THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE PREGNANT UTERUS OF THE RAT

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INTRODUCTION

The pregnant uterus and foetal membranes are known both on chemical (Busse, 1936) and histochemical (Dempsey & Wislocki, 1946) grounds to contain large quantities of alkaline phosphatase, but apart from Hard's (1946) work on the guinea-pig, the distribution of this enzyme in any one species has not been studied from the beginning to the end of pregnancy. Wislocki & Dempsey (1945, 1946*a*, *b*) and Wislocki, Deane & Dempsey (1946) in the course of a large-scale histochemical survey of the pregnant uterus have established the fact that there are major species differences in the distribution of the enzyme. Their work, therefore, emphasizes the need for each species to be studied separately and in detail.

The fundamental significance of alkaline phosphatase during pregnancy is not known with any certainty, but one reasonable hypothesis is that it is concerned in the transference of materials to and from the foetus. Data about the distribution of the enzyme in organs such as the decidua, yolk-sac and allantoic placenta, which play major roles in foetal nutrition, should therefore be of value in deciding for or against this hypothesis.

It was therefore decided to make a detailed histochemical investigation of the uterus of the rat for alkaline phosphatase throughout pregnancy.

MATERIAL AND METHODS

The rats used in this investigation were bred from albino and black strains of *Mus norvegicus*. The date of copulation was determined from daily vaginal smears, and the day after copulation was counted as the first day of pregnancy. The rats were killed at oestrus and at daily intervals thereafter until the end of pregnancy. The uterus and a piece of small intestine were removed at the same time and fixed in absolute alcohol, the latter organ serving as a control. The larger gestation swellings were slit anti-mesometrially to facilitate penetration by the fixative.

Sections were cut in paraffin in the usual way, a standard thickness of 8 μ being maintained throughout the investigation. Sections were floated on 90 % alcohol before mounting. After removal of the wax the sections were coated with 0·5 % celloidin in alcohol-ether before proceeding to water. They were incubated for 2 hr. at 37° C., with periodic agitation, in a substrate mixture

composed of: 2% sodium glycerophosphate, 1 part; 2% calcium nitrate, 1 part; distilled water, 6 parts; and sufficient 0·1% sodium hydroxide to adjust the pH of the solution to 9·5. The precipitate of calcium phosphate at sites of alkaline phosphatase activity was then made visible by converting it to cobalt sulphide. It will be realized that this procedure is essentially that of Gomori's (1939) and Takamatsu's (1939) original techniques. Results were not so good, however, when floating on alcohol and coating with celloidin were omitted. The addition of M/1000-magnesium chloride to the substrate mixture did not materially affect the results.

On examination of the stained sections, sites of enzyme activity appear as light brown to black deposits of cobalt sulphide. Provided the cell is not overstained, the deposit can be located with great accuracy in such parts as the nuclear membrane, nucleoli and peripheral cytoplasm, and the colour of the deposit gives an indication of the degree of activity at each site. A black deposit tending to obliterate cell details because of its density was regarded as indicative of intense enzymic activity; a dark brown deposit, of moderate activity; light brown, of slight activity; and just perceptible staining, of minimal activity. It was not justifiable to carry quantitative analyses further than this. Throughout this paper the terms 'intense', 'moderate', 'slight' and 'minimal' will be used in these restricted senses, though occasionally 'heavy' may be substituted for 'intense' and 'light' for 'slight' where the words qualify 'staining' rather than 'activity', although these two latter terms will be regarded as equivalent and interchangeable.

Of course, if incubation is carried on long enough, previously moderate staining gives place to intense staining, and so on. It was found by experience, however, that maximum separation, or contrast, was obtained with 8 μ sections after 2 hr. incubation at 37° C., and at a pH of 9·5, provided that the substrate was agitated at 15 min. intervals.

The terminology employed for the decidua and foetal membranes is due to Krehbiel (1937) and Mossman (1937) respectively.

RESULTS

The alkaline phosphatase activity of the pregnant uterus showed three well-defined phases: (1) before implantation (5th day) overall activity was slight, although the surface and glandular epithelium, leucocytes and vascular endothelium (Pl. 1, fig. 1) were heavily stained; (2) from implantation until the foetal vascularization of the allantoic placenta at the 11th day, the decidua showed intense activity (Pl. 1, figs. 3–5); (3) from the 11th day until full-term, activity was chiefly confined to the foetal membranes (Pl. 2, figs. 6–9).

Compared with that of the decidua and foetal membranes, the enzyme activity of the embryo and foetus was slight until the last week of pregnancy, when the developing bones and some other organs exhibited intense activity (Pl. 2, fig. 9).

Within the periods mentioned the overall distribution of activity did not vary greatly. Individual tissues, however, showed progressive changes in the details of their enzyme distribution. The results are therefore best considered tissue by tissue rather than in strict chronological order.

MATERNAL TISSUES

Surface epithelium of the uterus

From the time of copulation until the 4th day the nuclei showed slight activity, while the cytoplasm adjacent to the lumen showed a band of intense activity (Pl. 1, fig. 2). Within the nucleus the nuclear membrane, nucleoli, chromosomes and chromatin granules were most heavily stained, so that appearances were very similar to those of cells stained with haematoxylin. At the 5th day the enzyme disappeared from the epithelium, not only in the implantation crypts, but also generally throughout the uterus. At the 13th day the enzyme reappeared and was thereafter to be found at similar sites to those just described until full term. It is to be noted that this waning and waxing of enzyme activity paralleled the decreasing and increasing of the height of the epithelial cells which Selye, Collip & Thomson (1935) have described as part of their 1st and 2nd progestational responses, and which are attributable to changes in the oestrogen/progesterone ratio in the circulating blood.

