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COMPARATIVE HISTOCHEMICAL STUDIES ON IMPLANTATION AND PLACENTATION

VOL. 1.

TEXT.

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INTRODUCTION

INTRODUCTION

The first introduction of the term "placenta" was made by Realdus Columbus (quoted by Amoroso, 1952) in the early 16th century, who described that of the human as an "affusion" of residual matter in the form of a circular cake. For many years the term continued to be used in reference to the placentae of rodents and man - all having the form of a flattened circular cake - and it was only gradually that it came to be applied to any structure involved in the transfer of materials between mother and developing young in either direction.

In an attempt to clarify the meaning of the term Mossman (1937) described the placenta as "any intimate apposition or fusion of the foetal organs to the maternal (or paternal) tissues for physiological exchange" thus including the anal nutritive processes observed in the Goodeidae family of teleost fishes (Turner, 1933) or the branchial attachment seen in Salpa (Saint-Hilaire, 1912). He then modified this for normal mammalian species by stating "The normal mammalian placenta is an apposition or fusion of the foetal membranes to the uterine mucosa for physiological exchange".

There can be no doubt that the above definition covers adequately all types of mammalian placenta. If strictly applied, however, it does not include other structures involved in foetal nutrition for which the term has, by common usage, come to be accepted. Two examples spring immediately to mind, the conditions in the ovioviviparous

fishes - live-bearing, but with the foetal nutrition coming probably exclusively from the yolk contained in the egg - and those in the developing chick, where the maternal side of the placenta has been completely eliminated, the foetus receives its nutrition via the yolk-sac "placenta", and passes out its waste products of metabolism into the allantoic sac where they are stored. While these structures are not true placentae, as defined by Mossman, it is of interest to include them under that general term in this thesis, as it is hoped that, by a study of the enzymes involved in their presumably rather simpler processes of absorption and/or excretion, one may obtain, by comparison, some clue to the functions of the yolk-sac placenta of the rodents.

During the latter half of the nineteenth, and earlier part of the twentieth centuries much interest was focussed on the placenta, and many papers were published relative to its anatomy, gross or microscopic, and to its development from the time of implantation onwards. As this thesis is concerned with the histochemical anatomy of the placenta rather than its morphology these studies will not be reviewed here, but the reader is referred to the excellent papers by Mossman (1937), Amoroso (1952) and the recent reviews by Wimsatt (1962) and Wynn (1964) for details.

Some of the earliest work on the histochemistry of the placenta is that of Heinricius (1889, 1891, 1914) who was concerned with that

of the cat and dog, as was Bennet (1902) and Melissinos (1906). By far the biggest volume of work on this subject, however, is contained in the series of papers by Wislocki and his associates over the period from 1944 to 1947 which are partially summarised in Wislocki, Dempsey and Fawcett (1948) and which describe the distribution of a number of compounds and enzymes in a variety of species (vide infra). Their work has been supplemented and extended more recently by studies of further enzyme systems, with particular reference to the human placenta, although some comparative investigations, e.g. Fahmy (1953) have been made. A review of these is given here, and in extended form, according to species, in the Discussion where the results of this study are compared with those detailed in the papers referred to below.

Histochemically the substances described in placentae can be divided into two broad classes - firstly materials other than enzymes, and secondly enzymes. The second class can be further subdivided into (1) the hydrolases, which includes lysosomal enzymes (de Duve, 1959) - acid phosphatase, β-glucuronidase, cathepsin (which Hess and Pearse, 1958, considered can be demonstrated as a C esterase by the indoxyl-acetate method), acid ribonuclease, acid de-oxyribonuclease, and aryl sulphatase - and esterases (non-specific); (2) the alkaline phosphatases, which includes non-specific alkaline phosphatase, the nucleoside phosphatases - 5'-nucleotidase or adenosine monophosphatase (AMP ase) and adenosine triphosphatase (ATP ase) - and glucose-6-

phosphatase (G-6-Pase); and (3) the dehydrogenases concerned with the oxidation of lactate, malate, isocitrate, succinate, glucose-6-phosphate, α-glycero-phosphate, β-hydroxy-butyrate and recently the Δ^5-3β-hydroxy-steroids. The materials other than enzymes which have been described are glycogen, acid mucopolysaccharides, lipid, RNA - either as cytoplasmic basophilia which may be due to other substances, e.g. protein, or as enzymatically identified RNA -, and various inorganic materials including calcium and iron.

