Some Histochemical Observations on the Endometrium and the Yolk-sac Placenta of *Erinaceus europea*

by B. Morris

*From the Department of Zoology, Nottingham University*

WITH THREE PLATES

INTRODUCTION

The placentation of *Erinaceus europea* has been described by Hubrecht (1889). An account of the development and structure of the avascular and vascular yolk-sac placentae which are formed in this species has been presented (Morris, 1953). Reichert's membrane is formed in the wall of the yolk-sac and it persists to term. A further study of the yolk-sac placentae, Reichert's membrane, the decidua and the endometrium of *Erinaceus*, involving the use of several histological and histochemical techniques, is reported herein.

MATERIAL AND METHODS

The material consisted of the uteri of three hibernating females obtained in January and February, and the uteri of seven non-pregnant females caught at the beginning of the breeding season in Nottinghamshire in May 1954. Twenty-four gravid uteri and twelve uteri from non-pregnant lactating females were obtained between May and September.

At autopsy the uteri of the non-pregnant animals were removed and small portions were fixed in various fluids. The implantation swellings of the pregnant uteri were separated and similarly treated. The conceptuses from animals in mid and late pregnancy were slit open laterally and the embryos removed, whereupon the uterine wall, placenta, and attached foetal membranes were fixed in one block. Occasionally small portions of the foetal and maternal tissues were dissected from the conceptuses and fixed and embedded separately.

PAS reagents and Best's carmine method were used on material fixed in Rossman's fluid at 0° C. These techniques were performed on sections with and without preliminary exposure to saliva at 37° C. for 1 hour, to differentiate between glycogen and other carbohydrates.

1 *Author's address:* Department of Zoology, University Park, Nottingham, U.K.

The methyl-green pyronin method for RNA was employed on material fixed in Zenker's fluid. RNA was extracted from control sections by immersion in 10 per cent. perchloric acid at 4° C. for 16 hours.

Material fixed in cold acetone and formalin fixed frozen sections were used to show alkaline phosphatase (Gomori, 1941). Turnbull blue reagents and Perl's method for ferric iron were applied to material fixed in 10 per cent. neutral formalin.

Frozen sections were cut from gelatine embedded material which had been fixed in either 10 per cent. formalin or 10 per cent. formal-calcium. Selected sections were stained with Sudan black, and the Fettrot method for neutral fat was also applied. The acid haematin method and the pyridine extraction test (Baker, 1946) were used on some material.

The above procedures were carried out on most of the non-pregnant uteri and on all the early implantation stages of pregnancy that were collected. A series of ten of the later stages of pregnancy were selected and similarly treated. The smallest uterine swelling in this series contained an embryo measuring 2 mm. (crown-rump length), and the largest an embryo measuring 51 mm.

Some material was fixed in Champy's fluid, and a little, after Champy fixation was post-osmificated at 37° C. for periods varying from 24 to 72 hours. Pieces of the yolk-sac splanchnopleur from later stages of pregnancy were fixed separately in Helly's fluid, postchromed, and stained by Kull's triple stain.

Certain of the later stages of pregnancy in which Reichert's membrane is present were specially treated. The following techniques and stains were applied to material which had been fixed in either Zenker's fixative, Bouin's fluid, or 10 per cent. formalin: Weigert's resorcin fuchsin for elastic tissue; Gomori's silver method for the impregnation of reticulum (1937); Lillie's allochrome method for connective tissues (1951); Masson's trichrome stain, orange G; and Van Geison's stain.

Frozen sections from material fixed in formalin were mounted in water or glycerogel, or mounted and dried, and were examined in polarized light. Stained and unstained sections of material which had been variously fixed and embedded in paraffin wax were similarly examined.

RESULTS

The endometrium

In the uteri of the hibernating, oestrous, and lactating animals that were examined the cytoplasm of the sub-epithelial connective tissue is almost devoid of RNA. In the uterus of the hibernating hedgehog the cytoplasm of the uterine glands and surface uterine epithelium is stained faintly pink by the methyl-green pyronin reagents, indicating that these tissues contain very little ribonucleic acid. In the oestrous uterus, and in the uterus of the lactating animal, the staining
reaction of the cytoplasm of these tissues is moderate, indicating an increase in the amount of RNA present.

Stainable glycogen is entirely absent from the endometrium of the non-pregnant uterus of *Erinaceus*. In certain sections stained by the PAS method some small areas of the interglandular connective tissue in the deeper parts of the uterine mucosa stain intensely red, and this reaction is saliva resistant.

The presence of lipoidal inclusions in the endometrium was demonstrated by the use of Sudan black and osmic acid. With Sudan black a faint dusting of the distal and basal cytoplasm of the cells of the uterine glands and the uterine epithelium occurs. Very occasionally distinct small droplets of fat can be discerned, usually more easily visible in the distal cytoplasm. There is no noticeable difference in the amount of lipoidal material present in the epithelial elements of the uterus of hibernation, the oestrous uterus, and the uterus of the lactating female. The observations made on material fixed in Champy’s fluid, some of which was post-osmificated, were essentially similar to those described above.