Glandular epithelium

The distribution of the enzyme of this epithelium was similar to that of the surface epithelium. The enzyme, however, did not disappear from the former until the 6th day and did not reappear until the 14th day. The enzyme was also present in the lumina of some of the glands, which suggested that it might be a secretory product. On the other hand, it may have been carried there by leucocytes which had passed through the epithelium, for degenerating polymorphs were frequently to be seen within the ducts.

Uterine stroma

The nuclei of the stroma cells showed slight activity during the first 3 days of pregnancy. On the 4th day they became more heavily stained in the immediate neighbourhood of the glands and surface epithelium. With the onset of the decidual reaction on the 5th day the staining of the nuclei and later of the cytoplasm became intense (Pl. 1, fig. 3; Pl. 3, figs, 10, 11 and 13). Between the decidual swellings, however, the stroma remained in the same state as regards phosphatase activity as that found during the first 4 days.

Muscularis

Staining of the plain muscle of the uterine wall was minimal at all stages of pregnancy.

Vascular endothelium

Until the 5th day the nuclei and cytoplasm of the vascular endothelium in all parts of the uterus stained intensely. The vessels of the muscularis and non-decidual stroma continued to show activity throughout pregnancy, but those of the decidua lost their activity, except mesometrially, where the endothelium continued to stain in a narrow zone adjacent to the muscularis (Pl. 2, fig. 6). At the time of formation of the metrial gland in mid-pregnancy the vessels of the mesometrium also lost their activity.

Leucocytes

During the first 3 days after copulation the stroma showed a relatively heavy infiltration with polymorphonuclear leucocytes. Similar cells were present between the epithelial cells and within the lumen. They were characterized by intense nuclear phosphatase activity. In the middle stages of pregnancy the maternal sinuses of the decidua basalis also showed a moderate number of these heavily stained cells.

Decidua

The decidual reaction appeared on the 5th day and was characterized initially by intense staining of all its zones. At the periphery of each decidual swelling the staining passed off abruptly into that of the normal stroma.

Staining, however, did not remain uniform throughout the decidua (Pl. 1, figs. 3–5). Soon after the first appearance of the reaction a number of well-defined zones could be differentiated on the basis of differences in staining intensity and in the proportionate activity of nuclei and cytoplasm. By the 8th day (Pl. 1, fig. 4) these differences had become very well marked indeed. Apart from these criteria the zones were also distinguishable by differences in the packing of the cells, the size of the cells and the arrangement of their blood vessels. The zones, in fact, were almost identical with those described by Krehbiel (1937) on other morphological and histochemical grounds. They were as follows (Pl. 1, fig. 4): in sections cut transversely across the uterus, which included the blastocyst in its crypt, there were (1) a mesometrial subepithelial zone of intense activity, (2) an anti-mesometrial subepithelial zone of relatively light staining, (3) a horseshoe-shaped anti-mesometrial zone of intense activity, (4) wing-like lateral mesometrial zones of relatively slight activity, (5) a central mesometrial zone of intense staining and (6) a marginal zone, adjacent to the circular muscle, of extremely heavy staining. With later modifications in the decidua, zone (3) became the definitive decidua capsularis, while zones (4) and (5) combined to form the decidua basalis (Pl. 1, fig. 5).

On the 10th day enzyme activity was still intense in most parts of the decidua, the lightest staining areas being the glycogenic zones of the basalis. On the 11th day the decidua normally begins to atrophy, and it was observed that at this time the phosphatase activity was rapidly lost from all regions

except the central mesometrial zone and the more peripheral parts of the basalis where the endothelium of the maternal blood vessels stained intensely. By the 12th day the decidua had lost all its activity.

It is important to note that those decidual zones (viz. (2) and (4)) which from the beginning showed least phosphatase activity were precisely those which Krehbiel (1937) showed to accumulate glycogen, while the decidua capsularis, which stained very heavily for phosphatase, stored lipoids but was wholly devoid of glycogen.

Another feature of note was that when phosphatase activity became pronounced in the decidual cells on the 5th day, it was confined to the nuclei (Pl. 3, fig. 10). On the 6th day the cytoplasm began to stain in a diffuse manner, but less heavily than the nuclei (Pl. 3, fig. 11). On the 7th day conditions were reversed and cytoplasmic staining was heavier than nuclear; moreover, the enzyme now became concentrated at the surface of the cells. On the 8th day this change had progressed to the point where all cytoplasmic activity was peripheral and the nuclei stained but slightly (Pl. 3, fig. 13). Not all aspects of the cell surface showed a similar degree of enzyme concentration, however; in many places it was most marked on the side facing a blood vessel, but this relationship was by no means absolute.

Metrial gland

Between the circular and longitudinal muscle layers at the attachment of the mesometrium the perivascular accumulations of binucleated cells which constitute the metrial gland of Selye & McKeown (1935) did not show phosphatase activity. The normal vascular endothelium here likewise showed no activity. However, coursing in a spiral manner through the centre of the gland, there were normally one or two wide vessels lined irregularly by three to four layers of very large cells. These cells showed intense activity by contrast with the endothelium of the normal vessels which showed none (Pl. 3, fig. 15). In serial sections cut tangentially these specialized blood vessels could be traced through the centre of the decidua basalis into the spongy zone of the foetal placenta where their lining cells were continuous with the trophoblast. The large cells mentioned have usually been regarded as hypertrophic maternal endothelial cells (Mossman, 1937), but from the evidence given it seems more likely that they are of trophoblastic origin, especially since they stain for phosphatase with similar intensity to that of the trophoblastic cells of the spongy zone (Pl. 2, fig. 6).