Substances other than enzymes

(1) Glycogen

Since Claude Bernard's (1859) observation of glycogen in the placenta of the rabbit the occurrence of this substance has been intensively investigated. It has been reported in the placenta of man by Wislocki and Dempsey (1946a, 1948), Mancini (1948), Wislocki (1951), Fahmy (1953), McKay, Adams, Hertig and Danziger (1955), Hübner (1955), McKay, Hertig, Adams and Richardson (1958), Lobel, Deane and Romney (1962), Hertig (1962) and Hayakawa (1964), and its distribution there has been compared with other species by Fahmy (1953) and Fahmy and Huggett (1954), who described its distribution in the placentae of mice, rats, rabbits, guinea-pigs, cats, sheep and goats. Huggett (1957) reports its occurrence in the placenta of the rhesus monkey. In the mouse and rat, glycogen is also described in the placenta by Wislocki, Deane, and Dempsey (1946), Bridgman (1948a, 1948b), Schiebler

and Knoop (1959), Bulmer and Dickson (1960) and Padykula and Richardson (1963); in the cells of the metrial gland in the rat by Wislocki, Weiss, Burgos and Ellis (1957), Dallenbach-Hellweg, Battista, and Dallenbach (1965), Dickson and Bulmer (1961) and Bulmer and Peach (1965); and in the early decidua, and other structures surrounding the implanting rat embryo by Krehbiel (1937), Bridgman (1948a,b), Wislocki and Padykula (1953), Kulay and Moraes (1965) and Christie (1966). Its distribution in relation to the implanting mouse embryo is described by Finn and Hinchcliffe (1965). Its concentration in the rat placenta has been compared and correlated with that in the foetal liver by Brasseur, Isaac-Mathy, and de Meyer (1958), and the changes in placental concentration have been followed biochemically by Padykula and Richardson (1963). Glycogen in the rabbit placenta has been examined by Bernard (1859), Wislocki, Deane and Dempsey (1946), Tuchmann-Duplessis and Bortolami (1954a,b) and Fahmy and Huggett (1954), who also described its occurrence in the placenta of the guinea-pig and hamster, as did Wislocki, Deane and Dempsey (1946) and Davies (1956), Davies, Dempsey and Amoroso (1961). Amongst species having an endothelio-chorial placenta glycogen has been described in the cat, Wislocki and Dempsey (1946b), and in the ferret, Buchanan (1966), while its occurrence in the syndesmo-chorial placenta is detailed by Fahmy and Huggett (1954) - sheep and goat, Wimsatt (1951) - sheep and cow, and Greenstein, Murray and Folley (1958) - cow. The epithelio-chorial

placenta of the pig also contains glycogen - Wislocki and Dempsey (1946c). Some of the less common species in whose placentae glycogen has been described are the bat, Wimsatt (1949, 1958), Stephens (1962); the hedgehog, Morris (1957); North American Shrews, Wislocki and Wimsatt (1947); and the insectivore Crocidura caerulea, Owers (1951).

(2) Mucopolysaccharides

Diastase-resistant polysaccharide materials have also been described including the acid mucopolysaccharides thought to be associated with morphogenesis, Chiquoine (1957), Walker (1961). Their distribution in human placenta is detailed in Wislocki and Dempsey (1948), Wislocki (1951), Hübner (1955), McKay et al (1955) and Majewski (1962); in the mouse by Finn and Hinchcliffe (1965); in the rat by Wislocki and Padykula (1953), Schiebler and Knoop (1959), Bulmer and Dickson (1960), Dickson and Bulmer (1961), Kulay and Moraes (1965), and Dallenbach-Hellweg et al (1965) - metrial gland. In the guinea-pig they are described by Davies et al (1961); in the rabbit by Davies (1956); in the ferret by Buchanan (1966); in the sheep and cow by Wimsatt (1951) and cow by Greenstein, et al (1958); and in the bat by Wimsatt (1958), Stephens (1962).

(3) RNA

Ribonucleoprotein distribution has also been described in some detail in the human placenta by Wislocki and Dempsey, (1946a), Wislocki, Dempsey and Faucett (1948), Wislocki (1951), Shipunova (1953), Ortmann

(1955), McKay, et al (1955), Remotti (1956), McKay, et al (1958) and Weber (1961) who correlated its occurrence with gonadotrophin production. In the rat it is described by Wislocki, Deane and Dempsey (1946), Dickson and Bulmer (1960), Bulmer and Dickson (1961) and by Mayer, Blanquet, Canivenc and Capot (1953) and Schiebler and Knoop (1959) both of whom used the electron microscope to study the intracellular localisation of RNA, as did Wislocki, et al (1957) in their study of the RNA content of the metrial gland cells. RNA has also been described in the placenta of the rabbit, guinea-pig and hamster, Wislocki, Deane and Dempsey (1946); guinea-pig, Davies, et al (1961); rabbit, Zhemkova (1952), Tuchmann-Duplessis and Bortolami (1954), Davies (1956); cat, Wislocki and Dempsey (1946b); ferret, Buchanan (1966); sheep and cow, Wimsatt (1951); cow, Greenstein, et al (1958); pig, Wislocki and Dempsey (1946c); hedgehog, Morris (1957); shrews, Wislocki and Wimsatt (1947); and bat, Stephens (1962).