In the uterus of the hibernating hedgehog alkaline phosphatase has been observed in the cells of the uterine epithelium. It occurs mainly at the apical ends of these columnar cells. Lesser amounts are encountered in the distal cytoplasm of the epithelial cells of the necks of the uterine glands, but alkaline phosphatase is absent from the deeper coiled portions of these glands (Plate 1, fig. A). A considerable increase in the amount of alkaline phosphatase is noticeable in these epithelial elements of the endometrium of the non-pregnant oestrous uterus, and it is also present in large amounts in the apical cytoplasm and in the lumina of the deeper coiled portions of the uterine glands (Plate 1, fig. B). In the uterus of the lactating animal alkaline phosphatase is present in amounts comparable to that of the oestrus uterus, and its distribution is essentially similar.

No positive reactions for iron were obtained in the uterus of hibernation, nor in the oestrus uterus of the hedgehog. However, in transverse sections of the non-pregnant uteri of two animals which had only recently given birth to their first litters of the season, iron was found to be present in a crescentic zone on the anti-mesometrial side of the uterus in the inter-glandular connective tissue adjacent to the inner muscle tissue. No positive reactions were obtained in the uterine glands of these forms.

**The early yolk-sac placenta and decidua**

In the pregnant uterus the tissues adjacent to the interstitially implanting blastocyst become rapidly converted into decidual tissue of a distinct kind, the trophospongia (Hubrecht, 1889). The ducts of the uterine glands become occluded, but the epithelial elements of their deeper coiled portions can still be discerned. Early in development a decidua capsularis is formed which separates the blastocyst from the uterine lumen. In transverse sections the uterine lumen appears as a narrow crescentic cavity (Text-fig. 1).

In the early stages of pregnancy the cytoplasm of the endoderm and tropho-
blast, and of the adjacent trophospongia, is particularly rich in RNA. The enlarging endothelial cells of the maternal vessels present in the decidual tissue adjacent to the blastocyst also give an intense reaction. These enlarging endothelial cells contribute to the formation of the trophospongia, a compact zone of tissue surrounding the blastocyst. Elsewhere the cytoplasm of the decidual tissue appears to contain little RNA: in fact it tends to be slightly eosinophilic. The distribution of RNA remains essentially the same in all the implantation stages which were available for study—these ranged from a stage in which the embryonic knob was forming to late embryonic plate stages in which mesoderm had differentiated and amniogenesis commenced.

Glycogen is entirely absent from the foetal tissues of the implanting blastocyst. In transverse sections of the earliest stages examined, which had been treated with PAS reagents or stained with Best's carmine, glycogen is mainly located in a circular zone around the blastocyst, comprising some of the outer trophospongia and its adjacent decidual tissue. Lesser amounts are present in the deeper decidua. In such stages the decidua capsularis appears to be formed mainly of inner trophospongia and is generally lacking in glycogen. Glycogen is absent
from the epithelial elements of the deeper coiled portions of the uterine glands, but some saliva resistant and PAS positive material is present in the apical cytoplasm of some of the cells of the uterine epithelium.

In later embryonic plate stages, in which mesoderm has been differentiated and the blastocyst has enlarged considerably, the distribution of glycogen in the decidual tissues is as described above, but the staining reaction is much more intense, and glycogen in quantity is now present in the inner region of the trophospongia. The enlarging endothelial cells of the maternal vessels in the decidua appear to be devoid of glycogen.

Lipoidal material is equally abundant in the trophoblast and inner trophospongia of early and late implantation stages. Here it occurs in most of the cells as irregularly shaped dense clusters of moderately sized droplets. This material, which is absent from the endodermal epithelial cells of the bilaminar wall of the yolk-sac, is irregularly arranged within the cells, and is not indicative of any special polarity (Plate 2, fig. F). In the cells of the outer trophospongia lipoidal material is less abundant, and some scattered droplets are present in the cytoplasm of the cells of the adjacent decidual tissue. Lipoidal droplets are absent from the enlarging maternal endothelial cells, and there is no noticeable increase in the amount of sudanophil material in the epithelial elements of the deeper coiled portions of the uterine glands. The Fettrot method reveals that almost all of this material is neutral fat, but a little acid haematin positive material is present in the trophoblast and trophospongia, indicative that some phospholipid is present in these tissues.

In the earliest stage of implantation in which alkaline phosphatase was shown by Gomori’s calcium-cobalt method, the blastocyst measured 0.25 mm. across its greatest diameter. At this stage the embryonic knob is beginning to form. Only very slight traces of alkaline phosphatase were found in the cytoplasm of the endoderm, trophoblast, and trophospongia—at this stage the latter is in an early phase of its development. However, some alkaline phosphatase is present in the nuclei—particularly the nucleoli—of the endoderm and trophoblast (Plate 1, fig. C). In early embryonic plate stages the amount of alkaline phosphatase in the cytoplasm of these tissues has increased (Plate 1, fig. D). In later stages, when the mesoderm is being differentiated, the endoderm and trophoblast of the blastocyst are particularly rich in alkaline phosphatase, but the reaction obtained in the cytoplasm of the trophospongia is much less intense (Plate 1, fig. E). The occurrence of the enzyme in the uterine epithelium and in the deeper coiled portions of the uterine glands is similar in amount and distribution to that described for the oestrous uterus.