FOETAL TISSUES

Blastocyst

Until the 9th day the blastocyst showed little phosphatase activity (Pl. 1, fig. 3). After prolonged incubation (12 hr.) most nuclei showed slight activity, but after the standard 2 hr. incubation, staining was minimal. On the 9th day the ectodermal cells, both at the surface and within the blastocyst, began to show increased activity. The entoderm remained unstained.

The heaviest staining occurred in the mononuclear giant cells lying adjacent to the decidua. Here not only the nuclei but also the thin cytoplasmic processes of the cells were heavily stained. In view of later findings it is important to note that this first indication of cytoplasmic activity in the trophoblast occurred in cells which are bathed by maternal blood, and which form a part of the yolk-sac placenta whose functional importance at this stage of pregnancy has been clearly demonstrated (Everett, 1935).

At the 10th day (Pl. 1, fig. 5) the moderate to intense staining of the trophoblast was in marked contrast with the minimal staining of the embryo (excepting the neural tube), yolk-sac entoderm and allantoic mesoderm. The mononuclear giant cells showed intense nuclear and peripheral cytoplasmic activity. The spongy zone showed intense nuclear activity throughout, but intense cytoplasmic activity only at the surface of cells bathed directly by the maternal blood stream. The nuclei of the chorionic-plate trophoblast were also intensely active at this stage.

Allantoic placenta

On the 11th day (Pl. 2, fig. 6) the allantoic mesenchyme and blood vessels had begun to invade the chorionic plate. This mesenchyme differed from that of the allantoic stalk in that the former showed moderate nuclear activity while the latter was unstained. The distribution of activity in the trophoblast was similar to that of the previous day, the most marked feature again being the intense cytoplasmic activity of those parts of the trophoblast bathed with maternal blood.

Labyrinth

Between the 12th and 16th day the labyrinth consisted partly of syncytial trophoblast lining maternal blood channels, and partly of islands of cytotrophoblast (Pl. 4, fig. 16). The former continued to show intense cytoplasmic staining, while the latter gradually lost its nuclear activity. At the 16th day every maternal blood channel was clearly defined by lines of intense staining against an unstained background (Pl. 4, fig. 17). After the 16th day the staining of the syncytium became less regular but no less intense. At full term, under high magnification, it was seen to have lost its evenness and to consist of discrete clumps of precipitate of varying size which were chiefly concentrated around the nuclei. Between the nuclei, however, there was sufficient stain to make it possible to assert that there were no gaps in the syncytium. In the light of this observation it is difficult to agree with Mossman (1937) that a haemo-endothelial relationship exists in late pregnancy in the rat. On similar grounds Hard (1946) was unable to accept the view that the guinea-pig's placenta was haemo-endothelial.

Spongy zone

As in the labyrinth, the maternal blood channels of the spongy zone remained clearly outlined throughout pregnancy (Pl. 2, fig. 8 and Pl. 4, fig. 18). Unlike the labyrinth, however, some activity persisted in the nuclei until full

term. The binucleated glycogenic cells which normally accumulate in immense numbers in this zone between the 14th and 18th days never showed more than minimal activity. On morphological and histochemical grounds, therefore, these cells were identical with those of the metrial gland, an observation which supports Szendi's (1933) and Selye & McKeown's (1935) claims that all glycogenic cells have a common origin in the adventitia of the mesometrial blood vessels, and reach the spongy zone by migration.

Giant cell zone

Throughout pregnancy these cells showed strong cytoplasmic and moderate nuclear activity.

Allantoic mesoderm

After their initial invasion of the labyrinth on the 11th day, the foetal connective tissue and vascular endothelium rapidly lost their phosphatase activity. From the 14th day the labyrinth showed clear areas occupied by foetal connective tissue alternating in a striking manner with heavily outlined maternal channels (Pl. 4, fig. 17).

Entodermal sinuses

Beginning at the 14th day the entering and emerging allantoic vessels and their connective tissue coverings acquired double-walled omphalopleuric sheaths. Reichert's membrane and the entodermal cells comprising these sheaths showed slight activity until full term.

Yolk-sac

(1) *Omphalopleure.* The giant cells showed intense, but the entodermal cells only slight, activity.

(2) *Splanchnopleure.* Until the 13th day the entodermal cells of the inner wall of the yolk-sac did not stain, but on that day their nuclei began to stain strongly (Pl. 3, fig. 12). This change coincided with the normal hyperplasia of the entoderm which results in the formation of villous projections over the mesometrial half of this part of the yolk-sac. On the 14th day the distal cytoplasm of the entodermal cells began to stain intensely, and this continued to be the case (Pl. 3, fig. 14) until the 19th day, when staining became less intense and more diffuse. Nuclear staining became less intense after its initial appearance on the 13th day (cf. Pl. 3, figs. 12, 14).

Amnion

During the latter half of pregnancy the amnion stained in an irregular manner. Sections gave the impression that the enzyme had been absorbed at the surface of the membrane rather than of having been manufactured locally.

FOETUS

The location of phosphatase activity in the embryo and foetus throughout pregnancy was not subjected to detailed investigation. Only a few points can be made here.

