along the line of the arterial/

/arterial wall - the masses being separated from each other by new fibrous tissue which is replacing the wall. The "encapsulated" giant cells are to be found both within the remains of the wall and to a lesser extent in the surrounding endometrium. Even when separated from the wall they retain their covering of P.A.S. positive material and can thus be readily identified. Blood clot in the endometrium and in the artery show evidence of organisation (Fig. 10). The endometrial stroma in the region of the site remains condensed and cellular while that of the rest of the mucosa is still markedly oedematous. The glycogen containing decidual cells have disappeared from the placental region.

The uterus from 18 to 24 hours post-partum.

The reduction in size of the uterus continues although the folding of the endometrium persists (Fig. 9). The uterine horns in the placental region are now about 3.5 mm. wide. The placental site is extensively re-epithelialised; in an occasional specimen completely so. The new epithelium consists of hypertrophic cells which are similar to those that have already been described. Fig. 10 and Fig. 11 are of a section taken through a placental site of an animal killed twenty-four hours after littering. They show/

/show, in this case, that the site is completely re-epithelialised. The new epithelium consists of a layer of hypertrophic cells one cell thick. It covers over a mass of blood clot and is continuous with the adjacent columnar epithelium that bordered the site. The hypertrophic cells still retain their enlarged nuclei. As before, they show a diminished cytoplasmic basophilia while glycogen continues to be absent. There is no indication of a basement membrane deep to these cells.

Further changes have occurred elsewhere in the uterine epithelium. By twenty-four hours the eosinophilia at the bases of the cells has become more marked than in the twelve hour specimen and, in this region, the cell outlines are unrecognisable. Many of the cells are vacuolated. The vacuoles occur mainly in the supranuclear region. In size these may be as large or even larger than the nucleus and many of them contain debris (Fig.12). The debris does not stain with the P.A.S. technique. The degree of vacuolation is not uniform but varies indifferent regions of the epithelium. In some places the cells have a normal appearance while in adjacent regions vacuolation may be extensive. In these regions the cell outlines are obscure and in haematoxylin and eosin preparations it is difficult to identify the boundary between epithelium and endometrial stroma although/

/although with P.A.S. material a basement membrane can always be seen. The cells remain tall but in some places the epithelium appears to be pseudo-stratified. Mitoses were not found in the epithelium.

The breakdown of the stromal part of the placental artery continues. Hyaline material from the wall can still be identified as can "encapsulated" giant cells, but the wall tends to be thinner and in places is fragmented. Organisation of blood clot and thrombi has progressed and in places smaller clots have been almost completely replaced by cells. The endometrial stroma is becoming more cellular but the density of cell population remains greater in the region of the placental sites than elsewhere in the endometrium. The dilatation of the blood vessels which was apparent at the time of littering is still clearly visible (Fig.13). The leucocytic infiltration remains well marked. Polymorphs are abundant in the stroma immediately deep to the epithelium. Many are present at the placental sites and are also to be found in the blood clot that is present both in the stroma and in the uterine lumen.

The/

The uterus from 30 to 36 hours post-partum.

The uterus has decreased further in size and by thirty-six hours the horns are about three millimetres wide at the placental regions. The folding of the endometrium persists but the folds are fewer and larger (Fig. 14). In all the specimens studied the bare area of the placental site was completely re-epithelialised by thirty-six hours although many of the covering cells have not attained a columnar shape and are still oval or pear shaped.

In the rest of the epithelium the vacuolation is much more marked. A greater number of cells show vacuoles, most of which contain debris, but there are, here and there, patches of normal epithelium (Fig. 15). In the regions where the cells show a great degree of vacuolation, the boundary between epithelium and stroma is indefinite in material which is stained with haematoxylin and eosin. The basement membrane is, however, clearly visible in P.A.S. stained sections so that the distinction between epithelium and stroma can be readily made (Fig. 16). There were no mitoses visible within the epithelium.

Very little of the placental artery remains within the stroma. Small amounts of hyaline material from the wall and a number of "encapsulated" giant cells persist, but the track of/

/of the artery has been nearly entirely replaced by endometrium. The endometrium of the placental zone is still denser than that of the rest of the uterus, but the oedema of the remainder of the stroma has decreased and its cellularity is greater than in the eighteen to twenty-four group.

The leucocytic infiltration of the stroma and epithelium continues but is less marked than in earlier groups. The stromal vessels are less prominent and their dilatation is subsiding.

The uterus at 48 hours post-partum.

The uterus has decreased considerably in size, and in the region of the placental sites the horns are now about two and a quarter millimetres in width. The endometrium is no longer folded and the lumen has been reduced to a narrow slit which runs from the mesometrial to the antimesometrial border (Fig. 17). At the placental site the epithelial cells are now similar to those lining the remainder of the lumen. The epithelium is made up of low columnar cells which have central nuclei and from which all trace of vacuolation has gone (Fig. 18). A few of the cells show mitotic figures. The "rod" like cells which were present at the time of littering have reappeared.

The/

/The stroma now forms a thick layer between the epithelium and the myometrium. It is more cellular than before but the cell density still remains greatest in the regions of the placental sites. The part of the placental artery that lay within the endometrium has now been extensively replaced by stromal cells. Some hyaline remnants of its wall can be found and also "encapsulated" giant cells. These may lie either near to or at some distance from the old course of the vessel. Along the course of the vessel there are cells, sometimes singly, sometimes in groups, which contain small granules of a brown coloured pigment. These granules stain with the P.A.S. technique. Granule containing cells are to be found not only in the endometrium but also in the metrial gland. In the third section of this thesis the nature of the brown pigment will be described. There is little evidence of the leucocytic infiltration and only an occasional polymorphonuclear leucocyte can be found in the epithelium or in the stroma. Likewise the dilatation of the stromal vessels has subsided and the vessels are much more difficult to detect.

<u>The uterus from 3 to 5 days post-partum.</u>

Few additional changes have occurred during this period.

The/

/The uterus remains small. The cells lining the lumen continue to be cuboidal in shape. By the fifth day the stromal cell population has become the same at the placental site as elsewhere. In some cases the remains of the placental artery have completely disappeared from the stroma in the region of a site while in others a small fragment may remain. Figure 19 shows one of the larger of these. "Encapsulated" giant cells are to be found at nearly all of the sites within the endometrium. These cells retain their covering of eosinophilic P.A.S. positive material and they appear to be quite healthy. There are more pigment containing cells present and the individual pigment granules are larger in size so that they can be more easily detected in haematoxylin and eosin preparations. Pigment containing cells are numerous at the sites where they tend to lie in the endometrium close to the myometrium but small groups or single pigment cells may be found scattered anywhere in the endometrium. As will be described later similar cells are to be found within the metrial gland.

DISCUSSION/

DISCUSSION

In the material examined, the placental site clearly seemed to be epithelialised by a spread of cells from the surrounding uterine epithelium and not by a transformation of stromal into epithelial cells. The former view is supported by a number of the observations reported here. At no time were there any isolated patches of regenerating epithelium; indeed, at all stages the epithelium covering the placental site was continuous with the epithelium round it. The hypertrophy and varied form of the cells, particularly the flattening, are also characteristic of spreading epithelium (Florey 1954). Moreover, in many of the sections the new epithelium was resting on blood or fibrin clot and not directly on the stroma; an observation which in itself makes the theory of stromal transformation appear unlikely to be true. Where the regenerating epithelium was lying directly on the stroma there was an abrupt demarcation between epithelial and stromal cells and no difficulty was experienced in distinguishing the one from the other. Finally, there was no evidence of any transformation of stromal cells into epithelial cells, and despite a careful search of the material no forms intermediate between the two could be detected. It thus appears certain that Duval's

view/

/view of stromal cells changing into epithelial cells is incorrect and that Strahl is right in claiming that the placental site in the rat is re-epithelialised by epithelium spreading in from round its margin. In this respect uterine re-epithelialisation in the rat is similar to that in other species, and it resembles the general pattern of epithelial repair found in mammals.

In passing, it should be noted that as there are no glands at the placental site there is no question of glandular epithelium playing a role in the epithelialisation of the site. In this connection it will be recalled that in the human, where the surface layers of the decidua are shed, the new uterine epithelium lining the lumen is derived from glandular epithelium.

Although epithelial cells were dividing at the time of littering, no evidence of cell division was found in subsequent groups until forty-eight hours was reached when a few mitotic figures could be detected. During this forty-eight hour period the placental site was re-epithelialised and this occurred in the absence of cell division in the uterine epithelium as a whole and the spread epithelium in particular. These findings may be viewed in the light of the opinions put forward by McMinn and Johnson (1955). These authors point out that, on the/

/the basis of mitotic activity under ordinary conditions, two groups of epithelia can be described. One group, into which the epithelia of skin, cornea, and intestine can be placed, shows a high level of mitotic activity while the other, which is comprised of such epithelia as that of the ducts of exocrine glands, the vascular tree, serous cavities, gall-bladder, and urinary bladder, shows little activity. The behaviour during regeneration of these two types of epithelium differs. In the first group, skin wounds are re-epithelialised by epithelial spread, and this occurs without, at least in the early stage, any significant increase in mitotic activity (Arey, 1936). Likewise wounds of corneal epithelium are repaired, primarily, by a spread of cells over the bare area, with cell division taking a later and much smaller part (Friedenwald, 1950). Similarly, wounds of the mucosa of both the small and large intestines (McMinn & Mitchell, 1954, McMinn & Johnson, 1958b) are epithelialised by cell migration without the occurrence of any significant increase in cell division. In the other group mitosis begins early and mitotic figures are to be found within the spreading epithelium. Thus, Milstein (1960) found early proliferative activity during the repair/

/repair of the duct of the submaxillary salivary gland, while McMinn & Johnson (1955, 1957) report a high mitotic activity, with mitotic figures in both the normal and the migrating epithelium, in healing "artificial" ulcers of the urinary bladder and the gall bladder of the cat. These authors suggest that epithelia that normally have a high mitotic rate may rapidly repair defects by utilising cells that would otherwise be shed at the surface. On the other hand, in epithelia with a low resting mitotic rate, repair might be facilitated by an increase in the rate, with more cells becoming available for covering the raw area as a consequence. The observations on mitotic activity reported in the present paper would support this contention. While the mitotic activity of uterine epithelium may vary according to the endocrine state, and although no mitotic figures were found in any part of the epithelium while epithelialisation of the site was in progress, nevertheless large numbers of cells are available for covering the site. The uterus is rapidly decreasing in size during the first few days of the puerperium and the folding of the mucosa becomes less and less marked. This must result in a decrease in the surface area of the mucosa/

/mucosa with a consequent surplus of epithelial cells. Presumably some of these could migrate to cover the bare area at the placental site, which is in fact small, without any resort to cell division. Of course, in this case cells are available irrespective of the normal mitotic rate of the uterine epithelium.

Glycogen was absent both from the normal and from regenerating uterine epithelium. On the other hand, it is present in regenerating skin epithelium where it is abundant, particularly in the superficial layer of the stratum spinosum but is absent from the basal layer (Bradfield, 1951). A comparable distribution of glycogen was found in regenerating oesophageal epithelium where it is present in the superficial layers, but again absent from the basal layer (McMinn & Johnson, 1958a). These authors also found a relatively large amount of glycogen in the new epithelium covering the floor of an "artificial" ulcer in the wall of the gall bladder (McMinn & Johnson 1957) and small quantities in regenerating rectal epithelium (McMinn & Johnson, 1958b). The significance of these findings is difficult to assess even if the possibility of species variation is ruled out - the present work is on the rat, Bradfield's on the guinea pig, and McMinn & Johnson's on/

/on the cat. Even within a single species (cat) glycogen accumulation in regenerating epithelium varies from large quantities in the oesophagus to very small quantities in the rectum. So far, no theory has been advanced which accounts satisfactorily for the accumulation of glycogen in regenerating epithelial cells. Scothorne & Scothorne (1953), reviewing this field, criticise the main views, namely:-

(a) that it is a mechanism of carbohydrate storage,

(b) that it is a degenerative phenomenon, and

(c) that it is an adaptation to conditions of reduced oxygen tension.

They found none of these offered a satisfactory explanation of the phenomenon. As the significance of glycogen accumulation in regenerating epithelium is unknown, it is difficult to draw any useful comparison between the epithelia in which it occurs and uterine epithelium in which it does not.

In the migrating uterine epithelium cytoplasmic basophilia was diminished. As the cytoplasmic basophilia of normal uterine epithelium is due to ribonucleic acid, it may be concluded that there is a diminution of this substance in the migrating cells. It should, however, be noted that the migrating cells are hypertrophied, and even if the ribonucleic/

/ribonucleic acid content remains unchanged, the substance will be spread out through a greater volume of cell, and thus the staining reaction to suitable dyes will be less intense. However, the reduction in cytoplasmic basophilia was so marked as to lead one to believe that there is an actual reduction in the ribonucleic acid content of the cells. In contrast, skin epithelium shows an increase in ribonucleic acid (Scothorne & Scothorne, 1953) but this does not occur until the epithelium contains mitotic figures, and it will be recalled that there is no evidence of mitosis in the new uterine epithelium. On the other hand, regenerating oesophageal and gall bladder epithelia show no significant change in ribonucleic acid content (McMinn & Johnson, 1957, 1958a). Thus, as with glycogen different epithelia behave in different ways and this may represent some basic difference between the various epithelia, or may be due to species differences.

In the material investigated here all the bare areas of the placental sites were re-epithelialised within thirty-six hours after littering. This rapid repair can be correlated with the small size of the bare areas. The speed with which epithelialisation/

/epithelialisation occurs, along with other changes, results in the uterus returning quickly to its normal structure and to being able to support a further pregnancy. In this connection it will be recalled that in the rat there is an early post-partum oestrus with ovulation occurring about eighteen hours after littering. If fertilisation takes place, nidation will occur about four days later, although in lactating animals this is delayed. Nevertheless, there is a possibility of the uterus having to support further embryos within a very few days after littering and the rapid restoration of its normal structure enables it to do this.