Turnbull blue reagents and Perl’s method for ferric iron were applied to transverse sections of three implantation sites containing the following stages: an early blastocyst in a stage of development before the formation of the embryonic knob, and an early and a late embryonic plate stage. No positive reactions for iron were obtained in any of the foetal or maternal tissues.
The vascular omphaloidean placenta

In the earlier stages of development lacunae, in which maternal blood circulates, are present in the trophoblast and trophospongia. Later in development the trophoblast becomes converted into a spongy tissue in which more numerous lacunae are formed. Maternal blood circulates in these lacunae, which are continuous with the larger blood spaces present in the trophospongia (Hubrecht, 1889). Omphaloidean villi penetrate into the trophoblast and thus bring the foetal and maternal circulations into close proximity. Generally the circulations are separated from each other by two tissues only; the trophoblast which is generally only one cell thick and the foetal endothelium. Thus in Erinaceus the omphaloidean placenta is haemochorial in character (Morris, 1953).

The omphaloidean placenta attains its maximum development when the embryo is about 3 mm. in length. In such stages the cytoplasm of the trophoblast and inner trophospongia is rich in RNA, but the staining is not so intense as in the early implantation stages. A moderate reaction is also obtained in the endodermal cells of the yolk-sac splanchnopleur which, in some regions of this membrane, are cuboidal in form. In the outer trophospongia there is a noticeable decrease in the amount of RNA.

The distribution of glycogen remains essentially unaltered. Glycogen is present in the inner and outer trophospongia, and a moderate staining for glycogen is obtained in the remainder of the decidual tissue. Glycogen is absent from the trophoblast, mesenchyme, and yolk-sac endoderm.

The amount of lipoidal material present in the trophoblast has increased. The rounded droplets are a little larger than those described for earlier stages and they are more numerous. As in the earlier stages they are frequently massed together to form irregularly shaped clusters (Plate 2, fig. G). Even in those regions where the trophoblast is attenuated and intervenes, together with the foetal endothelium, between the maternal and foetal circulations, the same concentration of sudanophil material is found. Similar amounts are present in the inner trophospongia, whereas in the outer trophospongia and the adjacent decidua only scattered droplets are present. The endodermal epithelium of the yolk-sac is devoid of this material. The trophoblast of the avascular bilaminar omphaloidean placenta is far more compact than in this region described above, and the concentration of sudanophil material is correspondingly more intense. Whereas in the early implantation stages of pregnancy almost all of this lipoidal material was neutral fat, at this stage of pregnancy a very large proportion of the lipoidal material present in the trophoblast and the inner trophospongia immediately adjacent to it, is phospholipid.

Alkaline phosphatase is present in the nuclei and the cytoplasm of the trophoblast at this stage, but the reaction is not so intense as that obtained in late embryonic plate stages. This also applies to the more compact trophoblast in the avascular mesometrial region of the yolk-sac wall. As in earlier stages the
enzyme is present in the inner trophospongia, and in the outer trophospongia only the scattered giant nuclei, which are mainly located in this tissue, give a positive reaction. A faint reaction is obtained in the cytoplasm of the endodermal cells of the yolk-sac splanchnopleur.

No positive reactions for iron were obtained in the foetal or the maternal tissues at this stage of pregnancy. In slightly later stages of pregnancy (crown-rump length 5 mm.) a faint reaction is obtained in the trophoblast and in parts of the inner trophospongia after the application of Perl’s method for ferric iron.

The yolk-sac splanchnopleur

When the vascular yolk-sac placenta attains its maximum development some of the endodermal epithelial cells of the yolk-sac splanchnopleur are cuboidal in form. At such stages the cytoplasm of these cells gives a moderate reaction after the application of methyl-green and pyronin reagents, and a similar reaction is obtained in the latest stages of pregnancy examined (crown-rump length 51 mm.). In these late stages the RNA appears to be concentrated in the distal cytoplasm of these cells, which are cuboidal or low columnar in shape. These endodermal cells rest upon a distinct basement membrane which appears to be composed of argyrophilic fibres (Plate 2, fig. 1). The coelomic mesothelium of the yolk-sac splanchnopleur, which lines the membrane on its exocoelomic side is also impregnated by Gomori’s silver method (1937). The coelomic mesothelium and the endothelia of the vitelline vessels are stained a clear green colour by Masson’s trichrome stain and violet or red by Lillie’s allochrome method for connective tissues (1951). The mesenchyme, which in the earlier stages of pregnancy was of a loose texture, is now far more compact and contains scattered argyrophilic fibres.