(4) Lipid

Many studies of the distribution of lipid in the placenta exist. It has been detected histochemically in the human by Wislocki and Dempsey (1946a, 1948), Wislocki, Dempsey and Fawcett (1948), Wislocki (1951), Thomsen and Lorenzen (1956), Okuda (1960) who studied its transport across the placenta in man and rats after administration to the mother of a fatty emulsion, Lobel, Deane and Romney (1962), and Helmy and Hack (1964). Even more extensively studied has been the

rat in which lipid has been described in the term placenta, Wislocki, Deane and Dempsey (guinea-pig and hamster also) (1946), Bridgman (1948b), Schiebler and Knoop (1959), Bulmer and Dickson (1961), and Helmy and Hack (1964); in the uterus of early pregnancy, Everett (1935), Krehbiel (1937), Alden (1947), Bridgman (1948a), and Kulay and Moraes (1965); and in the metrial gland, Wislocki, et al (1957), Dallenbach-Hellweg, et al (1965) and Bulmer and Peach (1965). Lipid also occurs in the placenta of the bat, Stephens (1962); guinea-pig, Davies et al (1961); rabbit, Davies (1956); cat, Wislocki and Dempsey (1946b); ferret, Buchanan (1966) who divided it into neutral lipid, phospholipid and cholesterol; sheep, Wimsatt (1951); cow, in which its distribution is described in early pregnancy, Foley, Reece and Leathem (1954), Greenstein, et al (1958) and at term, Wimsatt (1951); pig, Wislocki and Dempsey (1946c); bat, Wimsatt (1948); hedgehog, Morris (1957); and shrew, Wislocki and Wimsatt (1947).

(5) Iron and calcium

The distribution of inorganic ions in the placenta has also received some attention. Iron and calcium are described in the human placenta by Wislocki and Dempsey (1946a), Rossi and Pescetto (1949), and by McKay, et al (1958) who describe the alterations observed in these substances during the course of pregnancy. Their occurrence in the rodents is described by Wislocki, Deane and Dempsey (1946); and in the bat by Wimsatt (1949). Iron alone is described in the placenta

of the bat by Stephens (1962); hedgehog by Morris (1957); in the shrew by Wislocki and Wimsatt (1947); in Crocidura caerulea by Owers (1951); and in the cat by Wislocki and Dempsey (1946b) who also described its distribution in the pig (Wislocki and Dempsey, 1946c). Haemosiderin has been identified in the ferret placenta, Buchanan (1966).

(6) Protein

Various histochemical tests for protein and amino-acids have been applied to the placenta, particularly that of early pregnancy in the rat, Wislocki and Padykula (1953), Bulmer and Dickson (1960, 1961), Kulay and Moraes (1965), and mouse, Finn and Hinchcliffe (1965) and that of the sheep and cow, Wimsatt (1951). The metrial gland of the rat has also received some attention with regard to the distribution of specific proteins in the granules of its cells, Wislocki, et al (1957), Dickson and Bulmer (1961), Dallenbach-Hellweg, et al (1965).

Enzymes

(1) Hydrolytic enzymes

Possibly because of the ease of the older methods for acid phosphatase, or possibly because of its assumed importance in placental absorption, the distribution of this enzyme in the placenta has been extensively studied. Its presence in the human placenta is reported by Wislocki and Dempsey (1946a, 1948), Wislocki (1951), Thomsen (1955), McKay, et al (1955, 1958), Lobel, Deane and Romney (1962), Wielenga and

Willighagen (1962), Curzen (1964), and Vacek (1965). In the rat its distribution has been described by Wislocki, Deane and Dempsey (1946) - mouse, rabbit and guinea-pig also -, Bejdl (1954), Sharov (1958), Padykula (1958) who correlated its histochemical distribution with biochemical determinations, and Bulmer (1965a), who described its occurrence and alterations throughout gestation in the placenta and metrial gland, the latter also being studied by Bulmer and Peach (1965). Activity has been reported in the rabbit by Tuchmann-Duplessis and Bortolami (1954) also. Wislocki and Dempsey have reported acid phosphatase activity in the placenta of the cat (1946b) and pig (1946c) and have reviewed its distribution acting on a variety of substrates in several mammalian placentae (Dempsey and Wislocki, 1947). Its distribution in the shrew has been described by Wislocki and Wimsatt (1947), and Wimsatt has considered its occurrence in the placentae of the bat (1949), (Stephens, 1962, also) and the ruminants (1951).

While it seems possible that the acid phosphatase acting on nucleic acid described by Dempsey and Wislocki (1947) was one of the lysosomal enzymes, the only other as yet unmentioned member of this class which has been studied in any detail is β-glucuronidase, whose distribution in rat placenta is described by Bulmer (1963) using the older techniques and again in 1965(b) using more specific methods. Its occurrence in the human placenta is described by Vacek (1965).

The other enzyme of the hydrolytic group which has been studied is non-specific esterase, whose cellular localisation is uncertain.