During involution, areas of the uterine epithelium show vacuolation. This can first be found at about eighteen hours, reaches its peak by thirty-six hours, and has subsided by forty-eight hours post-partum. There are two possible explanations. It may be that the vacuolation indicates degeneration of the epithelial cells consequent upon the decrease in size of the uterus with the resulting excess of cells. On the other hand, the vacuolation may be related to the first post-partum oestrus. Long & Evans (1922) described a similar vacuolation of the epithelial cells during oestrus and they referred to this as vacuolar degeneration. They described/

/described the vacuolation as appearing during their stage IV of the cycle. As ovulation takes place during stage III and vacuolar degeneration reaches its peak in stage IV, this corresponds to the events in the post-partum animal in which vacuolation reaches its maximum about eighteen hours after ovulation takes place. It should be noted that the vacuolar degeneration of oestrus begins after the distended uterus has collapsed so that it may be related to a surplus of epithelial cells.

Although vacuolar degeneration of the epithelial cells occurs, at no time, except at the placental site, were areas of endometrium found denuded of epithelium nor did there appear to be a universal shedding of epithelial cells into the lumen. In this respect the rat differs from the guinea pig (Hamilton, 1933). In this animal the uterine epithelium that lines the lumen before parturition is shed and replaced by new epithelium derived from the uterine glands. This shedding is apparent at about fifty-eight hours post-partum when epithelium in all stages of detachment can be found and when some areas of the endometrium are completely denuded. New epithelium can be seen extending as a single layer of cells from the mouths of the glands. This process of epithelial shedding/

/shedding and replacement can still be detected at eighty-four hours post-partum and is completed by between four to six and a half days after parturition. A similar process has been described by Bull (1949) in the rabbit. In this species degeneration of the epithelium commences late in pregnancy and is well marked at the time of parturition when, in some parts of the uterus, epithelium can be found separating into the lumen. This process is accompanied by the growth of a new layer of epithelium from that of the glands. By the end of the first day of the puerperium the replacement of the old by the new epithelium is complete. Thus, in three fairly closely related species three different patterns of behaviour of the uterine epithelium are to be discerned and this illustrates that it would be unwise to make any but the most general statement about species that have not already been investigated.

No evidence of the clearing of the placental site by the process described by Williams (1931) as exfoliation could be found. No undermining of the site by ingrowing endometrium with the subsequent separation and shedding of blood clot and placental vessels could be detected. Blood clot appeared to be/

/be organised and in many cases new epithelium was found spreading over it. The placental artery within the endometrium undergoes hyaline degeneration and is absorbed in situ. This is accomplished rapidly, usually by the fifth day, but it should be realised that only a small amount of the vessel lies within the endometrium. Bull (1949), in the rabbit, was unable to find any evidence of the undermining of thrombi and vessels by endometrium with their subsequent exfoliation. However, Deno (1937) in the mouse, states that there is "an exfoliation of polypoid masses of thrombosed vessels and other necrotic residium through undermining by the endometrium during the first few days post-partum". These findings, along with those noted in the previous paragraph, indicate that the pattern of post-partum repair varies considerably in different species. This may be related, at least in part, to differences in the details of placental development and to the time of occurrence of the first post-partum ovulation. Certainly it would be foolish to make any but the most general sssumptions about post-partum repair in a species that has not been studied.

SUMMARY/

SUMMARY

1. Rats were killed at intervals from 0 hours to 5 days after littering. Some of the changes that occur in the uterus during this period were studied, with particular attention being paid to the uterine epithelium.

2. The area of endometrium left devoid of epithelium by the separation of the placenta was re-epithelialised by cells which spread in from the existing marginal epithelium. There was no evidence of a transformation of stromal into epithelial cells.

3. The spreading epithelial cells did not contain glycogen and their cytoplasmic content of ribonucleic acid was reduced.

4. Re-epithelialisation of the site was completed within thirty-six hours.

5. There was vacuolation of the epithelial cells which was most marked at 36 hours post-partum. This is compared with the vacuolar degeneration that occurs during normal oestrus.

6. The behaviour of the non-placental epithelium in the rat is contrasted with that of the guinea pig and rabbit.

7. No evidence of exfoliation of the placental site was found.

PART II.

The involution of the metrial gland.

INTRODUCTION

It is to Selye & McKeown (1935) that one must turn for the first clear description of the structure in the rat to which they gave the name of 'metrial gland'. The metrial gland is "an enlargement in the wall of the uterus just below the placenta". It is situated in the mesometrial aspect of the uterine wall and in the adjacent part of the mesometrium. The typical cells of the metrial gland are arranged round the blood vessels which pass to the placenta. These cells are large and contain many granules. The granules are usually eosinophilic but sometimes there are also basophilic ones. Glycogen is always present in the cytoplasm. These characteristic cells will be referred to, for convenience, as metrial gland cells.

The gland begins to develop from about the 8th to 10th day of pregnancy. The metrial gland cells may arise from endothelium, from smooth muscle cells, or from fibroblasts.

During the puerperium metrial gland cells may behave in one of several different ways. Many of them degenerate; others may phagocytose pigment derived from blood clots or thrombi/

/thrombi, while still others become so loaded with lipoid material as to resemble cells of the corpus luteum or adrenal cortex. Round these lipoid containing cells the connective tissue becomes more and more hyaline in appearance. Lipoid containing cells are more numerous in lactating than in non-lactating animals. Likewise the hyaline connective tissue is better developed in lactating animals.

They suggest that the metrial gland should be regarded as a holocrine - endocrine gland. In applying the term endocrine they do not imply that the gland produces a hormone - indeed they were unable to find any true hormonal function although they think that its special development during lactation may indicate such activity - but use the term in its literal sense to imply liberation of products into the blood stream. They believe that the gland supplies nourishment to the embryo. They base this on the observation that if the embryo is removed and the placenta left in situ, then metrial gland cells accumulate in the lumen of the blood vessels and in the blood spaces of the maternal part of the placenta. They suggest that the cells accumulate because they would normally supply nourishment to the embryo but, that when the embryo is removed, there is nothing to use them up.

A/

/A structure similar to the metrial gland develops in the uterine wall during the involution of deciduomata - one metrial gland for each deciduoma. This finding indicates that the metrial gland cells are of maternal origin.

These original observations were extended by Selye, Borduas & Masson (1942) in an experimental study into the hormonal control of deciduoma and metrial gland development. On the morphological side, they noted that metrial gland cells are vacuolated. They found that the life span of the gland was markedly prolonged by the influence of progesterone. Indeed, evidence of proliferation of the gland could be found in animals treated with this hormone. On the other hand oestrogen, in the form of oestradiol, increased the rate at which involution of the gland took place. It thus appears that the metrial gland is under hormonal influence.

Bridgman (1948), in a paper devoted to the development of the rat placenta, notes the presence of metrial gland cells on the seventh day of pregnancy. They are few in number and are to be found in the central mesometrial region. In describing these cells, in addition to the characteristics already listed in the preceding paragraphs, she states that they/

/they are larger than other mesometrial cells, spherical and frequently binucleate. By the ninth day metrial gland cells show a tendency to cluster round the arteries. At sixteen days the gland is so large that it extends deeply into the mesometrium and forms a white coloured swelling on the surface. On the seventeenth day fat can be detected in the metrial gland cells. These cells are diminishing in number near to the placenta. A few of the cells, on the nineteenth day, took up trypan blue, a phenomenon which is, perhaps, related to their ageing.

Bridgman suggests that metrial gland cells may have two functions; one related to their high content of glycogen and the other to their granules. Like Selye and McKeown she states that the gland cells are to be found in the lumina of maternal arteries and that they may be carried down in the blood to the placenta where they give up their glycogen. This in turn may be stored in the placenta or transferred directly to the embryo. On the basis of their granularity she suggests that the cells may be hormone producers although there is no experimental evidence to support this. Both these views are similar to those put forward by Selye & McKeown on/

/on the function of the organ.

A study of the histochemistry of the gland, both during pregnancy and in the post-partum period, was reported by Baker (1948). He divides the life-cycle of the gland into three phases which overlap each other. The first phase is characterised by the presence of basophilia, the second by eosinophilic granules and glycogen, and the third by lipids. The basophilic phase begins on the sixth day of pregnancy when many of the connective tissue cells of the metrial gland area become round and begin to enlarge. At the same time the cytoplasm becomes basophilic and this is due to the accumulation of ribonucleic acid. Both the basophilia and the cell size increase during the next few days. Eosinophilic granules can first be detected in the cells on the eighth day and after the tenth day they accumulate rapidly. At this time the cells begin to form cuffs round the blood vessels in the mesometrium. Glycogen appears in the cells shortly after the granules. The glycogen is first found round the periphery of the cell while the eosinophilic granules lie near the centre, round the nucleus. Both granules and glycogen reach their maximum between the thirteenth to fifteenth days. As the second phase develops the cytoplasmic/

/cytoplasmic basophilia lessens and, by the time it reaches its peak, only a few scattered clumps of basophilic material are present and no young basophilic cells are to be found. After the fifteenth day, glycogen and granules are reduced in amount and by the twenty-first day the loss is significant. During this period, metrial gland cells may necrose in situ or be swept away in the arteries.

The third or lipid phase begins on the seventeenth day of pregnancy. Baker states that "lipid appeared at the periphery of the cuffs of granulated cells surrounding the metrial gland vessels". Thus lipid begins to appear as the eosinophilic granules and glycogen are declining. Baker does not make it clear whether lipid accumulates in metrial gland cells which have lost their granules and glycogen, or whether it is to be found in a second type of cell. This phase continues on into the puerperium when the lipid containing cells are located mainly in "the connective tissue between the blood vessels". From about the fifth to the twenty-second day of lactation the gland did not show any major change.

Velardo, Dawson, Olsen & Hisaw (1953) included an account of the life of the metrial gland in their study of the prolongation/

/prolongation of pseudopregnancy associated with deciduomata formation. They found that the metrial gland began to develop during the seventh or eighth day of pseudopregnancy and that it reached its maximum degree of development on the fourteenth day of pseudopregnancy. The gland maintained its size until the eighteenth day when small atrophic areas began to appear within it. By the twentieth day some regression of the gland was apparent and by the next day it was largely atretic with only a few typical metrial gland cells remaining. These writers do not mention any other type of cell in the gland.

Like Selye, Borduas & Masson (1942) they found that the administration of progesterone prolonged the life of the gland for approximately three days. They suggest that the gland may produce a hormone and that this may play some part in promoting the development of the mammary gland and thus enhancing lactation.

Little has been written about the comparative anatomy of the metrial gland. Selye & McKeown (1935) state that cells comparable to typical metrial gland cells have been described, under the names of "monster cells", "nephrophagocytes" and the like, in the uteri of many pregnant mammals including man, but that/

/that nearly always the cells are scattered throughout the uterus and do not form a compact organ as in the rat. Whether or not these scattered cells are identifiable with metrial gland cells is a matter of conjecture. Recently a comparative study has been made by Duke (1957). Unfortunately, he has not yet published a detailed account of his work and only a very short abstract is available. He found that there were histological changes in the meso-myometrial zone in a number of lagomorphs and rodents. The type, degree, and exact location of the changes varied from species to species. In some species a localised mass of large cells was found, while in others there was a hypertrophy of the cells of the perivascular connective tissue.

The present investigation is concerned with the metrial gland during the post-partum period. It will be recalled that Selye & McKeown (1935) thought that during the puerperium typical metrial gland cells either (1) degenerated, (2) phagocytosed pigment, or (3) became filled with lipoid. They also thought that there were more lipoid containing cells in lactating than non-lactating animals and that hyaline connective tissue was more abundant in the former. Apart from these observations on the puerperal gland there are only those/

/those of Baker (1948) who described the accumulation of lipid in some of the cells and who claimed that in lactating animals the gland of the twenty-second day was similar to that of the fifth day. The work reported here was carried out with the following object in view:-

1. To follow the structural changes that take place in the gland during its involution and to compare the findings with those already reported.

2. To determine the time taken for involution of the gland to occur.

3. To compare the glands in lactating and non-lactating animals in order to ascertain whether or not involution of the gland is influenced by lactation.

4. To study the staining reactions of typical metrial gland cells with the P.A.S. technique and with toluidine blue.

MATERIAL AND METHODS

For histological purposes the material employed in the last section was utilised. This was supplemented as follows. Virgin rats were mated and then removed from the male. After littering some animals were allowed to feed their offspring while/

/while from others the babies were removed. These rapidly ceased to lactate. Both lactating and non-lactating rats were killed at 5, 10, 15, 20, and 25 days post-partum. Portions of uteri and mesometrium containing metrial glands were excised and fixed in either Bouin's fluid or Rossman's fluid. Transverse sections were prepared and mounted in series. Series fixed in Bouin's fluid were stained either with haematoxylin and eosin or by Van Gieson's method. Rossman fixed series were stained with .5% toluidine blue or by the P.A.S. technique. Controls were prepared in the same way as in the previous section. A few specimens were fixed in absolute alcohol and after sectioning were subjected to the Gomori technique for alkaline phosphatase.

In a second set of experiments virgin rats were mated and then taken from the males. After littering the offspring were removed from some of the females so that both lactating and non-lactating animals were available. Animals of both types were killed at 5, 10, 15, 20 and 25 days post-partum. A number of animals were also killed immediately after littering. A minimum of three animals was employed in each group. As soon as possible after killing an animal, the uterus was removed and spread out on a cork mat. The size of the metrial glands/

/glands was then measured using a binocular dissecting microscope with a micrometer eye-piece on one side. The measurements are recorded in the units of the micrometer eye-piece's scale. At the magnification used, twelve of these units correspond to one millimetre. Two measurements were made on each gland. One was made of its length (i.e. in a direction parallel to the uterine horn) and one of its width (i.e. in a direction at right angles to the uterine horn).

OBSERVATIONS

1. The structure of the gland.

The metrial gland at the time of littering

At the time of littering the metrial gland is a more or less spherical structure, about four millimetres in diameter, which extends from the mesometrial aspect of the uterine wall into the adjoining mesometrium. It thus forms a substantial swelling which usually, like the rest of the uterine wall, is greyish in colour.