Glycogen is absent from the yolk-sac splanchnopleur throughout early development. However, in late stages of pregnancy glycogen is present in the endodermal epithelium and in the mesenchyme of the membrane. In both tissues it is present in the form of finely particulate material, usually occurring in the distal cytoplasm of the endodermal cells. In preparations stained with PAS reagents the basement membrane of the endodermal epithelium and the coelomic mesothelium show some variability in intensity of staining in different regions of the yolk-sac splanchnopleur. Generally both membranes are stained faintly pink by the PAS reagents, and this reaction is saliva-resistant.

The yolk-sac epithelium of the early implantation stages of pregnancy appeared to be devoid of lipoidal material, and this condition persists up to the stage at which the vascular omphaloidean placenta begins to retrogress. Subsequently lipoidal material stainable with Sudan black and Fettrot can be detected in the cytoplasm of these cells. The amount of sudanophil material present varies considerably from cell to cell, and its distribution within the cells is also variable. In some cells only a faint dusting of the cytoplasm results following the application of Sudan black, whereas in other cells large discreet droplets can be seen.
A similar result is obtained in Champy fixed material, and in Champy fixed material which was post-osmificated (Plate 2, fig. 1). Generally the larger droplets are found in the lower halves of the endodermal cells, and the finer droplets are scattered throughout the distal cytoplasm. Some lipoidal material is also present in the mesenchyme. Acid haematin positive material is present in the endodermal epithelium of the yolk-sac splanchnopleur of these late stages of pregnancy. It varies in amount from cell to cell, and under high magnification this material is seen to be granular. It occurs throughout the cytoplasm of the cells, but is more concentrated in the distal cytoplasm. The amount and distribution of this granular material corresponds to that of the strongly fuchsinophil material revealed in these cells by Kull's method for mitochondria.

At the 2–3 mm. stage a faint reaction for alkaline phosphatase is obtained in the endodermal cells of the yolk-sac splanchnopleur. In later stages there is no increase in the intensity of this reaction, and the mesenchyme of the membrane appears to contain little of the enzyme. No positive reactions for iron were obtained in the membrane.

The omphaloidean placenta of late pregnancy

The rapid enlargement of the embryo in later stages of pregnancy leads to the withdrawal of the vascular omphaloidean villi, together with their surrounding mesenchyme, from their sites in the spongy trophoblast. Concurrently the extension of the exocoel separates the yolk-sac splanchnopleur from the chorion over most of its extent, and the tissues of the uterine wall become stretched—particularly in the mesometrial region.

In such late stages the trophoblast and inner trophospongia of the mesometrial region of the conceptus are indistinguishable from one another, and form a compact narrow band of tissue (Morris, 1953). Reichert's membrane has been formed on the inner embryonic side of the trophoblast.

In late stages of pregnancy the cytoplasm of the trophoblast and inner trophospongia of the mesometrial region contains little RNA. Material treated with PAS reagents reveals that glycogen is present in this tissue (Plate 3, fig. M). Large blood spaces are present between the inner and outer trophospongia and a strong PAS positive reaction is obtained in the tissue of the outer trophospongia which, in these late stages of pregnancy, is fibrous in character.

Alkaline phosphatase is present in the mesometrial trophoblast and trophospongia but in greatly reduced amounts, and the sudanophil and acid haematin positive material which bulked so large in these tissues in earlier stages is now completely lacking. Negative reactions for iron were obtained in these tissues in all the late stages of pregnancy that were tested.

Reichert's membrane

In the later stages of pregnancy in the hedgehog Reichert's membrane is
formed between the somatic mesoderm and the trophoblast of the trilaminar omphalopleur. At first it is a very thin layer, but in very late stages it thickens and persists to term (Morris, 1953).

The PAS reagents stain Reichert's membrane from the time of its formation until term. At first, when the membrane is about 5 \( \mu \) thick, it is stained a crimson colour, but in very late stages when it has attained a thickness of 25 \( \mu \) along the lateral walls of the conceptus it is stained faintly pink (Plate 3, fig. M). A more intense reaction is obtained in the mesometrial region of the conceptus where the membrane remains relatively thin. In this region the membrane is stained a bright red colour (Plate 3, fig. N). These reactions are saliva resistant.

Gomori's silver method for the impregnation of reticulum (Gomori, 1937) impregnates the membrane in all the stages of pregnancy to which the technique was applied, though not as intensely as in the case of typical reticulum. In the lateral walls of the conceptus in late pregnancy, where the membrane is thickest, it is stained a pale brown colour with a narrow black region along its outer margin (Plate 3, fig. L), and it is stained black in the mesometrial region of the yolk-sac wall where the membrane remains relatively thin (Plate 3, fig. K).

In all stages of pregnancy Reichert's membrane is unstained by Weigert's resorcin fuchsin—the colour of the elastic tissue of the uterine vessels was used as a standard for comparison. It is stained readily by Van Gieson's stain for collagen. The membrane is clearly revealed by Lillie's allochrome method for connective tissue (1951). At the time of its first formation it is stained intensely red or violet, but in the latest stages examined the membrane is stained a pale blue colour.

In all stages of pregnancy the membrane is stained a clear green colour with Masson's trichrome stain (Plate 3, fig. O), and in preparations stained with eosin and methylene blue the membrane shows a selectivity for the acid dye. Its strong affinity for acid dyes is exhibited also in its deep staining with orange G.