Its occurrence in human placenta was first reported by Zacks and Wislocki (1953) and confirmed by Perrotta and Lewis (1958), McKay, et al (1958), Ricci (1963), Wachstein, Meagher and Ortiz (1963) and Vacek (1965). Other species have been less extensively studied but some observations exist on esterase activity in the rat, either in the placenta, Padykula (1958) who followed the variations in enzyme activity throughout gestation histochemically and biochemically, Bulmer (1963, 1965a), or in the metrial gland, Bulmer (1964), Bulmer and Peach (1965), Dallenbach-Hellweg, et al (1965). The other species in whose placentae esterase activity has been examined are the guinea-pig - non-specific esterase, Perrotta and Lewis (1958) - acetylcholine esterase, Goutier-Pirotte, and Gerebtzoff (1957), the cat, Zacks and Wislocki (1953), and the rabbit - acetylcholine esterase, Kehl, Dumont, Czyba and Germain (1960).

(2) Alkaline phosphatases

The enzymes found in this class can be divided into two groups. The first contains one enzyme histochemically - non-specific alkaline phosphatase, whose significance is uncertain, although it has been variously associated with growing areas of the embryo, or with areas of fluid exchange across cellular barriers, McKay, et al (1955). The other group includes several enzymes acting on specific substrates,

for example adenosine monophosphatase, adenosine triphosphatase, and glucose-6-phosphatase, whose distribution has generally been described as being different from that of non-specific alkaline phosphatase, although the latter enzyme can hydrolyse "specific enzyme" substrates to a variable extent dependent on the pH of the buffer used (Pearse, 1960).

Non-specific alkaline phosphatase has been described in the human placenta by Wislocki and Dempsey (1946a), Dempsey and Wislocki (1947), Botella and Cano (1950), Wislocki (1951), Vaczy and Juhos (1950), Fahmy and Huggett (1954) who compared its distribution there with that in the placentae of several other species, Thomsen (1955), McKay, et al (1955, 1958), Wielenga and Willighagen (1962), Wachstein, Meager, and Ortiz (1963), Curzen (1964), Sharov (1964), and Vacek (1965). In the rodents the classical papers of Pritchard (1947) - rat, Hard (1946) - guinea-pig, and Wislocki, Deane and Dempsey (1946) - rat, mouse, rabbit, guinea-pig, and hamster, have been supplemented more recently by those of Fahmy and Huggett (1954) - mouse, rat, rabbit, guinea-pig, Tuchmann-Duplessis and Bortolami (1954a,b), and Davies (1956) - rabbit, Foraker, Denham, and Mitchell (1954) - rabbit, Sharov (1958) and Padykula (1958) - rat, Finn and Hinchcliffe (1965) - mouse at the stage of implantation, Davies et al (1961) - guinea-pig. Placental types other than haemochorial have received some attention,

the distribution of non-specific alkaline phosphatase having been described in the endothelio-chorial placenta of the cat, Wislocki and Dempsey (1946b); Fahmy and Huggett (1954); in the syndesmochorial placenta of ruminants, Wimsatt (1951), Foley, et al (1954), and Fahmy and Huggett (1954); and in the epithelio-chorial placenta of the pig, Wislocki and Dempsey (1946c). Finally its distribution is described in the placenta of the bat by Wimsatt (1949, 1958), Stephens (1962); the shrew by Wislocki and Wimsatt (1947); and the hedgehog by Morris (1957).

The earliest observations on specific alkaline phosphatases in the mammalian placenta are those of Dempsey and Wislocki (1947) who describe, in the human, guinea-pig, cat and pig, the distribution of fructose-1:6-diphosphatase, lecithinase, and adenosine monophosphatase. The last named enzyme has been studied in detail since, its presence being reported in the placenta of the sheep and cow, Wimsatt (1951), and human, Thomsen and Panka (1956), McKay, et al (1958), Wielenga and Willighagen (1962), Wachstein, Meagher and Ortiz (1963), and Sharov (1964). The last three groups of authors also described the distribution of adenosine triphosphatase in the human placenta, and its presence in that of the rat is detailed by Padykula (1958). The remaining enzyme of this group whose presence in placenta has been described is glucose-6-phosphatase - human, Hertig (1962), Wachstein, Meagher and Ortiz (1963), Curzen (1964), Sharov (1964) -

rat, Padykula and Richardson (1963).

(3) Dehydrogenases - carbohydrate

Valuable as many of the early studies on carbohydrate distribution in the placenta no doubt are, their applicability to the problems of placental function are limited, without some knowledge of the use to which the carbohydrates, and particularly glycogen, are put, gained by knowledge of the presence or absence in the tissue of enzymes involved in their metabolic pathways. Thus our knowledge of placental function has gained recently, with the advent of methods for demonstrating these enzymes, methods which have been widely applied to the placentae of various species, but particularly to that of man at various stages of gestation. Certain of the enzymes for which methods are available are concerned with the introduction of lipid metabolic products into the carbohydrate pathway, or with divergence of metabolites of glycogen into ribose, and presumably RNA production. These too have been extensively studied.

Dehydrogenases concerned with the oxidation of α-glycero-phosphate and/or β-hydroxy-butyrate, the former certainly concerned with the metabolism of lipid breakdown products, the latter possibly so, have been studied in man only, by Boss and Craig (1962), Weilenga and Willighagen (1962) and Curzen (1964).