In the rat the outer, longitudinal muscle coat not only surrounds the uterus, but is continued for a considerable distance into the mesometrium. This occurs in such a way that there is a layer of muscle immediately deep to each surface/

/surface of the mesometrium, while separating the two muscle layers there is a layer of connective tissue. Nearly all the uterine blood vessels, particularly the larger ones as they pass through the mesometrium towards the uterus, come to lie in the connective tissue between the two layers of smooth muscle. Near to the uterus there is an increase in the amount of connective tissue between the two layers of the longitudinal muscle coat. This zone of connective tissue is limited at its uterine end by the intact circular muscle coat. In transverse section this mass of connective tissue is triangular in outline, and for this reason the zone has been called the mesometrial triangle (Selye & McKeown, 1935). It will be understood that the connective tissue of the triangle is bounded on two sides by the longitudinal muscle coat and on the third side or base by the circular muscle coat, (Fig. 20).

It is in the mesometrial triangle that the metrial gland chiefly develops. Here it surrounds the blood vessels traversing the mesometrium but it overflows from this region, and extends into the adjoining circular muscle coat (Fig. 21). At a placental site, fibres or small groups of fibres of the circular muscle coat are separated from each other, sometimes widely, by cells of the metrial gland. In transverse sections, these/

/these muscle fibres appear to form a trellis although in three dimensions it is probable that they have an arrangement which resembles rather a sponge with the spaces of the sponge filled with gland cells. The placental artery (or arteries) passes through a gap in the circular coat. Round the margin of the gap the muscle fibres have turned at right angles to their usual direction and run parallel to the course of the artery.

The placental artery can be differentiated from the other large blood vessels passing through the metrial gland by the abundance of eosinophilic, P.A.S. positive material in its wall. "Encapsulated" giant cells, similar to those found in its wall as it runs through the endometrium, and described in the previous part of this work, are present in or near to its wall. The endothelial cells lining its lumen are often large, as those of other vessels in the region may be. The artery, characteristically, has a mass of thrombus in its lumen. Other large and dilated vessels can be seen in the gland.

Typical granulated metrial gland cells, as described by Selye & McKeown, are readily identifiable within the gland. They can be recognised by their large size, vacuolation, and eosinophilic/

/eosinophilic granules while they are frequently binucleate (Fig. 22). No basophilic granules were found in the haematoxylin and eosin material. With toluidine blue, all the granules stain metachromatically (Fig. 23). The P.A.S. reaction colours many granules of varying size in the cell (Fig. 24). In control sections treated with diastase before staining, the cells still contain P.A.S. positive granules (Fig. 25) but these are less numerous than in the untreated sections. The residual P.A.S. positive granules correspond in size and number to the eosinophilic granules which are so characteristic of the cell, and it is undoubtedly these which stain in the control sections. The P.A.S. positive material that is lost by treatment with diastase is, of course, glycogen. In the specimens treated by the Gomori technique for alkaline phosphatase, no evidence of this enzyme was found in the metrial gland cells.

Typical metrial gland cells form only a relatively small proportion of the total cell content of the gland (Fig. 26). While they are more numerous round the vessels, they are to be found scattered irregularly throughout the gland. Large numbers of highly vacuolated cells, which are smaller than the/

/the metrial gland cells, are to be seen in the intervals between the vessels (Fig. 27). These cells, certainly, correspond to the lipid containing cells described by Baker (1948) and their vacuolation is undoubtedly due to the loss of lipid during histological processing. It is presumably to these cells that Selye and McKeown refer when they describe epitheloid cells in the gland. Scattered throughout the gland there are other cells which resemble those of the endometrium. These cells are not vacuolated nor do they have granules in their cytoplasm. They are particularly numerous in that part of the gland which extends into the circular muscle coat.

An occasional mast cell is encountered in the gland. These are easily distinguished from metrial gland cells. The mast cells are smaller, show no indication of vacuolation, and are tightly packed with granules which are most readily discerned when stained metachromatically with toluidine blue (Fig. 28).

<u>The metrial gland at one day post-partum.</u>

The metrial gland is much smaller at twenty-four hours than it was at the time of parturition. This can be seen by comparing figures 21 and 9, although allowance must be made for the fact that figure 21 is at a greater magnification than figure 9.

The/

/The blood vessels passing through the gland are large and dilated, as at the time of littering. The placental artery is easily identified and is filled with thrombus. The cells present in the gland are the same as those already described. Typical metrial gland cells are still most numerous near to the vessels, although they may occur anywhere within the gland. Many vacuolated cells are present, and as before these lie in the intervals between the blood vessels. There are also many of the smaller non-vacuolated cells and a few mast cells.

The metrial gland at two days post-partum

The decrease in size of the gland continues (compare figures 9 and 17 which are at the same magnification). The dilatation of the blood vessels, which was so marked earlier, is subsiding. The wall of the placental artery is undergoing destruction and there is organisation of the blood clot within its lumen. The fibres of the circular muscle coat in the neighbourhood of the gland are now nearer together. The hole in this coat for the placental artery is still present and the remains of the distal end of the vessel lie in it. "Encapsulated" giant cells are present in or near to the vessel's wall. The same cell types persist in the gland. However, typical metrial gland/

/gland cells by this stage are almost completely limited to the half of the gland farthest away from the uterus. They are still, in this region, fairly numerous and they remain close to the vessel walls (Fig. 20). Small granules of a brownish-yellow pigment are present in the cytoplasm of some of the small non-vacuolated cells. Similar pigment containing cells are present in the endometrium at the placental site and were mentioned in Part I. A full account of the pigment is given in Part III.

The metrial gland at four days post-partum

The gland has still further decreased in size. The blood vessels passing through the gland are less engorged. The disintegration of the placental artery is well advanced. In places the wall has entirely disappeared, while in others it is still complete (Fig. 31). The remains of the vessel wall consist largely of P.A.S. positive material. The distribution of "encapsulated" giant cells is unchanged. The fibres of the circular muscle coat are nearer together although the coat is still wider than in the intersite regions.

Typical metrial gland cells are much less numerous than in the earlier stages. They are now limited to the end of the/

/the gland farthest from the uterus (Fig. 30). Those that persist retain their close relationship to the vessels. There are many vacuolated cells present but the individual vacuoles appear to be smaller. There are also many more pigment containing cells. They are to be found, especially, round the remains of the placental artery and lie along the track of the artery through the circular muscle coat. The individual pigment granules are larger and many of the cells are more densely packed with them.

The metrial gland at five days post-partum

Specimens from both lactating and non-lactating animals were available for this and for subsequent stages. At five days the glands from the two types of animal are of about the same size and are similar to each other in structure, which closely resembles that of the four day glands. The involution of the gland is well advanced and the great reduction in size that has taken place since the time of littering can be seen by comparing figures 21 and 32. Apart from a slight decrease in size the main difference between five and four day glands is that the former contain many fewer metrial gland cells. The few that remain are limited to the part of the gland distant from the uterine wall.

The/

The metrial gland at ten days post-partum

The glands from lactating and non-lactating animals at this stage are similar to each other (Fig. 33). Very few blood vessels, none of a large size, are present in the gland. This forms a marked contrast with the original highly vascular nature of the organ. The placental artery has almost completely disappeared but a few fragments of its wall - formed of P.A.S. positive material - remain in both lactating and non-lactating animals (Fig. 34). No typical metrial gland cells could be found and vacuolated cells are much fewer in number. Pigment containing cells are by far the commonest type of cell in the gland. They may contain only a few granules but, for the most part, they are densely filled, frequently with large particles. Their distribution is as before and they can be traced from the gland through the opening in the circular muscle coat for the placental artery into the endometrium. An occasional "encapsulated" giant cell was found within the gland but they were more often encountered in the adjacent endometrium.

The metrial gland at fifteen days post-partum

Glands from lactating and non-lactating animals continue to resemble each other (Fig. 35). Involution is nearly complete and the glands are very much reduced in size. Viewed with the naked/

/naked eye a gland forms a very small brown swelling in the mesometrium adjacent to the uterine wall. They consist almost entirely of pigment containing cells, but a very small number of vacuolated cells are present. There are also one or two "encapsulated" giant cells in the gland although these may be fairly numerous in the endometrium (Fig. 36). When present these appear to be healthy and they retain their P.A.S. positive "capsule". The placental artery has completely disappeared although its course is marked, at least in part, by new connective tissue round which pigment cells are congregated.

The metrial gland at twenty days post-partum

There is no difference between lactating and non-lactating animals (Fig. 37). The glands have completely involuted but the position they occupied in the mesometrium is marked by a mass of brown pigment. The sections show very large numbers of pigment containing cells in these zones. No vacuolated or "encapsulated" giant cells were found.

The metrial gland at twenty-five days post-partum

The appearances are the same as those at twenty days. Indeed, the pigment containing zones which mark the sites of metrial glands may persist for very long periods, possibly for the rest of the animal's life.

TABLE I.

	LACTATING			NON-LACTATING		
Age	No. of sites	Mean Length	Mean Width	No. of sites	Mean Length	Mean Width
0 days	54	44.4±6.3	31.1±4.6	---	---	---
5 days	58	30.2±6.3	19.4±4.2	45	31±6.2	21±5.2
10 days	44	19.8±2.4	12.8±1.9	46	19.3±3.7	12.7±2.4
15 days	33	16.8±2.4	12±2.3	39	16.3±2.9	13.2±3
20 days	52	15.1±2.6	10.5±2.2	38	14.3±2.1	10.6±1.7
25 days	46	15.1±3.6	8.5±2.3	31	15.5±2.6	11.2±1.6

2. Measurements of the metrial gland

These are given in Table I. It should be noted that the measurements for the twenty and twenty-five day animals refer not so much to the metrial gland but to the dimensions of the pigment zone which mark its site.

DISCUSSION

It is clear, both from the histological sections and from the measurements, that the involution of the metrial gland is the same in both lactating and non-lactating animals. At any given time the histological appearance of the glands is similar in the two groups. There is no evidence to support Selye & McKeown's view that lipoid containing cells are more plentiful in the glands of lactating animals or that hyaline connective tissue is more abundant in this group. An inspection of Table I shows that the mean length and width of glands of lactating and non-lactating animals of the same post-partum age closely correspond to each other. This suggests that involution is occurring at the same rate in the two groups.

The involution of the gland takes place quickly. This is illustrated by the measurements given in Table I and by the sections. The gland rapidly decreases in size during the first/

/first few days post-partum and by the tenth day it is about half as long and wide as at the time of littering. By fifteen days involution is almost complete although a few "encapsulated" giant cells and occasional lipoid containing cells still persist. At twenty days involution has finished and the site of the gland is now marked by a brownish patch of pigment. These findings do not support Baker's contention that the fifth and twenty-second day glands in lactating animals closely resemble each other.

Typical metrial gland cells contain glycogen, the loss of which during histological preparation presumably results in the vacuolation which is so often found in these cells. The specific metrial cell granules are eosinophilic, metachromatic with toluidine blue, and strongly P.A.S. positive. The intense staining of the granules with the P.A.S. technique suggests that they contain a mucopolysaccharide. This could also account for their metachromasia. The marked eosinophilia of the granules is presumably due to the present of basic groups. Since these conclusions were reached the papers of Ellis (1957) and Wislocki, Weiss, Burgos, and Ellis (1957) have appeared. They also found the specific granules to be P.A.S. positive and metachromatic with toluidine blue and suggest that they contain a mucopolysaccharide/

/mucopolysaccharide conjugated with an alkaline protein.

All the specific granules in metrial gland cells were eosinophilic. No basophilic ones were found. Selye & McKeown (1935), however, claimed that both eosinophilic and basophilic granules could occur together in the same cell when stained with eosin and aniline blue. As Wislocki et al. (1957) point out, this assertion was based on the misconception that aniline blue is a basic dye. In fact, aniline blue is an acid dye and will thus stain basic substances. That it stains some of the granules, while most are stained by eosin, merely indicates that the iso-electric point of the individual granules varies somewhat from one to another so that their affinity for individual acid dyes varies.

At the time of littering typical metrial gland cells form only a small proportion of the total cell population of the gland, while none is found after the fifth day of the puerperium. They disappear from the uterine end of the gland first and persist longest at the opposite end. There was no indication that typical metrial gland cells ever phagocytose pigment or become lipoid bearing in the way suggested by Selye and McKeown. While disintegrating and dying cells of this type were not identified, it must be presumed that they degenerate as the gland/

/gland regresses. The lipoid containing cells are quite distinct from the metrial gland cells. They are smaller, never contain granules, and are always uninucleate. Ellis (1957) suggests that they may arise from fibroblasts or fibroblast-like cells within the gland. The third main type of cell found in the gland is that which takes up the pigment. These should be classified as macrophages and are similar to the macrophages described in the uterus of the mouse by Deno (1937).

It is not possible, with the present material, to determine whether the "encapsulated" giant cells are of foetal or maternal origin. They arise, in particular, from the wall of the disintegrating placental artery but, as they are to be found at the time of littering, it is possible that they may have arisen from trophoblast that has extended along the vessel. Mossman (1937) states that giant cells may remain in the uterine wall for long periods after parturition. "Encapsulated" giant cells persist for about two weeks. The intensive P.A.S. positive reaction of the "capsule" (which is not abolished by diastase) and the fact that it is non-metachromatic with toluidine blue indicates that it is formed by a neutral mucopolysaccharide. Cells of this type have not, hitherto, been described.

It will be noted that the placental artery, as it passes through/

/through the gland, disintegrates and is replaced by connective tissue. There is no evidence of the recanalisation of the vessel in the way described by Goodall (1910) in man.

The present investigation offers no suggestion as to the function of the gland. As, however, the gland regresses rapidly during the puerperium it may be presumed that it is concerned with the pregnancy itself rather than with the conditions occurring in the post-partum period, such as lactation.

SUMMARY

1. Metrial glands of lactating and non-lactating rats involute in the same way.

2. Involution of the gland is rapid in the early stages, is nearly complete by fifteen days, and is finished by the twentieth day of the puerperium.

3. The specific granules of the metrial gland cells possibly contain a mucopolysaccharide joined to an alkaline protein.

4. Metrial gland cells form only a small proportion of the total cell population of the gland. They do not phagocytose pigment or become lipoid containing.