On the 10th day the nuclei of the neural tube showed moderate activity. On the 12th day the anterior horn cells and their axons were conspicuously stained compared with the rest of the nervous system. The Wolffian duct and the entoderm of the alimentary canal also showed some activity. On the whole, however, the tissues of the foetus proper were poorly stained as compared with the foetal membranes.

On the 14th day the condensed mesenchyme in the position of the future mandible and maxilla showed intense nuclear activity. When ossification commenced at these sites on the 16th day both the nuclei and the cytoplasm of the osteoblasts stained with great intensity, and the enzyme was also present intercellularly. At this stage hypertrophic cartilage also showed great activity. There was little activity in the remainder of the embryo. On the 19th day (Pl. 2, fig. 9), in addition to centres of ossification, organs such as the kidney, lung, salivary glands and pancreas, in which rapid proliferation of ducts was taking place, showed intense activity (Pl. 4, fig. 19); developing hair and teeth and the spinal ganglia were also active. In all these instances staining was predominantly nuclear and rivalled in intensity that of the foetal membranes. The activity of the liver was patchy, being heavy in areas showing active haematopoiesis, but light elsewhere.

DISCUSSION

The only other observations on the distribution of alkaline phosphatase in the decidua and foetal membranes of the rat which have been reported are those contained in papers by Wislocki & Dempsey (1945) and Wislocki *et al.* (1946), and with their findings, so far as they go, the observations made in this paper are in substantial agreement, although it has not been possible to subscribe fully to all their interpretations, particularly those concerning the role of the enzyme in glycogen synthesis. This point will be discussed later. The data given by Hard (1946) for the guinea-pig and by Wislocki & Dempsey (1945, 1946*a*, *b*), Wislocki *et al.* (1946) and Dempsey & Wislocki (1945, 1947) for man, the mouse, rabbit, guinea-pig, hamster, cat and sow, make it clear that the distribution of the enzyme varies greatly from species to species. The only constant finding was the presence of the enzyme in substantial amounts in either the trophoblast or the maternal endothelium between the foetal and maternal circulations. From this it might be inferred that alkaline phosphatase is intimately concerned in the transport of materials to and from the foetus. The enzyme, however, is not confined to sites where such exchange is taking place, so that it is necessary to consider other explanations of its presence.

Moog (1944) and Brachet (1946) found that alkaline phosphatase was most active at two stages in the life history of cells: (1) during proliferation and differentiation and (2) during specific functional activity. The observations reported here are in agreement with this view, but, in addition, they bring out the fact that during the former stage the enzyme was mainly active within the nucleus, while in the latter stage the activity was almost exclusively

cytoplasmic. Thus, in the decidua, trophoblast, inner wall of the yolk-sac and in the skeletal, glandular and nervous tissues of the foetus, the stage of rapid cellular multiplication and differentiation was associated with intense nuclear activity. On the other hand, the mature decidua, and those parts of the trophoblast and yolk-sac engaged in the transference of materials to and from the foetus, were characterized by intense activity of the peripheral cytoplasm. Where functional activity followed rapidly upon cellular proliferation, an intermediate stage was observed, in which heavy nuclear staining was combined with moderate, diffuse cytoplasmic staining. It would appear from this that the significances of nuclear and cytoplasmic phosphatase should be separately evaluated.

(1) The role of the nuclear phosphatases appears to be bound up with the metabolism of nucleoproteins, which in turn are intimately concerned in protein synthesis and cell division (Brachet, 1946). Thus the enzyme which breaks down nucleotides to nucleosides is an alkaline phosphatase (Sumner & Somers, 1947). It has been shown that the intensity of alkaline phosphatase activity runs parallel with the rate of synthesis of desoxynucleic acid (Brachet, 1946). A similar enzyme is present in large amounts in the chromosomes where its distribution corresponds closely with that of the Feulgen-staining desoxynucleic acids (Willmer, 1942; Krugelis, 1942; Danielli & Catcheside, 1945). In the developing chick Moog (1943) found a general correspondence between alkaline phosphatase activity and the rapidity of cell division, though in some cases the activity was better correlated with the stage of cellular differentiation than with mitosis.

Summing up we can say that the intensity of nuclear phosphatase staining is a useful index of the morphogenetic activity of the cell by virtue of the participation of the enzyme in the manufacture and degradation of nucleoproteins.

(2) The functions accorded to cytoplasmic phosphatases are numerous and varied, and in many cases appear to be specific for the cells concerned.

In certain glandular and other cells fine granules can be centrifuged off which are very rich in both ribosenucleic acids and alkaline phosphatase (Brachet & Jeener, 1944). In regenerating nerves the resynthesis of the nucleoprotein-containing Nissl substance is accompanied by intense acid phosphatase activity of the cell body (Bodian & Mellors, 1945). The phosphatases are not confined to the formed nucleoprotein elements of the cytoplasm, however, for they are also found free in the general cytoplasm (Brachet & Jeener, 1944). Recently, Jeener (1947) has suggested that the alkaline phosphatase of the cytoplasm is more particularly concerned in the manufacture of fibrous proteins such as keratin, collagen and myosin, than with general protein formation.

In ossifying and calcifying tissues an alkaline phosphatase has been shown to be responsible for the provision of inorganic phosphate ions from an unknown precursor (Robison & Soames, 1924). The enzyme is nuclear, cytoplasmic and intercellular in its distribution (Bourne, 1943).