The same three groups of authors have also studied the distribution of enzymes concerned with ribose production via the "Pentose

Shunt", namely glucose-6-phoshpate dehydrogenase, and 6-phospho-gluconate dehydrogenase, as did Lobel, Deane, and Romney (1962) and Ginsburg (1964) again on human material.

Dehydrogenases acting on some or all of the following substrates of the glycolytic and respiratory metabolic pathway - lactate, isocitrate, succinate, and malate - have been described in the placenta by a number of authors, including, in the human, the five groups of authors already cited above, and Telkkä and Lehto (1954), Marazzinni and Tessari (1957), Villee and Hagermann (1958), Troen and Gordon (1958), Wachstein, Meagher and Ortiz (1963), Helmy and Hack (1964), and Vacek (1965). Far fewer studies have been made on other species, and succinic dehydrogenase alone has been described in the rat by Padykula (1958), and Helmy and Hack (1964), and in the rabbit by Foraker, Denham, and Mitchell (1954).

With the exception of succinic dehydrogenase, all of the enzymes mentioned previously in this section require the presence in the tissues of an enzyme - a diaphorase - capable of transferring hydrogen from the reduced co-factor formed by their action, either reduced nicotinamide adenine dinucleotide, - NADH (DPNH), or reduced nicotinamide adenine dinucleotide phosphate - NADPH (TPNH), to the dye used as an indicator. Thus studies of enzyme localizations in tissues by these methods are of limited value unless accompanied by examination of the tissue sections for the presence or absence of the necessary diaphorase. Only in human

material has the study of carbohydrate dehydrogenases been accompanied by simultaneous search for the diaphorase, by Boss and Craig (1962), Wachstein, Meagher, and Ortiz (1963), Curzon (1964) and Ginsburg (1964).

Another enzyme concerned indirectly with carbohydrate metabolism - with the entry of amino-acid into the respiratory cycle, or with divergence of α-keto-glutaric acid into amino acid synthesis - glutamic dehydrogenase, has been described in human material by Boss and Craig (1962).

(4) Dehydrogenases - steroid

It is well known that removal of the ovaries, following the establishment of the chorio-allantoic placenta, is not necessarily, depending on the species studied, associated with termination of the pregnancy. From this it has been deduced, and biochemically confirmed, that the placenta is the site of production, in these species, of hormones which help in the maintenance of pregnancy, and these hormones have been divided into two groups - chorionic gonadotrophin, and steroids.

The localisation of chorionic gonadotrophin production to the cytotrophoblast has been carried out by tissue culture, Gey, Seegar, and Holman (1938), and to the cells of the basal plate of the placenta by Weber (1961) the latter on very flimsy evidence. More recently, however, using immuno-fluorescent techniques Midgeley and Pierce (1962) have localised the hormone to the syncytiotrophoblast, although

Thiede (1965) has observed some fluorescence in cytotrophoblast also. Thus the problem of the site of formation of this hormone remains unresolved, and, as the techniques used in this thesis cannot contribute to it, will not be discussed further.

The problem of the site of production of steroid hormones has been investigated in this thesis, however. Early workers in this field localised steroids in the placenta by a so-called "cumulative histochemical test", Wislocki, Dempsey and Fawcett (1948), Ashbel and Seligman (1949) in the human, Wislocki and Wimsatt (1947) in the shrew, and Wimsatt (1951) in the sheep. However, this "method" can be called into question on two counts, firstly as regards specificity, Karnovsky and Deane (1955), and secondly because even if specific, which is doubtful, it demonstrates preformed steroid in the placenta, and not sites of steroid production. Thus the elucidation of the problem of the localisation of steroidogenesis in the placenta has had to await more modern methods.

Within the last few years, techniques for the demonstration of enzymes dehydrogenating steroids of various molecular configurations have become available. Initially only enzymes acting on steroids with the Δ^5-3β-ol configuration could be detected, Wattenberg (1958), but more recently, partly due to refinements in technique, partly to the availability of new substrates, the range of enzymes

detectable has been increased, and enzymes have been demonstrated acting on 11 β-hydroxysteroids, Baillie, Ferguson, Calman and Hart (1965), and on 3α-, 6β-, 11α-, 12α-, 16α-, 16β-, 17α-, 21-, and 24-hydroxysteroids, Baillie, Calman, Ferguson, and Hart (1966).

By far the vast majority of the papers published in this field have been concerned with the human placenta, Kellogg, and Glenner (1960), Lobel, Deane and Romney (1962), Adams, Jarabak, and Talalay (1962), Goldberg, Jones and Turner (1963), Koide and Mitsudo (1965), Deane and Rubin (1965), and Hart (1966a,b,c,). The only other species so far investigated is the rat and mouse, Deane, Rubin, Driks, Lobel, and Leipsner (1962), Deane and Rubin (1965), Botte, Materazzi, and Chieffi (1966).

(5) Glycogen synthetic and degradative enzymes

One paper exists, to my knowledge, on the enzymes phosphorylase and UDPG-glycogen synthetase in the decidual cells of the rat, Bo, Smith and Colborn (1964).