5./

5. "Encapsulated" giant cells persist for about two weeks.

6. The placental artery is absorbed in situ.

PART III

Some characteristics of the uterine pigment

INTRODUCTION

Selye & McKeown (1935) and Baker (1948) have noted the presence of pigment in the metrial glands of the uterus of the rat after littering. They found that it contained iron and concluded that it was haemosiderin. Baker also stated that some of it stained with Sudan black in frozen sections. Attention has been drawn to this pigment in the earlier sections of this thesis. It has been pointed out that the pigment appears in the region of the placental sites, both in the endometrium and in the metrial glands, on about the second day post-partum and that it is positive to the P.A.S. test. As haemosiderin is not usually considered to be sudanophil or P.A.S. positive this investigation was undertaken in order to study the characteristics of the pigment in greater detail. Of the various techniques employed, many are such as could be expected to give a positive result with a substance containing lipid.

MATERIAL AND METHODS

Two rats were killed on the 10th day after littering and two on the 20th. On examining their uteri there was a small brown discolouration of the mesometrial part of the uterine horn/

/horn and mesometrium at each placental site. Portions of the horns containing placental sites were removed and fixed in neutral 10% formalin for 24 hours. They were dehydrated in alcohol, cleared in cedarwood oil followed by benzene, and embedded in paraffin wax. Sections were cut, mounted, and treated with one or more of the following techniques:-

1. For routine histological study haematoxylin and eosin staining was used.
2. Staining for 10 minutes in 0.5% aqueous toluidine blue.
3. Perl's reaction for ferric iron.
4. The P.A.S. test, with diastase-treated sections as controls.
5. The performic acid/Schiff (PFA) reaction, as described by Pearse (1951).
6. The peracetic acid/Schiff reaction, by Lillie's technique (1954).
7. Oxidation for one hour in 4% chromic acid followed by 45 minutes in Schiff's reagent.
8. Exposure of a section without previous oxidation to Schiff's reagent for periods up to 18 hours.
9. Staining in a saturated solution of Sudan black in 70% alcohol for 3 hours, rinsing in 70% alcohol, and mounting in/

/in glycerine jelly.

10. The long Ziehl-Neelsen method as employed by Pearse (1953).

11. Gomori's chrome alum/haematoxylin method.

12. Mallory's technique for haemofuscins.

13. The ferric ferricyanide reduction test, by Lillie's method (1954).

14. (a) Bromination, and (b) treatment with 5% chromic acid for 1 hour, followed by the ferric ferricyanide reduction test.

15. The Masson-Fontana alkaline silver technique.

16. The Gmelin test for haematoidin.

17. Bleaching in hydrogen peroxide for 48 hours.

18. Exposure to ultra-violet light for fluorescence.

OBSERVATIONS

The distribution, appearance, and properties of the pigment were the same in all the specimens that were examined, whether from the 10 day or 20 day rats. Pigment was found in large quantities at each of the old placental sites. All the pigment was intracellular and the cells containing pigment/

/pigment were grouped either in the remains of the metrial gland situated in the mesometrial triangle or in the mesometrial half of the endometrium, where they lay close to the myometrium (Fig. 34). The pigment was yellow to light brown and formed granules that varied in size from fine particles to those that were 10 or more microns in diameter.

In assessing the results of the various techniques that were employed, allowance must be made for the original colour of pigment because it is on to this that any second colour is superimposed. For instance, a method which gives a blue colour as a positive may with the pigment produce a green or greenish-blue shade. The observations described in detail below are summarised in Table II.

The pigment was not stained by either haematoxylin or eosin, but with toludine blue it became a deep greenish-blue, in parts appearing almost black. Perl's test for ferric iron gave a strong positive reaction and resulted in all the pigment granules becoming a deep blue, while a lighter bluish-green shade was imparted to the cytoplasm of the cells containing pigment. The pigment reacted strongly with the P.A.S. technique, the granules becoming a deep brownish-red which could be readily distinguished from their normal colour.

The/

/The same colouring of the pigment was also found in the diastase controls, and thus the possible presence of glycogen was excluded. The pigment remained uncoloured after exposure to Schiff's reagent for 18 hours when no previous oxidation was employed. This shows that the P.A.S. method was giving a true positive reaction and was not responding to the presence of preformed aldehyde groups in the pigment. The P.F.A. reaction, the peracetic acid/Schiff reaction, and oxidation with chromic acid followed by Schiff's reagent all failed to produce any colouring of the pigment. It was sudanophil, most of the particles giving a greyish colour with Sudan black, although some of the larger ones became a deep black. It was acid-fast, resisting the acid alcohol differentiation in the long Ziehl-Neelsen method, so that while the background was a pale pink the granules were a deep brownish-red. With the chrome alum/haematoxylin technique of Gomori the pigment took up some of the stain, and although the original yellow colour was not completely abolished, the effect of the haematoxylin could be easily recognised. After alcoholic differentiation in Mallory's technique for haemofuscins some of the basic fuchsin was retained by the pigment granules which/

/which became a light reddish-brown. With the ferric ferricyanide reduction test the section as a whole was coloured a pale green but the pigment was more markedly affected. Nearly all the granules were greenish-blue. A positive reaction with this test is normally indicated by a blue colour. Nevertheless, the granules are regarded as being positive because they colour (although for the most part in green) much more deeply than the background. After bromination and the chromic acid treatment the granules remain uncoloured by the ferric ferricyanide method. Treatment with alkaline silver resulted in slight darkening of the granules which became browner. The Gmelin test for haematoidin was negative while the pigment was not bleached by hydrogen peroxide. There was no fluorescence under the microscope when ultra-violet light was employed.

DISCUSSION

This investigation confirms the findings of Selye & McKeown and of Baker that the pigment contains ferric iron. These writers were also undoubtedly right in regarding the pigment as haemosiderin as this substance is by definition a pigment which exhibits one or more of the reactions of ferric/

TABLE II

Technique employed	Colour of Pigment	Comments.
Haematoxylin and eosin	unchanged	--
Toluidine blue	deep greenish-blue	basophil with a basic dye
Perl's method	deep blue	ferric iron present
Periodic acid/Schiff	brownish-red	a strong positive
Schiff's reagent	unchanged	no preformed aldehyde group present
Performic acid/Schiff	unchanged	negative
Paracetic acid/Schiff	unchanged	negative
Chromic acid/Schiff	unchanged	negative
Long Ziehl-Neelsen	brownish-red	acid fast suggesting a lipid
Sudan black	grey to deep black	suggests presence of a lipid
Gomori's chrome alum/ haematoxylin	greenish-blue	a positive reaction
Mallory's technique	pale reddish-brown	a weak positive
Ferric ferricyanide	green to greenish-blue	a positive reaction
Bromination and ferric ferricyanide	unchanged	negative
Chromic acid and ferric ferricyanide	unchanged	negative
Alkaline silver	brown	a weak positive
Gmelin test	unchanged	no haematoidin present
Hydrogen peroxide	unchanged	an absence of bleaching
Fluorescence (U.V.)	--	no fluorescence observed.

/ferric iron (Lillie, 1954). Also, the uterine pigment resists bleaching by hydrogen peroxide and this is a characteristic of haemosiderin. According to Lillie (1954) three varieties of haemosiderin can be recognised, one of which stains with basic dyes as does the uterine pigment with toluidine blue. Haemosiderin is one of the breakdown products of haemoglobin (Florey, 1954) and the pigment in the rat's uterus is certainly derived from the blood, as it is found almost entirely at the old placental sites into which haemorrhage occurs at the time of separation of the placenta.

Unlike the pigment in the rat's uterus, human haemosiderin is not P.A.S. positive (Lillie, 1950). According to Pearse (1953) haemosiderin is not acid-fast, does not stain with Sudan black nor colour with Gomori's chrome alum/haematoxylin technique, is negative in the ferric ferricyanide test, and does not darken alkaline silver. All these techniques, as well as Mallory's technique for haemofuscins give positive results when applied to the uterine pigment. It is thus clear that while the uterine pigment may be regarded as a haemosiderin, it possesses several properties that are not usually associated with this substance.

The colouring with Sudan black suggests that there is a lipid/

/lipid element present, and other positive reactions, as with the P.A.S. reaction and the Ziehl-Neelsen technique, support this. A comparison may be made between the uterine pigment and the lipogenic pigments, the properties of which have been summarised by Gomori (1952), Pearse (1953), and Lillie (1954). These pigments, which are fairly widely distributed, are known by a variety of names such as ceroid, luteolipin, wear and tear pigment, and lipofuscin. They all arise as oxidation products of a lipid precursor (Pearse, 1953). Their reactions with different histological and histochemical techniques vary and this may be related to the degree of oxidation that they have undergone. Like the pigment in the rat's uterus they withstand lipid solvents and can be identified in paraffin sections. Again, many of the lipogenic pigments such as adrenal lipofuscin are acid-fast and P.A.S. positive, react with ferric ferricyanide, and colour with oil-soluble colouring agents in paraffin sections. They may darken with alkaline silver, colour with Gomori's chrome alum/haematoxylin, and retain basic fuchsin in Mallory's technique. In these respects the uterine pigment resembles them. It differs from some such as ceroid which is positive/

/positive to peracetic acid/Schiff and fluoresces in ultra-violet light. Nor are the lipogenic pigments usually associated with iron, although ceroid may occasionally contain a few granules of pigments containing iron.

The negative Gmelin reaction indicates that haematoidin, the iron free breakdown product of haemoglobin, is absent. Although the uterine pigment in some respects resembles melanin it is not melanin, for granules of the latter substance are darker in colour, do not stain with Sudan black, nor give the P.A.S. reaction, and are usually bleached within 48 hours by hydrogen peroxide.

It thus appears that the uterine pigment should be regarded as a variety of haemosiderin in which the substance containing iron is mixed or perhaps combined with a lipid component that has many properties in common with the lipogenic pigments.

SUMMARY

1. A yellowish-brown pigment was found at the old placental sites in rats killed at 10 and 20 days after littering.

2. The pigment contained ferric iron and therefore may be regarded as haemosiderin.

3./

3. Other properties of the pigment suggest that there is also a lipid component present. Lipid is not usually associated with haemosiderin.

4. The lipid component behaves in many ways like the lipogenic pigments.

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Fig. 1. Transverse section of the uterus immediately after littering. Note the blood clot at the placental site and the folding of the mucosa. H. & E.

x 6·5.

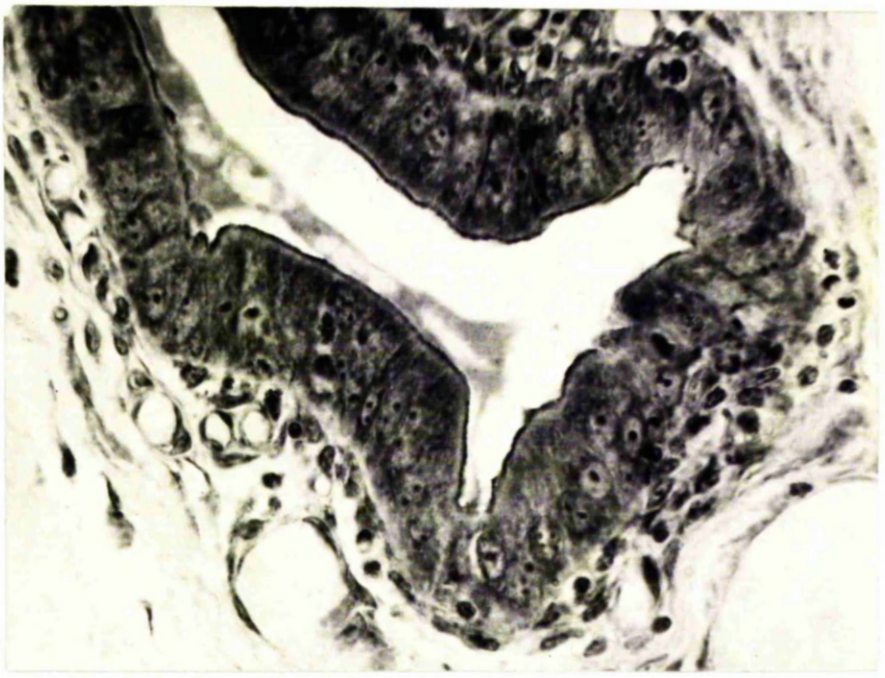

Fig. 2. Part of a transverse section of uterus at 0 hours post-partum stained with toluidine blue. Note the intense staining of the cytoplasm of the epithelial cells.

x 485.

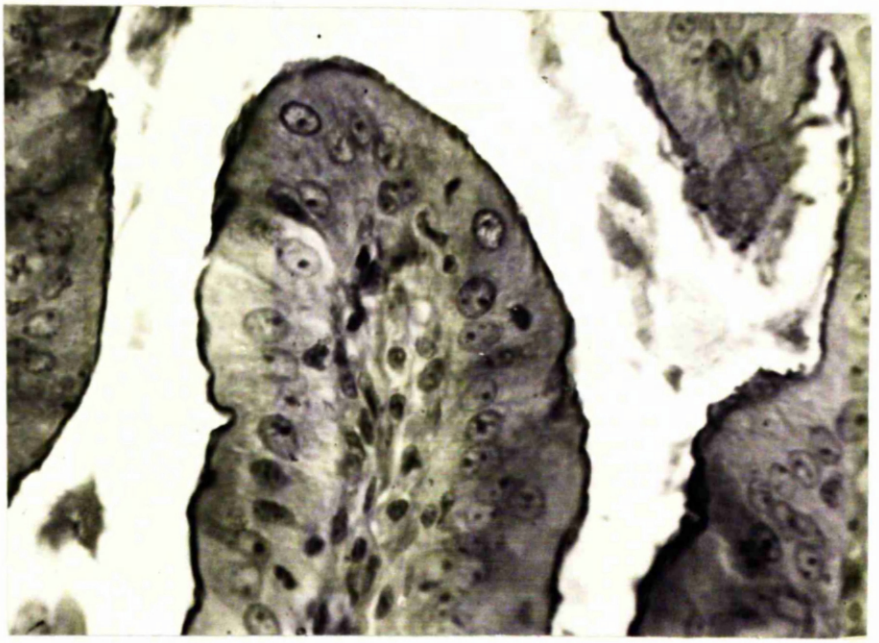

Fig. 3. Portion of a transverse section of uterus from the same animal as Fig. 2, stained with toluidine blue after extraction with perchloric acid. Note the decrease in cytoplasmic basophilia.

x 485.