In frozen sections treated with Sudan black, Reichert's membrane is completely unstained. Similarly, in material fixed in Champy's fluid and post-osmificated for varying periods, Reichert's membrane is unaffected (Plate 2, fig. H).

At the time of its formation Reichert's membrane exhibits only faint birefringence in deparaffinized sections cut from blocks fixed in Bouin's, Rossman's, and Zenker's fixatives. In unstained frozen sections cut from blocks fixed in 10 per cent. formalin and mounted moist in water or glycerogel the membrane exhibited strong birefringence, comparable with that of the reticular and collagenous fibres in the uterine wall, from the time of its formation to term. In deparaffinized sections from blocks which had been variously fixed the membrane is more strongly birefringent in very late stages of pregnancy than in earlier stages.
DISCUSSION

The occurrence of lipoids, glycogen, iron, and phosphatases in the lumina and epithelial cells of the uterine glands of a variety of mammals suggests that these substances are secreted by the glands for the purpose of nourishing the growing blastocyst during some part of the period of gestation (Wislocki & Dempsey, 1945). The 'uterine milk' of the ungulates has long been regarded as a nutritive fluid which contains lipoid, protein substances, and iron compounds. Wislocki has shown that in the sow iron and lipoid are present in the uterine glands and uterine epithelium suggesting that these substances are produced by the uterine glands and uterine epithelium, instead of being derived from erythrocytes and leucocytes which have undergone dissolution.

In Erinaceus the RNA content of the cytoplasm of the epithelial and sub-epithelial tissues of the uterus of hibernation is low, and there is only a slight increase in the intensity of staining in these tissues in the oestrus uterus and in the early implantation uterus. In the rat and guinea-pig an intensification of the basophilic staining of the cytoplasm of the glandular and surface epithelia of the uterus occurs prior to implantation and in many forms the subsequent conversion of the superficial stroma into decidua is accompanied by a reduction in the amount of basophilia, and the cytoplasm of the decidual cells of women, cats, rats, and guinea-pigs exhibit negligible staining with basic dyes (Wislocki & Dempsey, 1945). In the hedgehog the transformation of the sub-epithelial tissues into trophospongia is accompanied by a marked increase in the amount of cytoplasmic RNA, and this trophospongia tissue, which appears to be derived mainly from the enlarging cells of the maternal endothelium, stains as intensely as the trophoblast. The RNA content of the remainder of the decidual tissue is low.

In the hedgehog glycogen is entirely absent from the endometrium of the non-pregnant uterus, but is present in great amounts in the decidual tissues of the earliest implantation stages examined. Similarly, no positive reactions for iron were obtained in the endometria of non-pregnant uteri, and the application of Sudan black to non-pregnant uteri resulted in only a faint dusting of the distal and basal cytoplasm of the glandular epithelia. Some alkaline phosphatase is present in the surface epithelium of the uterus of hibernation and a considerable increase in the amount of this enzyme is evident in the surface and glandular epithelia, and in the lumina of the glands, of the oestrous uterus.

It is probable that the secretions of the uterine glands in all mammals contribute in some way to the nourishment of the early blastocyst, and in some degree to the support of the implanting blastocyst (Wislocki & Dempsey, 1945). Whereas in some animals such as the sow and other centrally implanting forms, the uterine glands may serve as a principal source of nutritive materials, in others they play only a subsidiary role. In Erinaceus, in which implantation is of the interstitial type, it appears that the uterine secretions are of minor importance since it seems unlikely that they are rich in nutrients. The secretions of the
glands are available to the blastocyst for only a short time, since the early establishment of the decidua capsularis effectively separates the foetal tissues from the uterine lumen and its fluid contents. Once implantation commences other means of obtaining nutritive materials become available.

In the early stages of pregnancy in Erinaceus the yolk-sac, which is a closed cavity, is dependent for any renewal of its fluid contents as far as maternal sources are concerned, on substances which enter it from the maternal blood, passing through the trophoblast and endoderm. Since numerous lacunae, in which maternal blood circulates, are present in the trophoblast and trophospongia of such early stages, the early omphaloidean placenta should be an important source for the transudation of fluid into the yolk-sac cavity. Everett (1935) contends that that proximity of circulating maternal blood to Reichert's membrane in the early stages of pregnancy in the rat accounts for the rapid appearance of vital dyes in the cells of the yolk-sac endoderm and in Reichert's membrane itself.

In all the implantation stages examined the trophoblast, trophospongia, and the enlarging cells of the maternal endothelia are rich in RNA. RNA is important in the process of protein synthesis (Danielli, 1953), and in these tissues it is probable that it plays some important role in the synthesis of protein during the early period of active growth of the blastocyst and decidua.