(6) Proteolytic enzymes other than acid phosphatase

The localisation of leucine amino-peptidase has been described for human placenta by Wielenga and Willighagen (1962), Kleiner and Wilkin (1963), Wachstein, Meagher and Ortiz (1963), and Curzen (1964); and for rat placenta by Hopsu, Ruponen, and Talanti (1961).

(7) Monoamine-oxidase

This enzyme has been described in human placenta by Wachstein, Meagher and Ortiz (1963) and by de Maria (1963).

(8) Cytochrome oxidase

This ubiquitous enzyme, or rather enzyme system has been described in human placenta by Dempsey and Wislocki (1944) and Wachstein, Meagher, and Ortiz (1963); and in the sheep, Wimsatt (1951).

(9) Carbonic anhydrase

Although this enzyme has been studied extensively biochemically in endometrium and placenta (see Hafez, 1964, for review) I have been able to find only one paper concerned with its histochemical localization, Bleyl (1964).

(10) Lipase

The specificity of methods for this enzyme is questionable, Pearse (1960). It has been described in the placenta of the bat, Wimsatt (1949), and sheep and cow, Wimsatt (1951).

(11) Vitamin C

The concentration of this substance, as detected histochemically, has been described in human placenta by Holzaepfel and Barnes (1947), and Kassabyan (1956) who followed its fluctuations in the decidua also.

(12) Tyraminase

This enzyme has been described in the human placental syncytium by Zampetti (1954).

(13) Relaxin

Recently, using immuno-fluorescent techniques, relaxin has been localized to the granules of the metrial gland cells in the rat, Dallenbach-Hellweg, et al (1965).

(14) Fibrinoid

This substance has been described by many authors in the human placenta, where its staining reactions to a mixture of methylene blue, and orange G have been described, Singer and Wislocki (1948), Sokoloff, Mund and Kantor (1951); and in the mouse placenta, where it has been examined histochemically and under the electron microscope, Bradbury, Billington and Kirby (1965).

(15) Placental transport

Few histochemical studies exist directly concerned with this subject, although there can be little doubt that much of the volume of work reported above is concerned directly, or indirectly, with the passage of materials across the placental barrier. As a few examples one might cite the studies on acid phosphatase, which, as a lysosomal enzyme, is probably concerned with the absorption and breakdown of materials for embryonic nutrition, or the localizations detailed for adenosine triphosphatase which is concerned either with trans-cellular transport, or, acting as a test for transphosphorylation, with detecting energy production.

One or two papers of a histochemical nature, are to be found, where the detection of placental transport has been the prime aim. In the human homologous serum proteins have been detected passing into the trophoblastic cytoplasm, using immuno-fluorescent techniques by Bardawil, Toy and Hertig (1958), and the passage of copper, iron, calcium, lipid, and glucose into the placental villi has been followed

either following their injection into women in labour or before termination of pregnancy, or in mice, rats, and rabbits, by Yamaguchi, Noda, Sugawara, Murakami, Yamada, Yamashita, Akiyama, Obuda and Imamura (1958). The results of the last paper have to be interpreted with caution, however, firstly as no attempt was made to compare the localisation of the substances detected with that in placentae not exposed to the excessive concentrations, and secondly as the techniques used for detection are not all specific.

Another line of approach to the problem of the histochemical study of trans-placental transport, has been to study simultaneously the histochemistry of the placenta and of the foetal mesonephroi. This has been done in the sheep by Davies (1952).

As can be seen from the above brief review, the volume of papers on placental function and metabolism, as detected histochemically, is enormous. However, a fact which emerges quite clearly, is that no comprehensive comparative histochemical study of placentation and/or embryonic nutrition has been made, using the modern techniques that are now available.

It is the aim of this thesis, therefore, to report the results of such an investigation. In it are studied various aspects of placentation - placental metabolism with respect to carbohydrate, lipid, and RNA accumulation and breakdown, following histochemically,

wherever possible, the metabolic pathways involved; and with respect to the enzymes involved in absorption and metabolism of substances for embryonic nutrition. Also examined are sites of steroidogenesis and steroid utilization; and the enzymes involved in the passage of substances across the placental barrier. For these purposes the main types of placenta, ranging from the six-layered epithelio-chorial placenta of the horse, to the three-layered haemo-chorial one of the rodents, and man have been studied, in the hope that significant differences in enzyme concentration and occurrence, correlating with the number of layers found in the placenta, might emerge. Wherever possible, also, the placental type under investigation has been studied at different stages of gestation to follow the changes in enzyme concentration which occur as it matures (and possibly ages).

It is also hoped to study the enzymes involved in the three phases of embryonic nutrition, i.e. histiotrophic nutrition before placentation, nutrition via the yolk-sac placenta either before establishment of the definitive chorio-allantoic placenta or after its development also, where the yolk sac persists in a functional state until term, and nutrition via the chorio-allantoic placenta. For this purpose the material already described above has been supplemented by studies of the process of implantation in the rat,

and rabbit, two species in which the process, while exhibiting similarities in the initial mode of attachment, shows considerable differences thereafter. From this material also, it has been possible to make some deductions on the function of the decidua, in two of its many varied types. Finally the placental types already detailed have been supplemented by study of the yolk-sac and chorio-allantoic "placenta" of the chick, and the yolk-sac "placenta" of the ovoviviparous fish.