Fig. 4. Transverse section of uterus from the same animal as Fig. 2, stained by the P.A.S. technique and showing cytoplasmic granules, surface P.A.S. positive layer and basement membrane.

x 485.

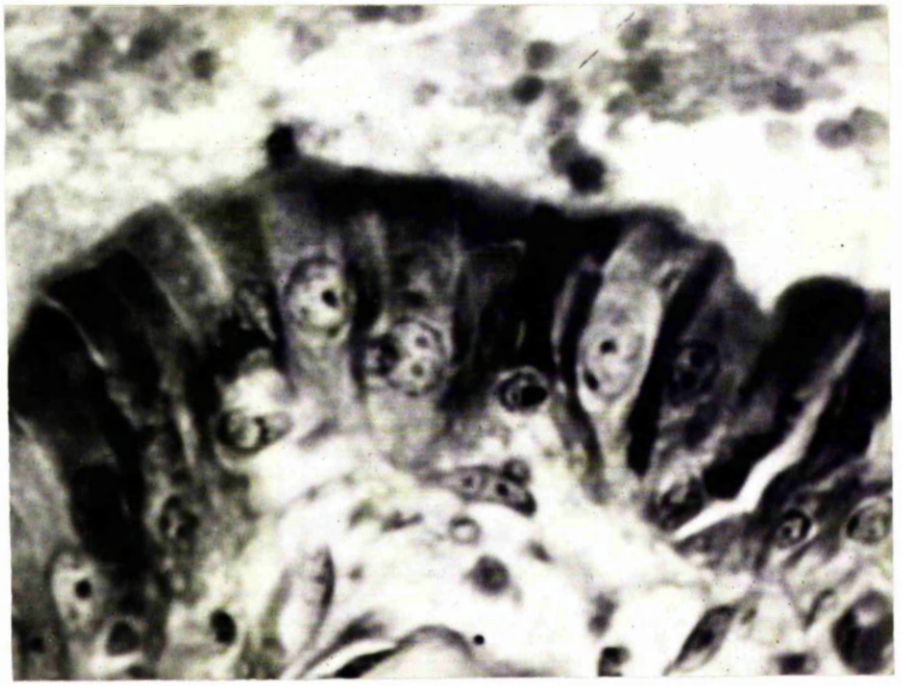

Fig. 5. Transverse section of uterus at 0 hour post-partum. Note the darkly stained "rod" cells. H. & E.

x 1050.

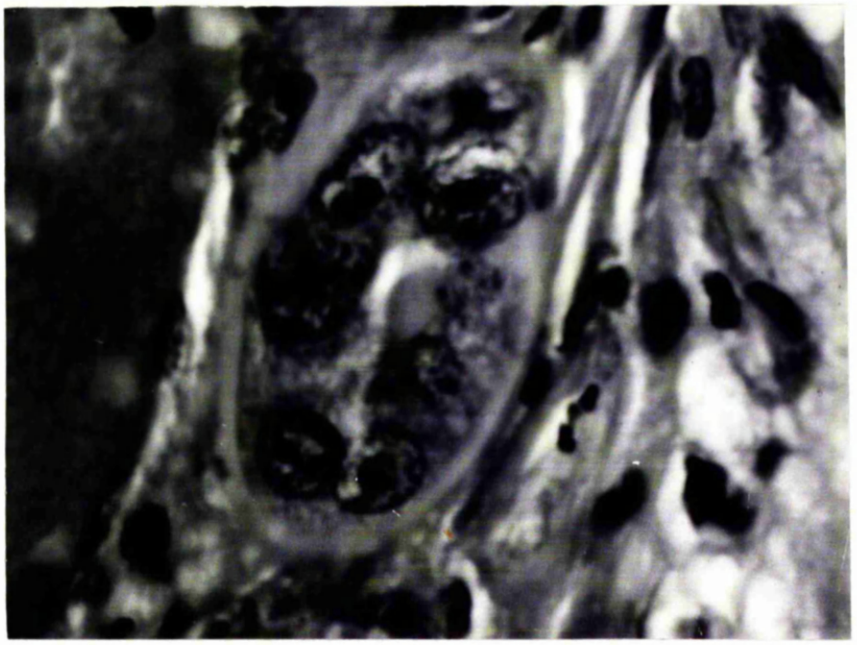

Fig. 6. An "encapsulated" multinucleate giant cell. H. & E.

x 1050.

Fig. 7. Part of a transverse section of uterus 6 hours after littering showing the edge of the "bare area" at the placental site. H. & E.

x 105.

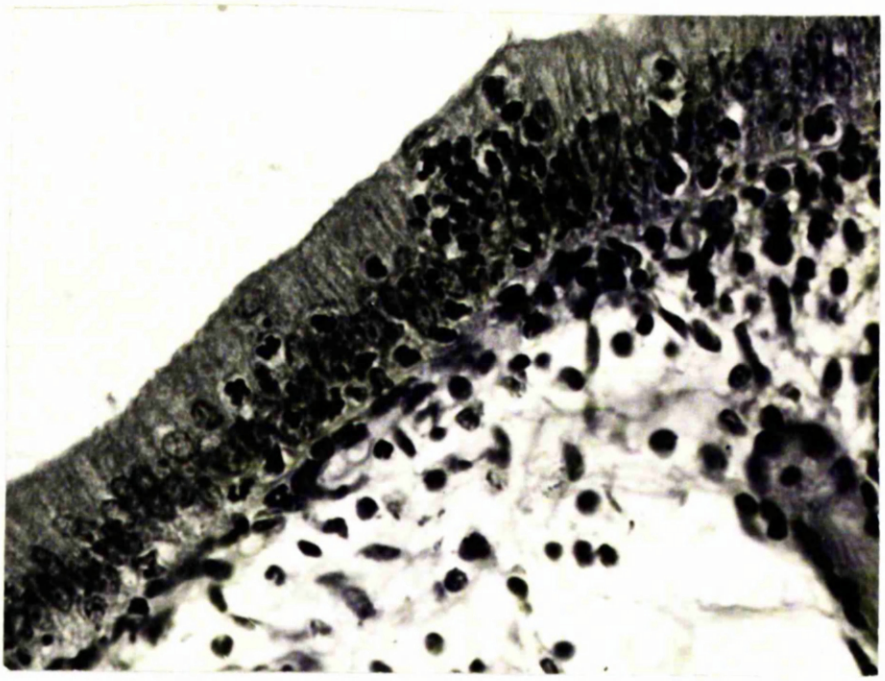

Fig. 8. Transverse section of uterus 12 hours post-partum. Note the infiltration of polymorphonuclear leucocytes in the epithelium. H. & E.

x 485.

Fig. 9. Transverse section of a uterus at 24 hours post-partum. The folding of the endometrium is clearly shown. H. & E.

x 12.

Fig. 10. Transverse section of a placental site at 24 hours post-partum. The site has been re-epithelialized and the hypertrophic epithelial cells are resting on a mass of blood and fibrin clot. Toluidine blue.

x 105.

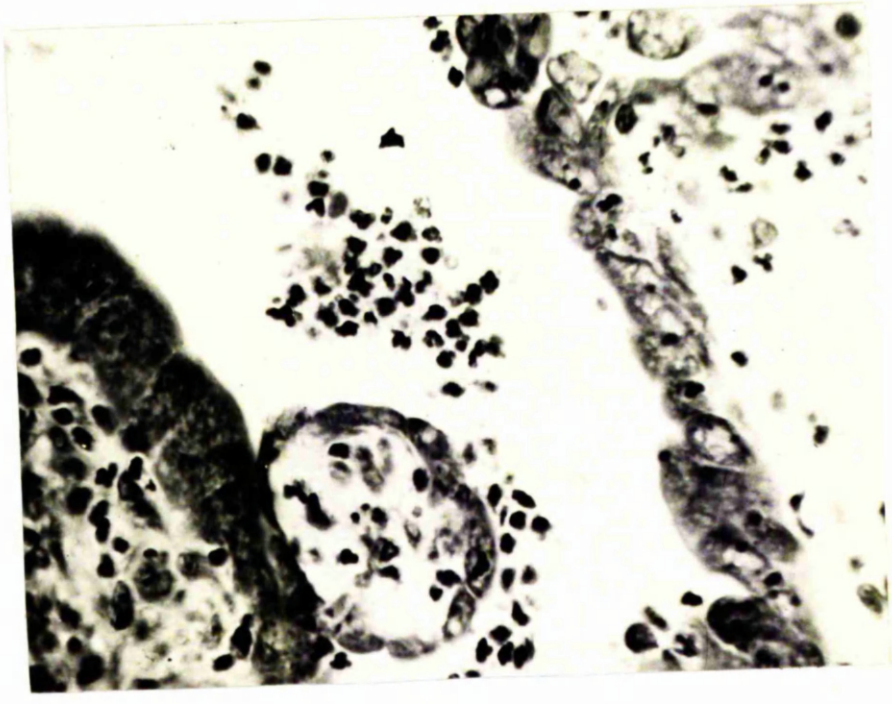

Fig. 11. Part of Fig. 10 at a higher magnification. Normal epithelium is to the left and hypertrophic epithelium is to the right. Note the reduced cytoplasmic basophilia of the hypertrophic cells. Toluidine blue.

x 485.

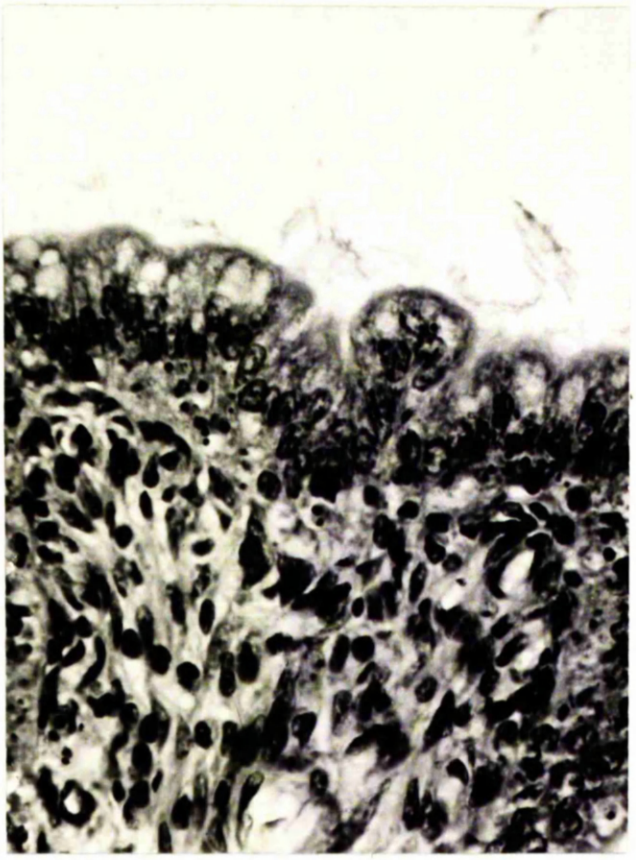

Fig. 12. Transverse section of uterus from the same animal as Fig. 10. The epithelial cells show early vacuolation. H. & E.

x 485.

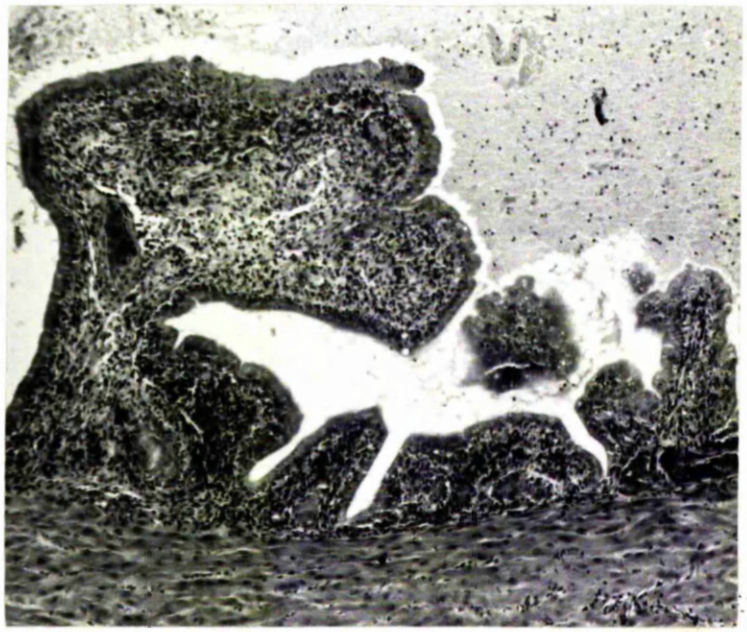

Fig. 13. Endometrium of a 24 hour post-partum animal to show dilatation of the blood vessels. H. & E.

x 75.