The phosphatase of the liver appears to be responsible for the final stage in the breakdown of glycogen to glucose (Cori, Cori & Schmidt, 1939). On the other hand, in developing hair (Johnson & Bevelander, 1946), teeth (Horowitz, 1942; Bevelander & Johnson, 1945) and in the placenta (Wislocki *et al.*, 1946), alkaline phosphatase stains most heavily in regions which are apparently actively storing glycogen. Wislocki *et al.* (1946) therefore suggest that the enzyme takes part in glycogen synthesis. This view, however, is improbable on thermodynamic grounds (Soskin & Levine, 1946), and in any case glycogen synthesis in both liver and muscle apparently does not involve either acid or alkaline phosphatases (Barron, 1943). In this connexion it is of interest that in the present investigation the glycogen-storage zones of the decidua showed much less alkaline phosphatase activity than the fat-storage zones, while in the metrial gland, where glycogenesis is very active, the enzyme was restricted to the lining of the specialized blood vessels.

In the intestinal epithelium the alkaline phosphatases of the brush border and Golgi zones are probably concerned in the absorption of sugars (Lundsgaard, 1933) and fatty acids (Jeker, 1936). In the proximal convoluted tubules of the kidney also there is evidence that the phosphatase of the brush border is concerned in the absorption of glucose from the glomerular filtrate (Lundsgaard, 1933).

In the placental trophoblast and yolk-sac epithelium, the alkaline phosphatase is strategically placed for the performance of similar functions to those ascribed to the intestinal and renal-tubular phosphatases. The enzyme may therefore play a vital role in the transference of sugars and fatty acids. It must not be forgotten, however, that the demands of the embryo and foetus for inorganic phosphate are very great and that therefore the enzyme may be primarily concerned with the liberation of this substance from organic precursors in the maternal blood stream. The fact that the level of inorganic phosphate is higher in the foetal than in the maternal blood, while the reverse is true in the case of organic phosphate compounds, is in agreement with this point of view (Timpe, 1931).

Finally, however, it must be stressed that alkaline phosphatase almost certainly does not act alone, but that in all probability it merely forms one of a battery of enzymes concerned in the transference and metabolism of nutritive substances. Until more information is available about the other members of the system, it is premature to speculate too far as to the functional importance of the one or two components which have been intensively studied.

SUMMARY

1. The distribution of alkaline phosphatase has been studied in the decidua and foetal membranes of the rat throughout pregnancy.

2. During the first 4 days the enzyme is present in the uterine epithelium, endothelium and leucocytes. From the 5th to the 11th days it is found in large amounts in the decidua. From the 11th day until full term it is present in

large amounts in the placenta and yolk-sac. The enzyme disappears from the uterine epithelium on the 5th day and reappears on the 13th day.

3. During the stages of cellular proliferation and differentiation the enzyme is predominantly nuclear in distribution.

4. Fully differentiated cells engaged in the transference of materials to and from the foetus show a heavy concentration of the enzyme in their peripheral cytoplasm.

5. The enzyme is least active in regions of the decidua, trophoblast and metrial gland engaged in the storage of glycogen.

6. Support is given for the hypothesis of Szendi and of Selye & McKeown that the glycogen cells of the spongy zone of the placenta have their origin in the metrial gland.

7. The so-called hypertrophic endothelium of the retro-placental maternal blood vessels is probably of trophoblastic origin.

8. The evidence is against Mossman's view that the placenta of the rat becomes haemo-endothelial in type late in pregnancy.

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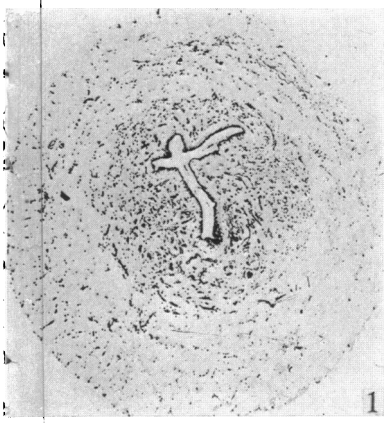