MATERIALS AND METHODS

TABLE 1

List of material used, and specimens obtained. (F = foetal placenta, M = maternal placenta, YS = yolk sac)

Placental type	Animal	Foetal age (days) or length (cm)	Specimen obtained
Epithelio-chorial	Horse	340 days	F
Syndesmo-chorial	Sheep	2.5 cm (B.R.)	F + M (cotyledon + inter-cotyledon area)
		15 cm (B.R.)	F + M " "
		25 cm (B.R.)	F + M " "
		40 cm (B.R.)	F + M " "
		55 cm (B.R.)	F + M " "
Endothelio-chorial	Cat	7 cm (C.R.)	F + M
		12.5cm (C.R.)	F + M
	Dog	24 days	F + M
		32 days	F + M
		45 days	F + M
		60 days	F
	Ferret	22 days	F + M

TABLE 1 (continued)

Placental type	Animal	Foetal age (days) or length (cm)	Specimen obtained
Haemo-chorial	Human	1.5 cm	F + YS
		1.8 cm	F
		5.5 cm	F
		7.5 cm	F + M (decidua + muscle)
		15 cm	F
		Term	F + M (decidua)
Haemo-chorial	Rat	10½ days	F + M
		14½ days	F + M
		17½ days	F + M
		20½ days	F + M
	Rabbit	13 days	F + M
		17 days	F + M
		20 days	F + M
		30 days	F + M
	Guinea-pig	20 days	F + M
		63 days	F + M

TABLE 1 (continued)

Placental type	Animal	Foetal age (days) or length (cm)	Specimen obtained
Haemo-chorial (implantation)	Rat	$4\frac{1}{2}$, 5, $5\frac{1}{2}$, 6, $6\frac{1}{2}$, 7, $7\frac{1}{2}$, $8\frac{1}{2}$, $9\frac{1}{2}$ days	F + M
	Rabbit	5, 6, 7, $7\frac{1}{2}$, 8, $8\frac{1}{2}$, 9 days	F + M
Yolk-sac	Chick	3 days	F (YS)
		5 days	F (YS)
		10 days	F (YS)
	Limia Maculata	(Mid-late pregnancy)	F + M

MATERIALS AND METHODS

Table 1 shows the material used in this study and the stages of gestation at which it was obtained (expressed in days where laboratory material was used, or in terms of foetal length where the material was collected in the field). Representatives of the main placental types, with the exception of the chorio-vitelline marsupial placenta which was not available, are present, the haemochorial placenta (rat, rabbit, guinea-pig, and human) being particularly well represented. In the sheep, rat, rabbit, and human an extensive series of material at different stages of gestation was collected, and some early stages of placental development in the dog were obtained through the kind co-operation of veterinary colleagues.

With the exception of the horse, and term dog (from both of which only foetal placenta was obtained) all material was collected either under ether anaesthesia (ferret, dog, rat, rabbit, guinea-pig, human) or within 2 to 3 minutes of maternal death (cat, sheep, limia maculata). In the horse, the area of placenta examined was removed while the membranes were still in situ in the uterus and attached to the foetus by the umbilical cord whose vessels were still patent. The term dog placenta was collected at the time of delivery. For the chick membranes, fertile eggs at appropriate stages of incubation were opened under warm saline, and the membranes immediately removed (while the heart was still beating) and frozen. Human material was

obtained during termination of pregnancy for non-obstetric reasons, or during elective Caesarian section for dystocia. All specimens used were collected from normal healthy animals, experiencing an adequate diet, and (in the case of laboratory animals) caged in uncrowded conditions with normal lighting.

In the rodents, where necessary, implantation sites were visualised, prior to obvious gestational swellings, by the intravenous injection of 2 ml. of a 2% solution of Niagara Blue 2B 10 minutes before laparotomy, following which treatment the implantation sites show blue colouration, while the rest of the uterus remains uncoloured. (Psychoyos, 1960a, b, 1961). Pre-implantation blastocysts in situ in the rabbit uterus at 5 days were localized by longitudinal sectioning of the uterus. For pre-implantation stages of the rat no attempt was made to find the blastocysts, but uteri were sectioned longitudinally and transversely to ensure that no alterations in enzyme activity occurred along their length.

Following collection all material was treated in one of 4 ways:-

(1) Fixation in Bouin's fixative, for routine histology and glycogen staining. For histology either Haematoxylin and Eosin, or the Masson staining method was used, the latter being found to differentiate between one tissue and another particularly well, for example the distinction between syncytiotrophoblast and cytotrophoblast in the rabbit which was much more pronounced with this stain than with the routine Haematoxylin and Eosin.