The trophoblast and trophospongia are rich in neutral fat although this material is absent from the yolk-sac endoderm. The individual droplets are frequently clustered together into large irregularly shaped masses, and no specific polarity is indicated by the distribution of this material within the cells. The amount of lipoidal material present in individual cells varies considerably. It is probable that these droplets are absorption products, and that the early omphaloidean placenta is concerned in the absorption of lipoidal material from the maternal blood. The vascular omphaloidean placenta is also important in this connexion, for in later stages of pregnancy (crown-rump length 2-5 mm.) the trophoblast and trophospongia contain increased amounts of lipoidal material, a large proportion of which is phospholipid.

The role of alkaline phosphatase in tissue metabolism is as yet inadequately known. It is thought that phosphatases are probably involved in a variety of chemical reactions which occur in tissue metabolism and the specificity of their actions cannot be demonstrated by histochemical means. It is not therefore possible to attribute a specific function to the tissues in which the enzymes are found. Probably phosphatases play some part in the conversion of glycogen into glucose (Cori, 1941), and there is some evidence to show that sugars are phosphorylated in their absorption into the epithelia of the intestine and kidney tubule (Davson & Danielli, 1943). It has been suggested that fats undergo a like phosphorylation (Bloor, 1943), and that the phosphate necessary in this scheme of sugar and fat absorption is presumably made available by phosphatases (Sumner & Somers, 1943). In view of these activities the sites of enzyme location
which merit attention as far as the yolk-sac placenta is concerned, are the trophoblast and trophospongia.

The concentration of alkaline phosphatase in the trophoblast increases as pregnancy proceeds, and the enzyme is present in this tissue in considerable amount at the late embryonic plate stage. A somewhat less intense reaction is obtained in the trophoblast at the stage in which the yolk-sac placenta attains its greatest development. The enzyme is also present, in lesser amounts, in the trophospongia. The alkaline phosphatase in these tissues may play some part in the absorption of sugars from the maternal blood in the early avascular yolk-sac placenta and in the vascular yolk-sac placenta which is developed later. In late stages of pregnancy the trophoblast and trophospongia of the yolk-sac wall contain very little of the enzyme, and this reversal of concentration suggests in itself a significant change in the physiology of the yolk-sac wall during the course of gestation.

In *Erinaceus* the trophospongia is very rich in glycogen and the amount of this substance present—judging by the intensity of the staining reaction with PAS reagents and Best's carmine—appears to increase between early implantation and late embryonic plate stages. The source of glucose for the elaboration of the glycogen molecule must be the maternal blood. It is probable that a similar uptake of sugars occurs in the trophoblast in which greater amounts of alkaline phosphatase are present, and that it is utilized by this tissue, for glycogen deposition does not occur in it until very late in pregnancy when the yolk-sac placenta is in an advanced state of retrogression.

It is generally considered that decidual tissue, which is invariably rich in glycogen and fat, plays an important part in the nourishment of the early blastocyst. It has been suggested (Hard, 1946) that the presence of alkaline phosphatase in this tissue may indicate the existence of a mechanism whereby these substances may be released and made available to the blastocyst. The relatively reduced amount of glycogen in the trophospongia of the vascular yolk-sac placenta—when compared with that of the avascular placenta of embryonic plate stages—may be correlated with this.

In *Erinaceus* the yolk-sac persists to term and in late stages the yolk-sac splanchnopleur is invaginated into the yolk-sac and becomes thrown into folds. At term the yolk-sac appears in transverse sections as a slit-like crescentic cavity, the bilaminar (mesometrial) wall of which is complete (Morris, 1953). The yolk-sac placenta of the hedgehog is concerned in the absorption of lipoidal material from the maternal blood, yet lipoidal droplets have not been detected in the endoderm of the yolk-sac until very late in pregnancy. It therefore appears unlikely that the lipoids absorbed by the trophoblast are transmitted into the yolk-sac cavity, and it is probable that the lipoidal material that accumulates in the endoderm and mesenchyme of the yolk-sac splanchnopleur in late pregnancy is derived from the foetal blood of the vitelline circulation. A similar consideration may be applicable to the late occurrence of glycogen in the membrane.
The yolk-sac splanchnopleur does not become fully differentiated until late in development when the vascularity of the mesometrial half of the conceptus is poor and the vascular yolk-sac placenta has regressed. Very little alkaline phosphatase is present in the endoderm of the membrane at the 2-mm. stage, and there is no apparent increase in the amount of the enzyme in the endoderm in later stages. Inorganic iron is absent from the membrane in all the stages that were tested.

It is unlikely that the yolk-sac fluid in late stages of pregnancy can be regarded as an important source of absorbable substances. It has been shown (Morris, 1953) that the concentration of protein nitrogen in the yolk-sac fluid increases in late pregnancy, and that this increase is probably due in part to the absorption of water from the cavity. Since this cannot account for the higher concentrations obtained, there must be a continued entry of protein into the yolk-sac in late stages. The probable route of entry is from the vitelline vessels of the yolk-sac splanchnopleur.