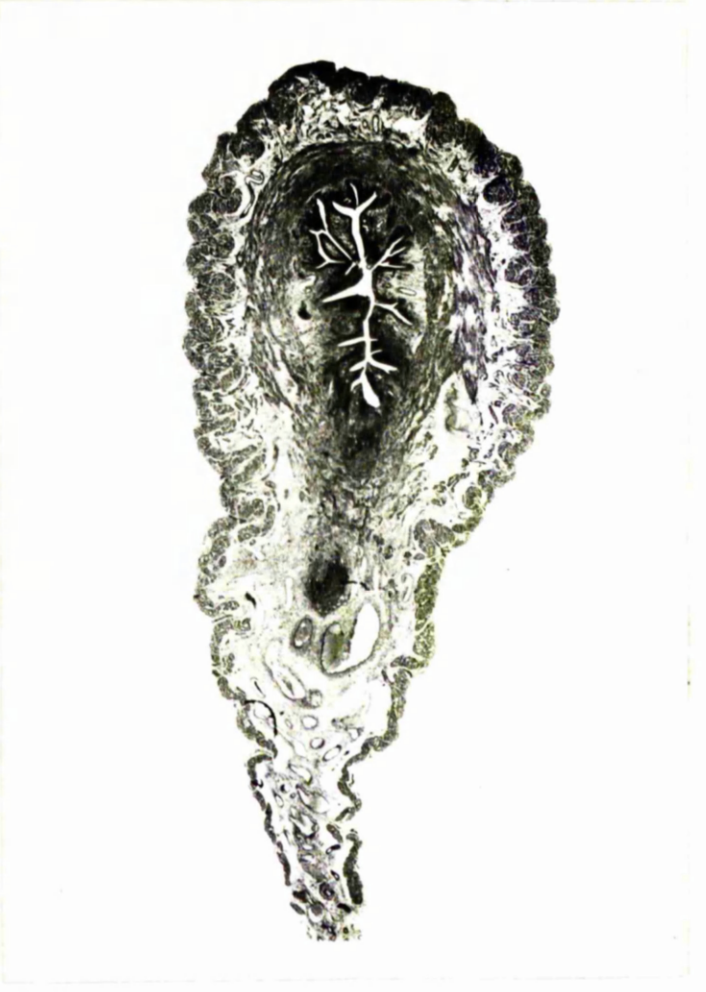

Fig. 14. Transverse section of the uterus at 36 hours post-partum, to show the folding of the endometrium. H. & E.

x 12.

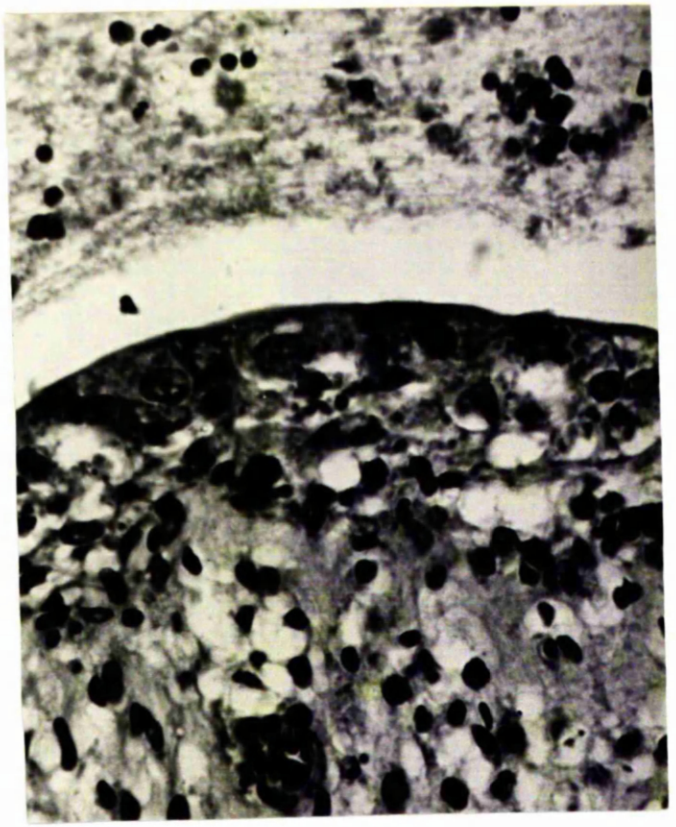

Fig. 15. Transverse section of the uterine epithelium at 36 hours to show the extensive vacuolation of the cells. H. & E.

x 485.

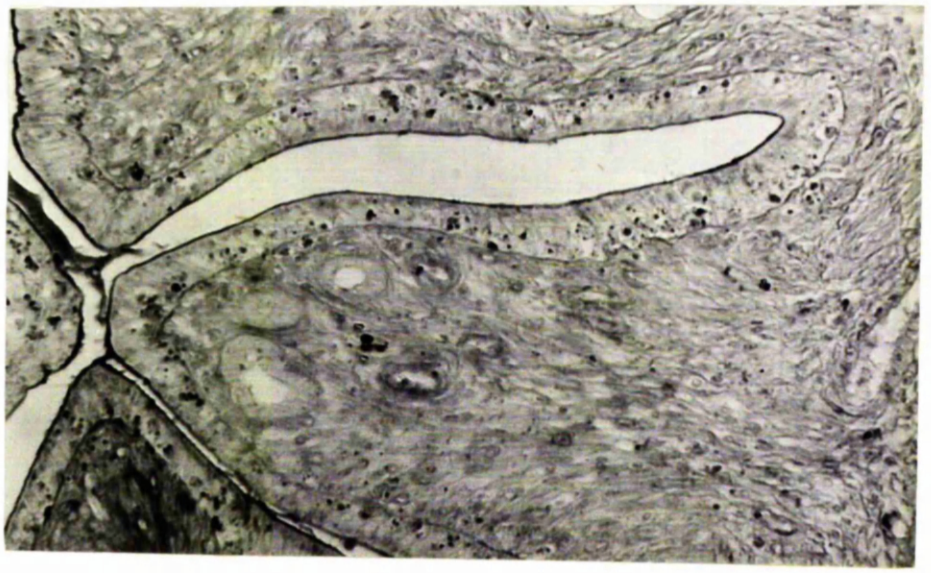

Fig. 16. Note the basement membrane revealed by the P.A.S. technique. Transverse section.

x 200.

Fig. 17. Transverse section of a uterus at 48 hours post-partum. Note the slit-like lumen and absence of folding of the endometrium. H. & E.

x 12.

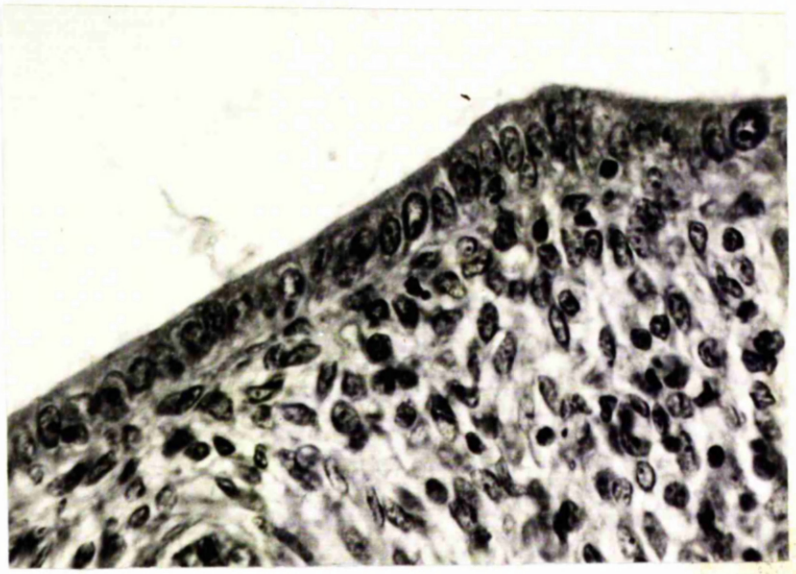

Fig. 18. Transverse section of uterine epithelium at 48 hours post-partum. Note the low columnar shape of the cells and the absence of vacuolation. H. & E.

x 485.

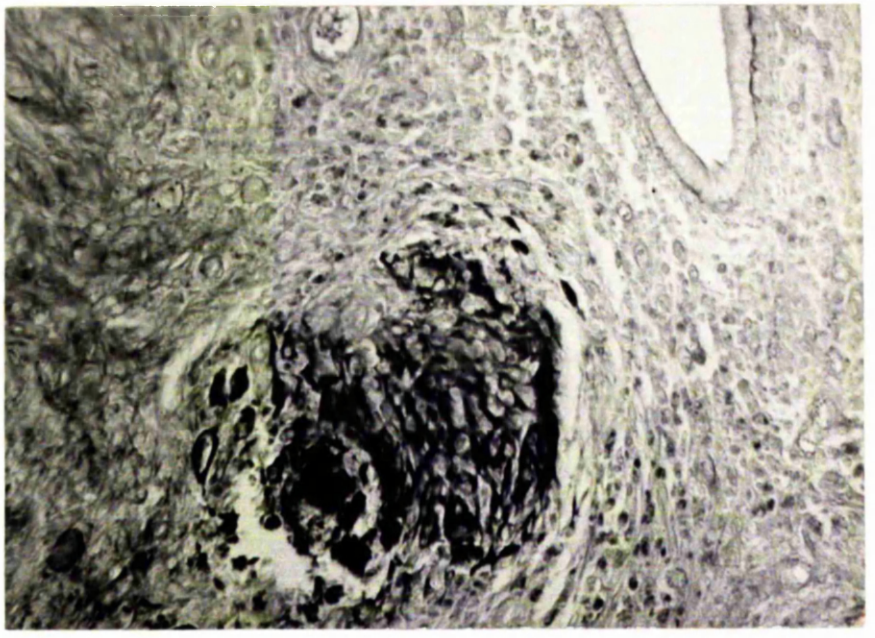

Fig. 19. The remnants of the placental artery in the endometrium at 4 days post-partum. P.A.S.

x 100.

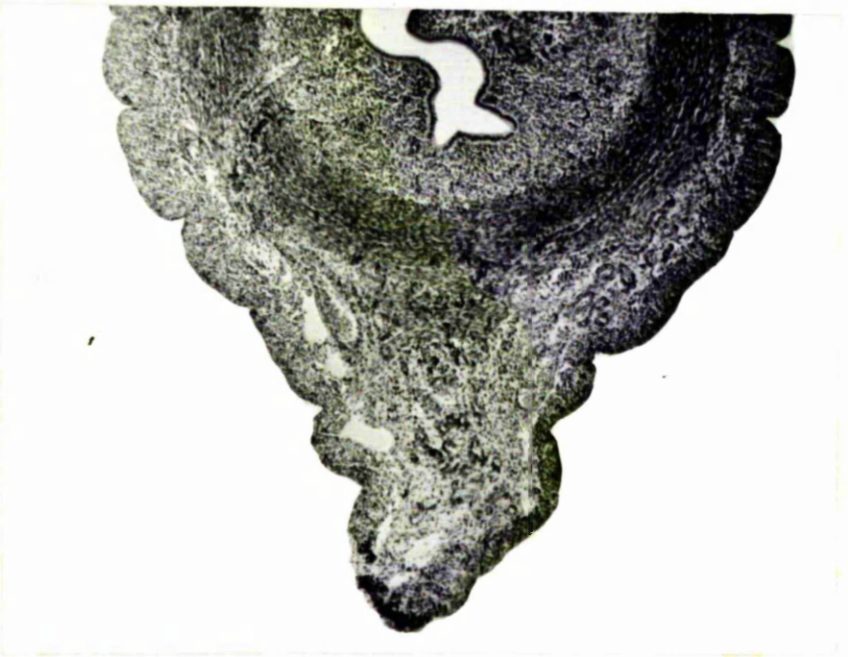

Fig. 20. Transverse section of uterus at the time littering in an intersite region to show the mesometrial triangle. H. & E.

x 50.

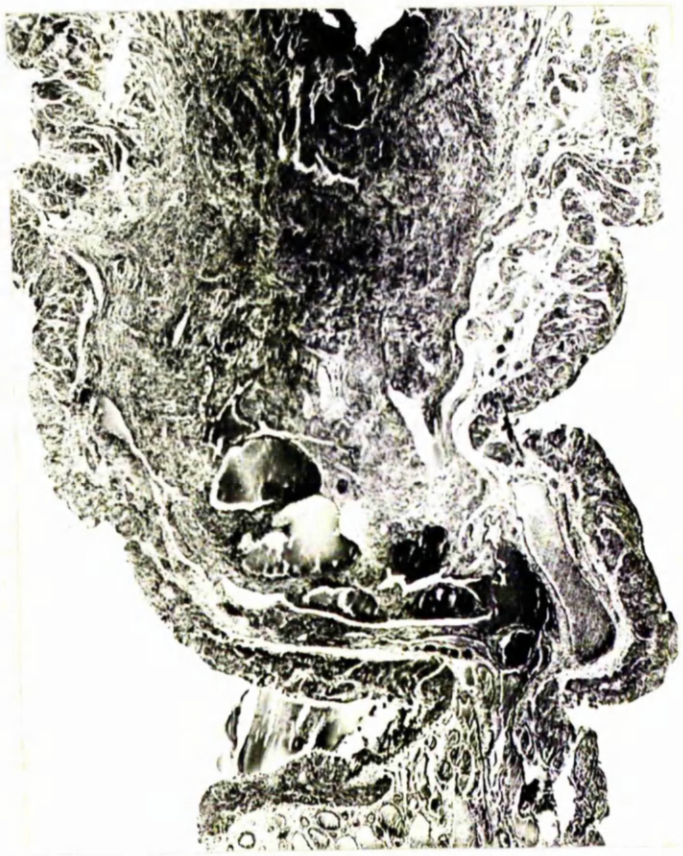

Fig. 21. A metrial gland at 0 hours. Transverse section. H. & E.

x 20.

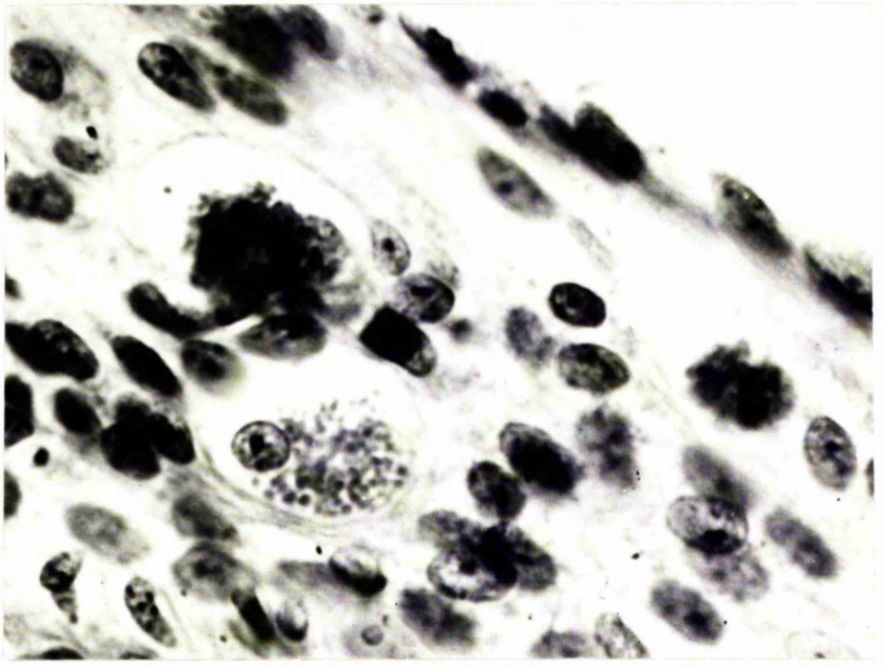

Fig. 22. Typical metrial gland cells from a 0 hours specimen. H. & E.

x 800.