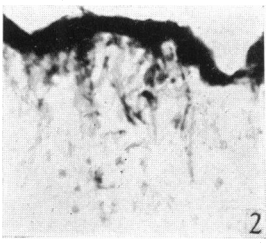

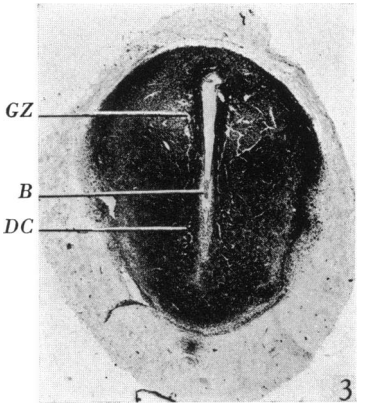

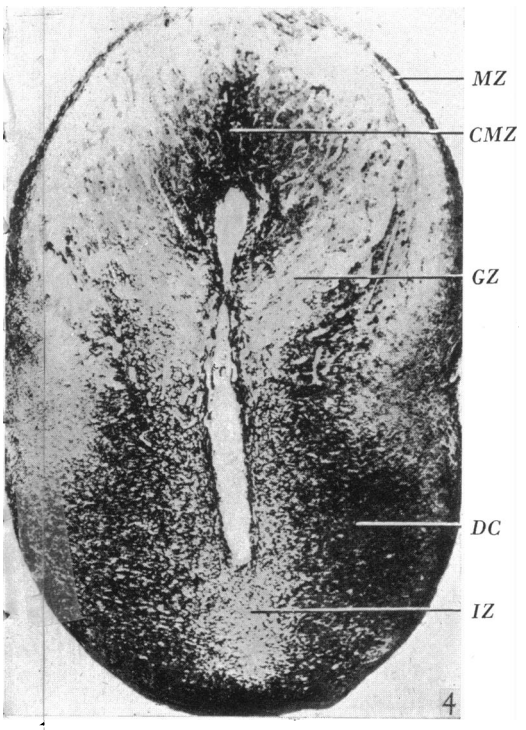

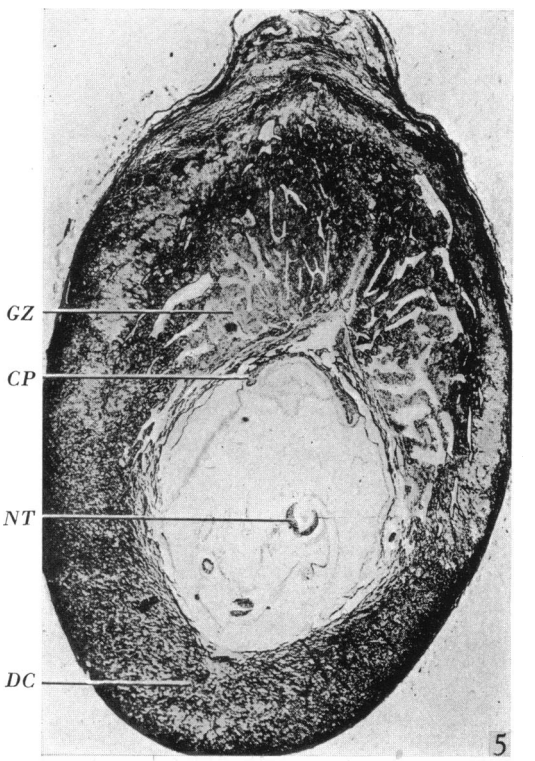

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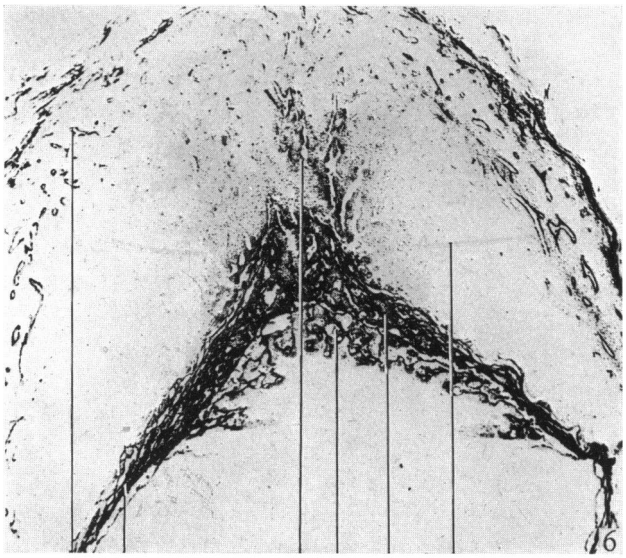
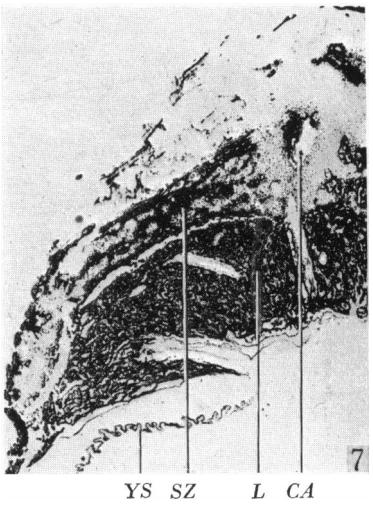
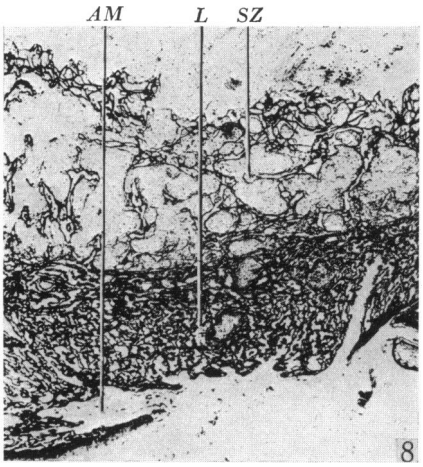
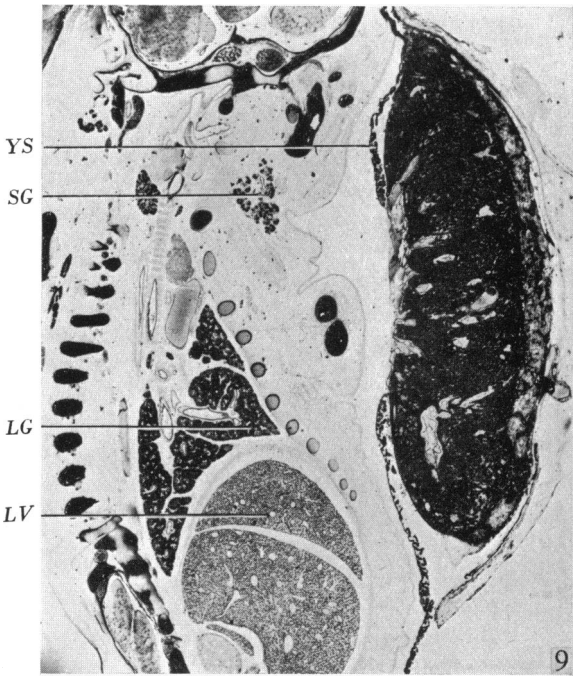
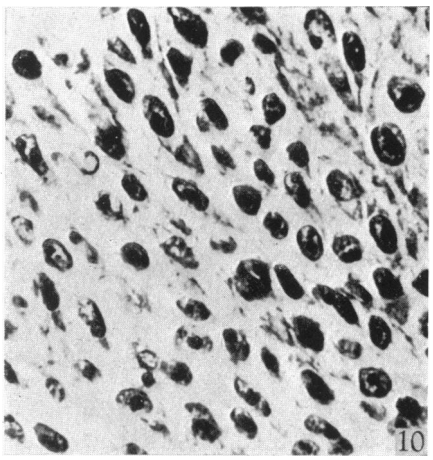