The endodermal epithelial cells of the yolk-sac splanchnopleur of rodents contain glycogen, fat droplets, and iron, and it has been shown in the rat and the hamster (Wislocki et al., 1946) that this epithelial layer is probably concerned in the absorption of iron. In those forms in which the yolk-sac becomes inverted, the vascular yolk-sac splanchnopleur is either bathed by the fluid contents of the uterine lumen, which may contain a variety of substances capable of being absorbed, or it may come to lie adjacent to the decidua capsularis. It has been suggested (Wislocki et al., 1946) that the yolk-sac splanchnopleur of these forms is actively engaged in the absorption of iron, fat, and protein from the uterine fluid or from the yolk-sac cavity, the bilaminar wall of which has degenerated. In the hedgehog it is probable that the yolk-sac splanchnopleur is not of any great importance as a placental structure.

In Erinaceus, as in some of the shrews (Brambell & Perry, 1945), Reichert's membrane is formed very much later in development than in the various rodents in which it occurs, and it first becomes noticeable between the somatic mesoderm and the trophoblast of the lateral wall of the conceptus (Morris, 1953). In so far as the yolk-sac serves as an absorptive organ the conditions under which substances may reach it will differ materially before and after the appearance of Reichert's membrane. The yolk-sac placenta is of very great importance in the nourishment of the growing blastocyst in early stages of pregnancy, and it continues to be of importance up to about the 7-mm. stage. Subsequently its importance rapidly declines as the extent of the vascular omphaloid placenta diminishes. In Erinaceus the formation of Reichert's membrane is more or less coincident with the onset of the retrogression of the omphaloid placenta, and in this species the membrane attains a far greater thickness than in the rat. In later stages of pregnancy the vascular flow in the omphaloid (mesometrial) region of the conceptus is being retarded. These factors strongly suggest that the entry of substances via the omphaloid placenta is very seriously
The histochemical properties and tinctorial reactions of Reichert's membrane have been described. The staining reactions of the membrane are, in some ways, similar to those described for Reichert’s membrane in the rat (Wislocki & Padykula, 1953). The failure of the membrane to stain with Weigert’s resorcin fuchsin indicates that it is not composed of elastic tissue. Its marked affinity for acid dyes, its reaction to Van Gieson’s stain, its argyrophilia, and its birefringence suggest that it is composed of collagenous material. In the rat the membrane does not stain typically as dense collagen or as reticulum (Wislocki & Padykula, 1953), whereas in Erinaceus the staining reactions of the membrane are far more typical and very similar to those of collagen.

The membrane is strongly PAS positive at the time of its formation, indicating that it probably contains some mucopolysaccharide or glycoprotein. This strong reaction persists to term in those regions of the mesometrial wall of the conceptus where the membrane remains comparatively thin (5-15 μ). However, along the lateral walls of the conceptus where it may reach a thickness of 30 μ, it is stained faintly pink with PAS reagents after saliva treatment. It has been assumed that reticular fibres are strongly PAS positive, whereas collagenous bundles are poorly stained. It is probable that the degree of PAS staining of collagenous tissues is dependent upon the presence of ground substance coating the connective tissue fibres as well as upon the fibres themselves (Lillie, 1952).

Silver impregnation techniques have been used in attempts to distinguish between reticulum and collagen in connective tissues. The reticular fibres are blackened by silver impregnation, whereas collagenous bundles are stained brown—the colour may vary from light golden brown to dark brown. In the later stages of pregnancy in Erinaceus to which Gomori’s silver impregnation method was applied, Reichert’s membrane is stained black in those mesometrial regions of the conceptus where the membrane is relatively thin, and a pale brown colour along the lateral walls of the conceptus where it is very much thicker. It has been shown (Irving & Tomlin, 1954) that the difference in the silver impregnation of reticulum and collagen is not a means of differentiating between two types of fibres, but only distinguishes between two types of fibre ground-substance complex, and that these different responses of reticulum and collagen cannot be held to contradict the conclusion that the fibres of reticulum and collagen are identical. Thus it seems probable that the presence of varying amounts of ground-substance complex—relative to the thickness of the membrane—may account for the different reactions of the membrane to PAS reagents and to silver impregnation in the mesometrial and lateral regions of the wall of the conceptus.

In the rat (Wislocki & Padykula, 1953) the membrane exhibits variable birefringence, which is not as constant or as strong as that of reticular and collagenous fibres. In Erinaceus the membrane exhibits birefringence at the time of curtailed from the stage of pregnancy in which Reichert’s membrane appears onwards.
its formation and it becomes more strongly birefringent in late stages of pregnancy. In such late stages its birefringence is comparable to that displayed by the reticular and collagenous fibres present in the uterine wall.

The origin of Reichert's membrane in the rat and mouse is uncertain. It may arise from the endoderm of the bilaminar omphalopleur or from the trophoblast. In some of the shrews (Brambell & Perry, 1945) and in Erinaceus (Morris, 1953), there is strong evidence that the membrane is of trophoblastic origin.

**SUMMARY**

1. An account is given of the distribution of RNA, fats, iron, glycogen, and alkaline phosphatase in the endometria of non-pregnant animals and in the foetal tissues and decidua of the yolk-sac placentae of pregnant forms.

2. The RNA content of the cytoplasm of the uterine and glandular epithelia of the oestrous uterus of Erinaceus is low, and little lipoidal material is present. Iron and glycogen are entirely absent. The uterine secretions must play only a minor role in the nourishment of the early blastocyst.