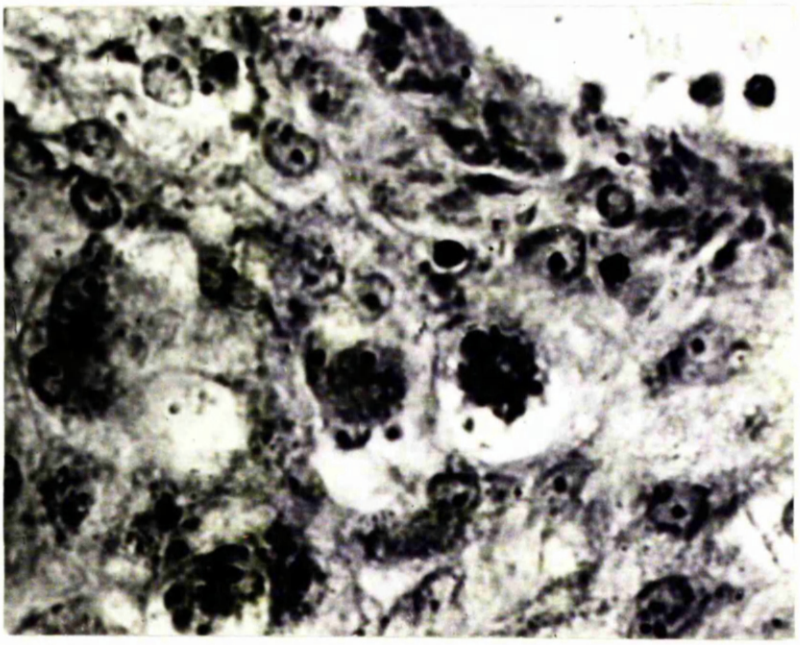

Fig. 23. Metrial gland cells from a section stained with toluidine blue. Note the presence of the granules.

x 800.

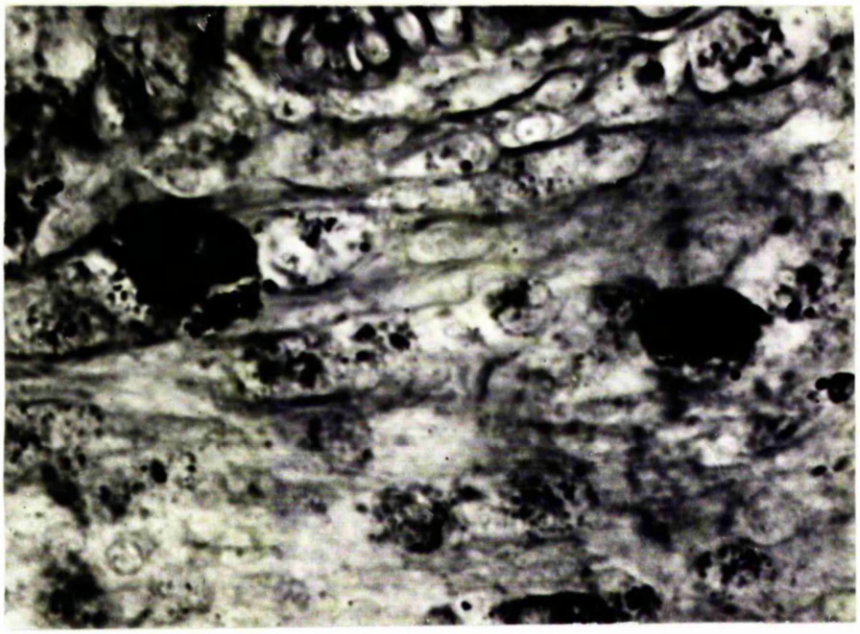

Fig. 24. Metrial gland cells stained by the P.A.S. reaction.

x 800.

Fig. 25. Metrial gland cells stained by the P.A.S. reaction after treatment with diastase.

x 800.

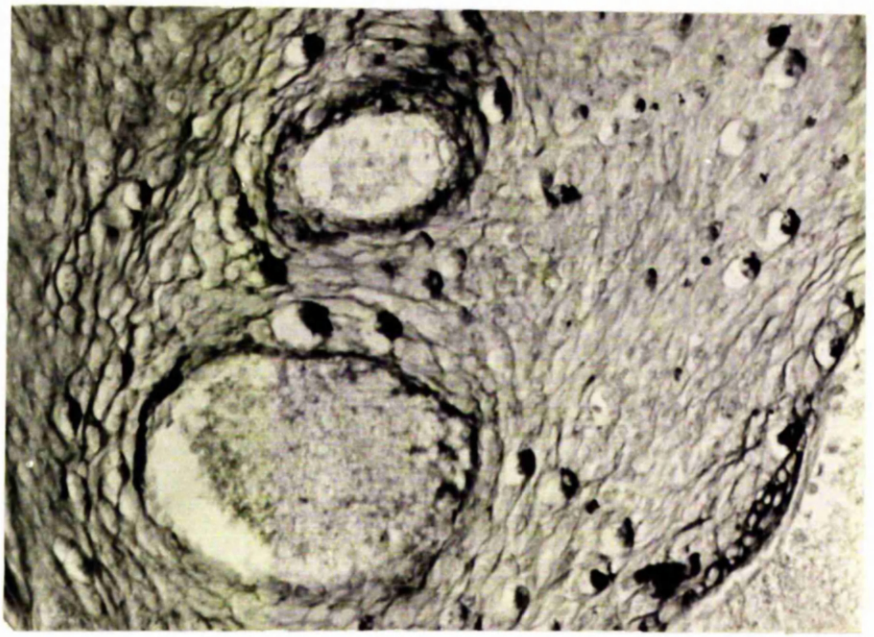

Fig. 26. Transverse section of a metrial gland at the time of littering to show the distribution of metrial gland cells. Stained P.A.S.

x 180.

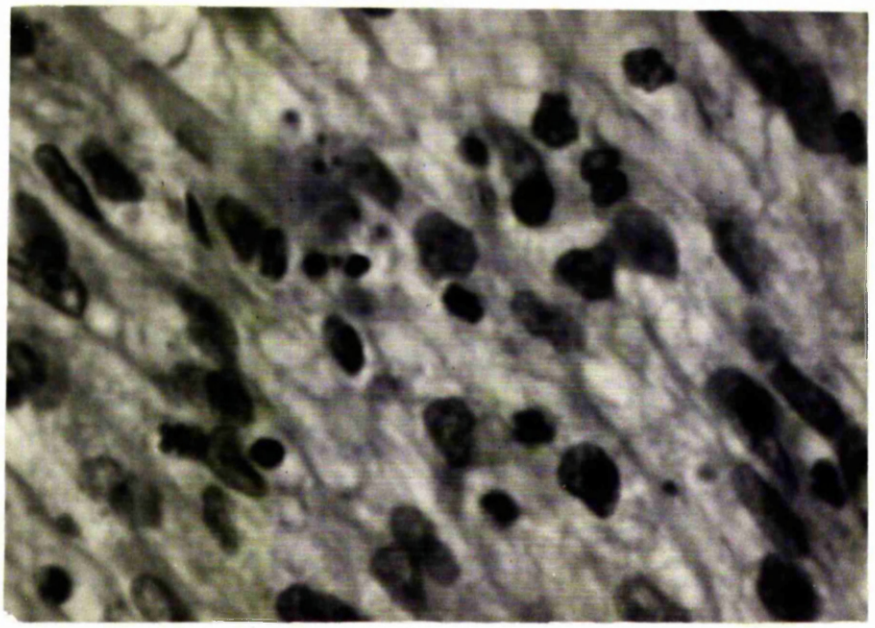

Fig. 27. Vacuolated cells in a metrial gland at 0 hours. H. & E.

x 1050.

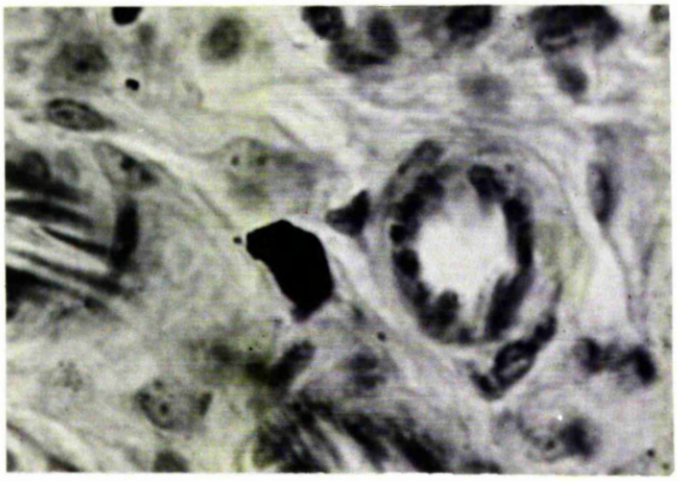

Fig. 28. A mast cell in same section as fig. 23. Note that the cell is so densely filled with granules as to appear as a black mass. Toluidine Blue.

x 800.

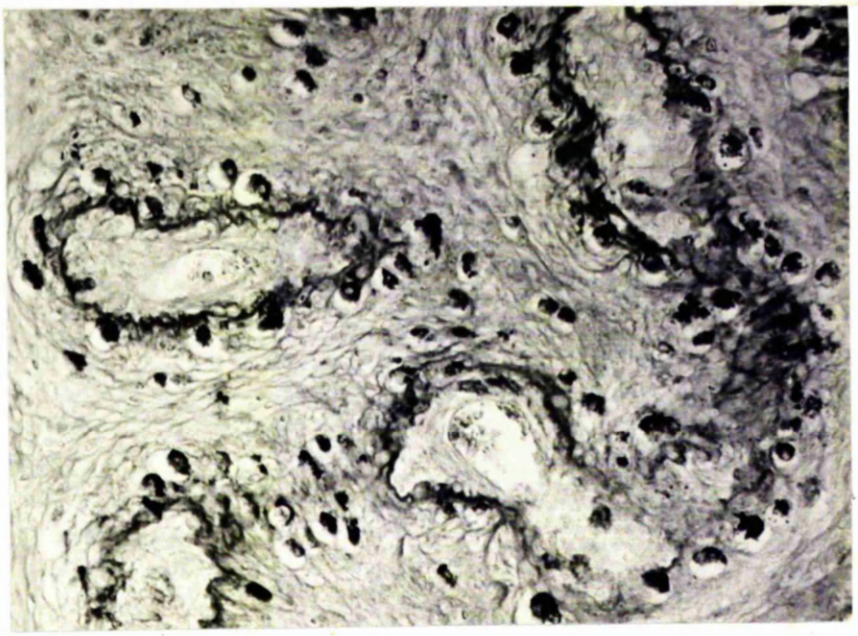

Fig. 29. A P.A.S. stained section to show metrial gland cells at 2 days post-partum.

x 180.

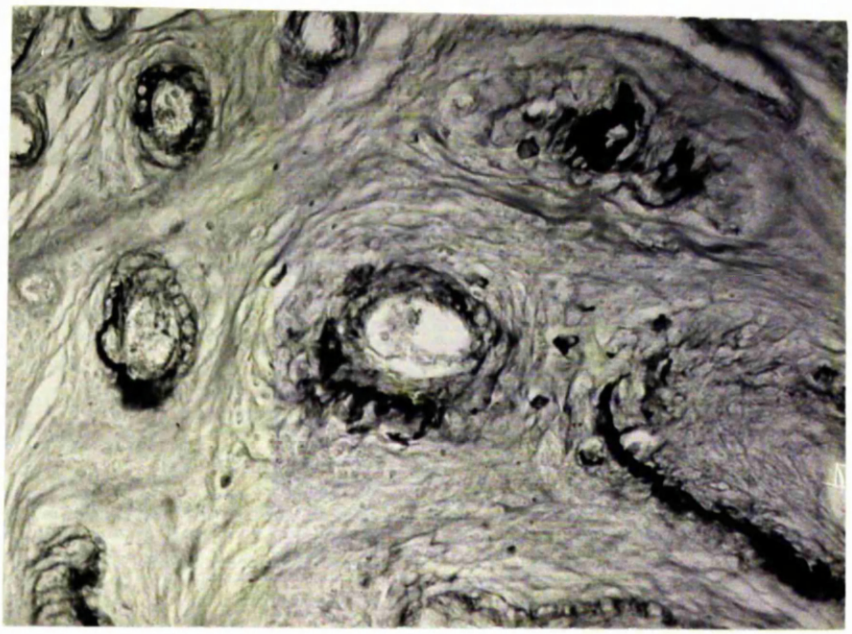

Fig. 30. Note the distribution and paucity of metrial gland cells in a 4 day specimen. P.A.S. technique.

x 180.