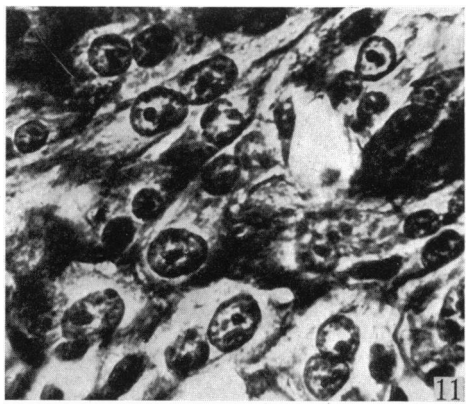


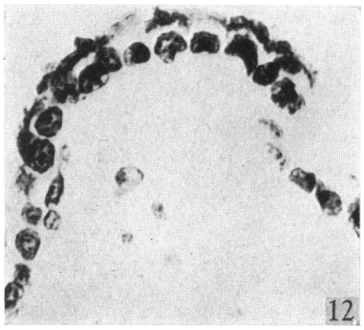


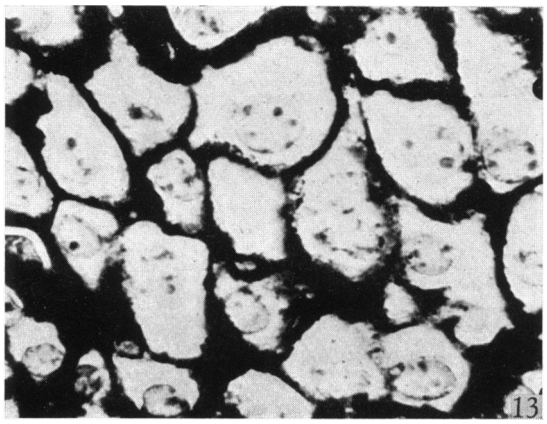


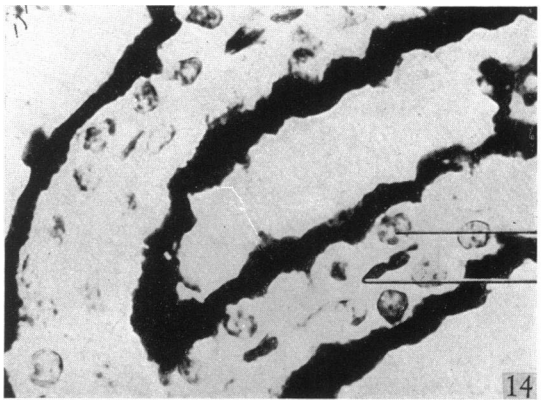
PRITCHARD—THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE PREGNANT UTERUS OF THE RAT