3. The histochemical reactions of the tissues which comprise the avascular yolk-sac and the vascular yolk-sac placentae are described. The yolk-sac placenta is concerned in the nourishment of the embryo in early stages of pregnancy—up to about the 7-mm. stage, for it is involved in the uptake of substances, particularly fats and sugars from the maternal blood. Subsequently, the yolk-sac placenta retrogresses. Whereas in early stages the trophoblast of the yolk-sac wall is rich in alkaline phosphatase in late stages very little of the enzyme is present in this tissue.

4. In Erinaceus the yolk-sac fluid of late stages of pregnancy cannot be regarded as an important source of absorbable substances, and it is improbable that the yolk-sac splanchnopleur, the histochemical reactions of which are described, is as important as a placental structure in this species as it is in the rodents.

5. The histochemical and tinctorial reactions of Reichert's membrane are described, and these reactions are compared with those that have been described for the membrane of Reichert formed in the rat. Since the membrane is unstained by Weigert's resorcin fuchsin elastic tissue is not one of its components. Most of its observed properties suggest that it is formed of collagenous fibres. In the hedgehog its formation is more or less coincident with the onset of the retrogression of the yolk-sac placenta.

I wish to express my thanks to Professor F. W. R. Brambell, F.R.S., for his advice.

**REFERENCES**


EXPLANATION OF PLATES

PLATE 1

Abbreviations:

<table>
<thead>
<tr>
<th>E.</th>
<th>Endoderm</th>
<th>M.</th>
<th>Maternal blood</th>
<th>M.</th>
<th>Mesoderm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS.</td>
<td>Maternal blood space</td>
<td>R.</td>
<td>Reichert's membrane</td>
<td>T.</td>
<td>Trophoblast</td>
</tr>
<tr>
<td>Tr.</td>
<td>Trophospongia</td>
<td>V.</td>
<td>Vitelline vessel</td>
<td>Y.</td>
<td>Yolk-sac cavity</td>
</tr>
</tbody>
</table>

Fig. A. Part of a transverse section of the uterus of a hibernating female showing the presence of alkaline phosphatase in the uterine epithelium and in the necks of the uterine glands. Cold acetone fixation. × 90.

Fig. B. Part of a transverse section of an oestrous uterus showing the distribution of the greatly increased amount of alkaline phosphatase. The enzyme is now present in the deeper parts of the uterine glands. Cold acetone fixation. × 80.

Fig. C. Part of the bilaminar wall of a very early blastocyst. Very little alkaline phosphatase is present in the endoderm, trophoblast, and trophospongia. Formalin fixation. × 450.

Fig. D. Part of the bilaminar wall of a later blastocyst showing the increased amount of alkaline phosphatase in the trophoblast and trophospongia. Formalin fixation. × 340.

Fig. E. Part of the bilaminar wall of a late embryonic plate stage in which the endoderm and trophoblast are particularly rich in alkaline phosphatase. Cold acetone fixation. × 320.
PLATE 2

Fig. F. Part of the bilaminar wall of an early blastocyst showing the distribution of lipoidal material in the trophoblast and trophospongia. Formalin fixation and stained with Sudan black. x 525.

Fig. G. Part of the vascular yolk-sac placenta at its greatest development showing the increased amount of lipoidal material present. Formalin fixation and stained with Sudan black. x 315.

Fig. H. Part of the lateral wall of a late conceptus showing Reichert's membrane and the absence of lipoidal material in the trophoblast and trophospongia. Champy fixation and post-osmification. x 370.

Fig. I. Part of the yolk-sac splanchnopleur of a late stage of pregnancy showing the lipoidal material present in the endoderm and mesenchyme. Champy fixation and post-osmification. x 500.

Fig. J. Part of the yolk-sac splanchnopleur of a late stage showing the argyrophilia of the basement membrane of the endoderm and of the coelomic mesothelium. Argyrophilic fibres are present in the mesenchyme. Formalin fixation and silver impregnation. x 400.

PLATE 3

Fig. K. Part of the mesometrial wall of the yolk-sac of a late stage of pregnancy. Reichert's membrane is coloured black after silver impregnation. Formalin fixation. x 450.

Fig. L. Part of the lateral wall of a late conceptus in which Reichert's membrane is coloured brown after silver impregnation. Formalin fixation. x 470.

Fig. M. Part of the lateral wall of a late conceptus. Reichert's membrane is stained faintly pink and glycogen is present in the trophoblast and trophospongia. Rossman fixation and PAS reagents. x 440.

Fig. N. Part of the mesometrial wall of a late conceptus. Reichert's membrane is stained crimson. Rossman fixation and PAS reagents after treatment with saliva. x 440.

Fig. O. Part of the yolk-sac wall showing Reichert's membrane, which is stained green, soon after it first becomes discernable. Bouin fixation and stained with Masson's trichrome. x 360.

(Manuscript received 2: vii: 56)
B. MORRIS

Plate 2
B. MORRIS

Plate 3