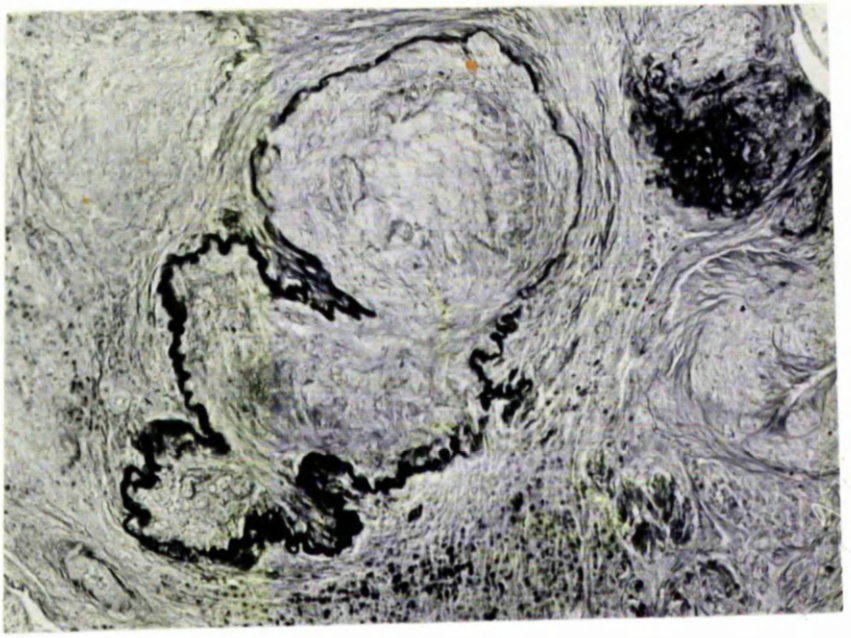

Fig. 31. The placental artery in the metrial gland shown by the P.A.S. technique in a 4 day specimen.

x 100.

Fig. 32. Transverse section through a metrial gland at 5 days post-partum in A lactating and B non-lactating animals. H. & E.

x 25.

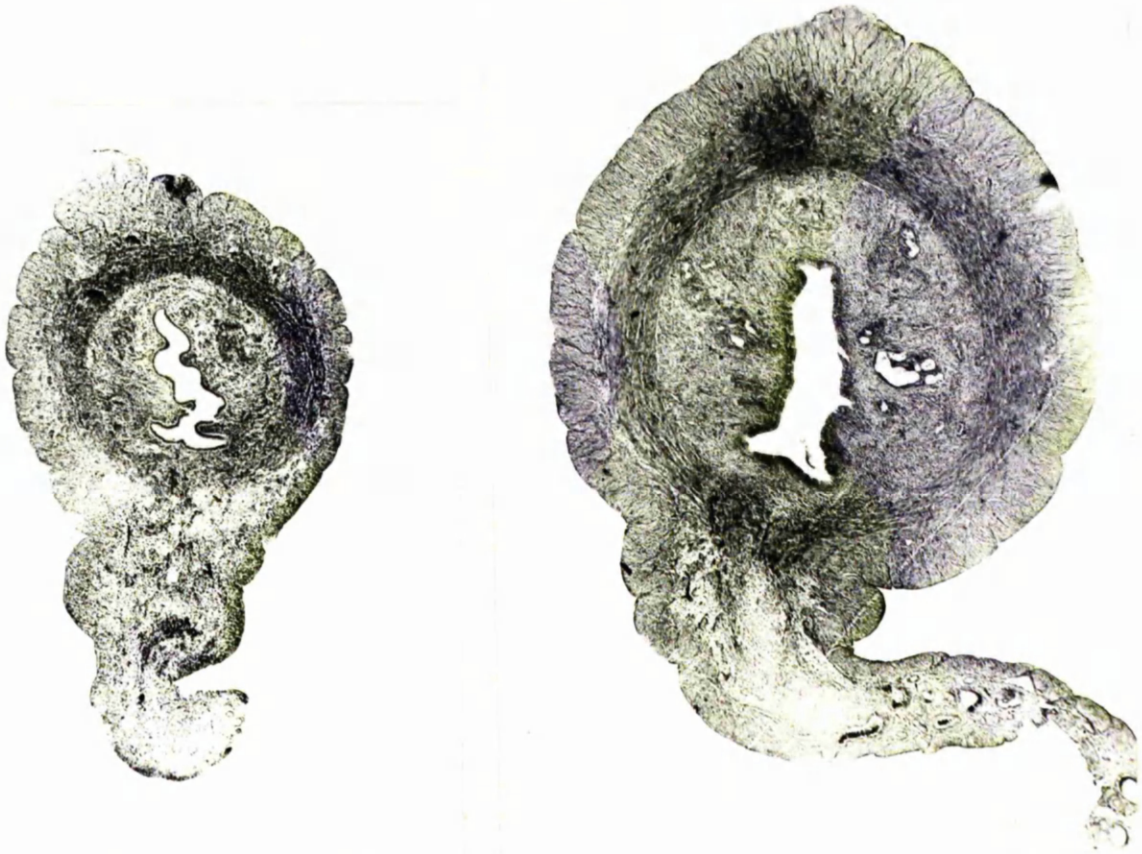

Fig. 33. Transverse sections through metrial glands in A lactating and B non-lactating animals at 10 days post-partum. The greater size of the uterine horn in the non-lactating animal is due to the re-commencement of oestrus cycles. H. & E.

x 25.

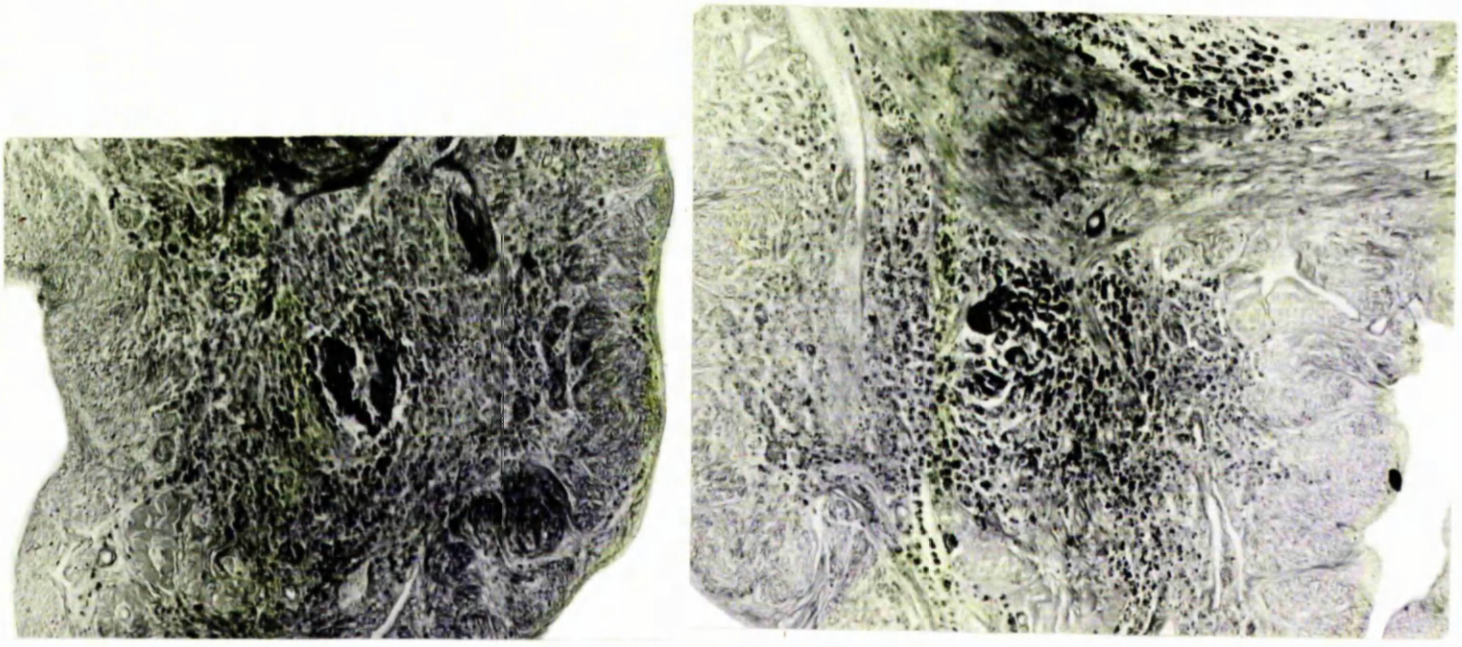

Fig. 34. Transverse sections of metrial glands at 10 days post-partum in A lactating and B non-lactating animals to show the remnants of the placental artery. P.A.S.

x 80.

Fig. 35. Metrial glands of A lactating and B non-lactating animals at 15 days post-partum. T. S. H. & E.

x 25.

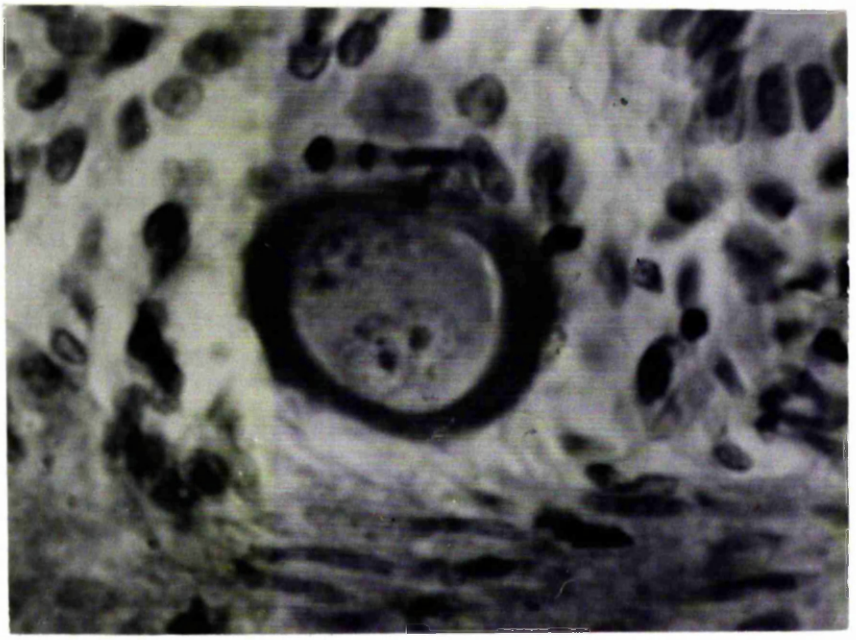

Fig. 36. An "encapsulated" giant cell in the endometrium at 15 days post-partum. Note the P.A.S. positive capsule. P.A.S.

x 1050.

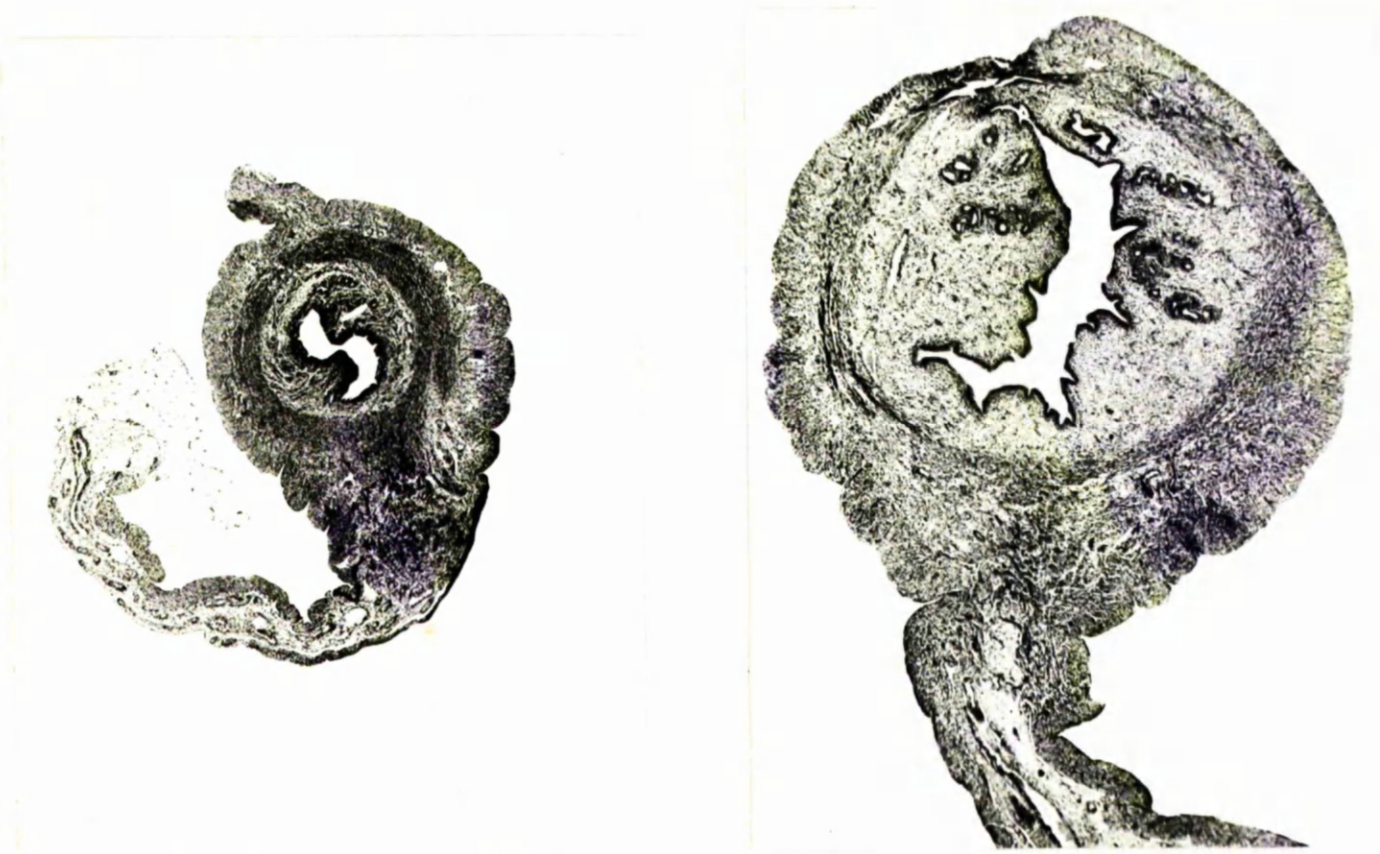

Fig. 37. Metrial glands from A lactating and B non-lactating animals at 20 days post-partum. T. S. H. & E.

x 25.