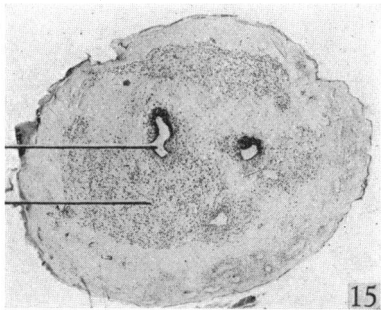




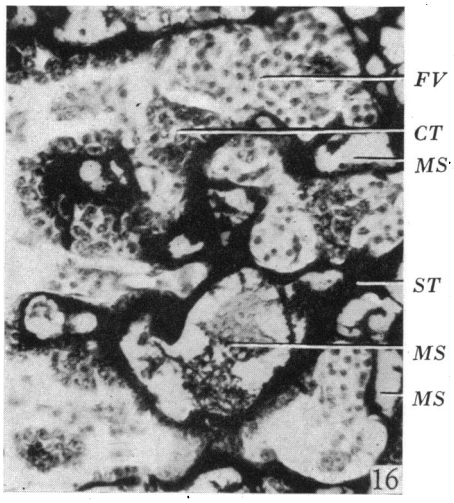








PRITCHARD—THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE PREGNANT UTERUS OF THE RAT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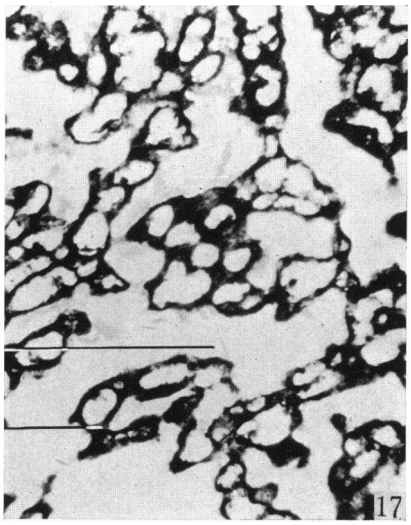
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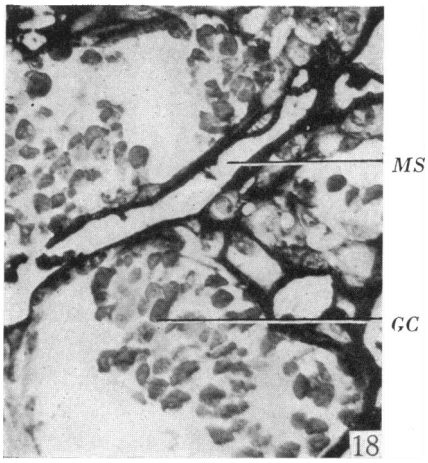
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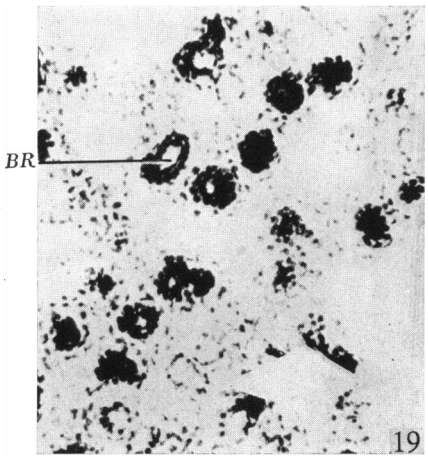
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EXPLANATION OF PLATES

All sections are stained solely for alkaline phosphatase, except for those shown in figs. 8, 15 and 18 in which glycogen has also been stained with Best's carmine.

PLATE 1

Fig. 1. Transverse section of uterus at the 1st day of pregnancy. ×8·3.

Fig. 2. Epithelium from part of section shown in fig. 1, showing intense staining of distal cytoplasm. ×700.

Fig. 3. Transverse section of uterus at the 5th day. The decidua is intensely stained, but only blood vessels stain in the muscularis. ×8·3.

Fig. 4. Transverse section of uterus at the 8th day. Only decidua stained. Contrast the intense staining of the central mesometrial zone, decidua capsularis and marginal zone, with the light staining of the glycogenic and implantation zones. ×8·3.

Fig. 5. Transverse section of uterus at the 10th day. In addition to the decidua, the trophoblast and the neural tube are stained. ×8·3.

PLATE 2

Fig. 6. Part of transverse section of uterus at the 11th day. Contrast the intense staining of the trophoblast with virtual absence of staining in the decidua basalis and allantois. ×8·3.

Fig. 7. Part of transverse section of uterus at the 17th day showing intense staining of the placenta and inner wall of the yolk-sac. ×8·3.

Fig. 8. Part of transverse section of uterus at the 15th day. Note the intense staining of the trophoblastic syncytium, both in the labyrinth and spongy zone. The glycogen cells of the spongy zone, stained with Best's carmine, appear as fine dots. ×8·3.

Fig. 9. Longitudinal section of foetus and placenta at the 19th day showing intense staining of the skeleton and glandular tissues, as well as of the placenta and yolk-sac. ×4·8.

PLATE 3

Fig. 10. Part of decidua capsularis at the 5th day showing intense staining of nuclei. ×594.

Fig. 11. Part of decidua capsularis at the 6th day showing intense nuclear staining combined with diffuse cytoplasmic staining. ×594.

Fig. 12. Part of inner wall of the yolk-sac at the 13th day showing intense nuclear staining with beginning distal cytoplasmic staining. ×594.

Fig. 13. Part of decidua capsularis at the 8th day showing intense staining of the peripheral cytoplasm with minimal nuclear staining. ×594.

Fig. 14. Parts of two yolk-sac villi at the 16th day showing intense staining of the distal cytoplasm with light to moderate nuclear staining. ×700.

Fig. 15. Transverse section of metrial gland at the 18th day showing two maternal blood channels with heavily stained lining cells. The glycogen cells, stained with Best's carmine, appear as fine dots. ×5·9.

PLATE 4

Fig. 16. Part of the labyrinth at the 12th day showing intense staining of the syncytio-trophoblast with moderate staining of the cytotrophoblast and foetal vascular endothelium and red cells. ×174.

Fig. 17. Part of the labyrinth at the 16th day, showing intense staining of the syncytium but absence of staining from the foetal blood vessels and mesenchyme. ×174.

Fig. 18. Part of the spongy zone of the placenta at the 15th day showing intense cytoplasmic staining of the trophoblast lining the maternal blood sinuses. Glycogen (grey) has been stained with Best's carmine. ×174.

Fig. 19. Part of the developing lung at the 19th day showing intense staining of the entodermal lining of the bronchi. ×149.

ABBREVIATIONS

AM allantoic mesenchyme
B blastocyst
BR bronchus
CA central artery
CMZ central mesometrial zone
CP chorionic plate
CT cyto-trophoblast
DB decidua basalis
DC decidua capsularis
EN entodermal nucleus
FE foetal endothelium
FV foetal blood vessel
GC glycogen cells
GZ glycogenic zone
IZ implantation zone
L labyrinth
LG lung
LV liver
MS maternal blood sinus
MV maternal blood vessel
MZ marginal zone
NT neural tube
O omphalopleure
SG salivary gland
ST syncytio-trophoblast
SZ spongy zone
YS yolk-sac