The question of the best fixative for glycogen is still debated (see Pearse, 1960) and was aggravated in this study by the delicacy of some of the membranes being studied. Bouin's fixative, being watery in nature, and containing acetic acid is known to produce minimal "streaming" artefact, although whether it preserves all of the glycogen content of the tissue (in particular the less highly polymerised glycogens) is doubtful. The mixture of 96% alcohol saturated with pieric acid (85 parts), 40% formalin (10 parts) and acetic acid (5 parts) at - 73°C, recommended by Lison and Vokaer (1949) and said by them to preserve all of the tissue glycogen content, was found to be so destructive in its action on embryonic tissues as to be of no value in this study. A number of other fixatives were tried including formol-saline, as recommended by Vallance-Owen (1948) but eventually it was decided that the best balance with respect to tissue and glycogen preservations was given by Bouin's fixative which was therefore used throughout. This decision was influenced to a certain extent by the results of a survey of glycogen in the tissues of the rat which was carried out using the histochemical methods described below, and in which Bouin, and Lison and Vokaer's fixative were compared. In this survey little difference between the results given by the two fixatives was observed.

Glycogen was detected in the tissues using a series of 4 adjacent sections, and the technique of dimedone blockade described by Bulmer

(1959). Sections were treated as follows:-

(A) PAS - procedure (McManus, 1946).

(B) PAS - procedure, following pretreatment of the sections with diastase (B.D.H.) (1% aqueous for 1 hour at 37°C). This section shows PAS - positive material other than glycogen, i.e. muco- and glyco-protein mainly in paraffin sections.

(C) Oxidation of the sections in the periodic acid of the PAS procedure, followed by exposure to dimedone (5% in absolute alcohol at 60°C for 6 hours), followed by the remainder of the PAS procedure. This section shows glycogen and mucin.

(D) Section treated as (C) but following diastase pre-treatment as in (B). This section shows mucin only.

The histochemical specificity of Bulmer's method was checked on the survey of normal rat tissues described above and it was found that mucin of the uterine and intestinal glands was stained following dimedone blockade as well as glycogen. Thus section D was introduced into the staining series described above as a check on the results in section C.

(2) Fixation in Lillie's (1954) acetic-alcohol-formalin fixative:-

40% formaldehyde	10 ml
glacial acetic acid	5 ml
absolute alcohol	85 ml

(fixation for 24 hours at 0°C)

Material fixed in this solution was stained for nucleic acids, which are well preserved but not made resistant to enzyme extraction (Pearse, 1960). Staining was by the chrome-alum gallocyanin technique at pH 1.64 which stains both DNA and RNA, and whose specificity for nucleic acids at that pH has been confirmed by de Boer and Sarnaker (1956), non-specific staining being negligible. For the localization of RNA sections were treated, prior to staining, with ribonuclease (Sigma) (0.1% solution in water for 1 hour at 37°C) following which nuclear staining only with vacuolated nucleoli was observed in the vast majority of the cells.

(3) Fixation in the fixative described by Williams and Jackson (1956):-

5-amino-acridine hydrochloride	0.4 gm
50% ethanol	100 ml

which is particularly effective in the preservation of acid muco-polysaccharides with which it forms highly insoluble complexes.

For staining of acid mucopolysaccharides several methods were employed.

(A) for implantation stages and all placental specimens the dialyzed iron method of Mowry (1958). Some staining of nucleo-protein (particularly DNA) was observed with this method despite reduction of the pH below 1.3. However, apart from that non-specific staining, which was not very intense, a dense blue reaction was observed in

sites of acid mucopolysaccharide location. Thus these could be observed with this method, if known non-specific staining was disregarded.

(B) for certain placental specimens (for comparative study) the above method was supplemented by:-

(i) Toluidine blue (all specimens), Kramer and Windrum (1955) in which both β and ϒ metachromasia are preserved.

(ii) Azur A (0.02% in 0.1M phosphate-citrate buffer at pH 1.5 or 4.5), Spicer (1960) - dehydrated through acetone.

(iii) Alcian blue (1% in 3% acetic acid) Mowry (1956).

(iv) Toluidine blue (Kramer and Windrum) following extraction in ribonuclease as in (2) above.

(v) Dialyzed iron following extraction in ribonuclease as in (2) above.

(vi) Azure A as in (ii) following "mild methylation"- pre-treatment for 4 hours at 37°C with pre-heated absolute methanol made 0.1N with H-Cl, Spicer (1960).

(vii) Dialyzed iron following "mild methylation".

Ribonuclease extraction in (iv) above was used to eliminate the alcohol-stable β metachromasia of RNA; in (v) above to eliminate the possibility of a certain amount of dialyzed iron staining being due to nucleo-protein, it being found that, in placental material, no non-specific staining of this type occurred.

The methylation in (vi) and (vii) was carried out to investigate, to a certain extent, the chemistry of the compounds giving positive reactions with the stains employed.

Following fixation in each of the three fixatives detailed above, the tissues were dehydrated through ascending grades of alcohol, cleared through chloroform and benzene, and embedded in paraffin wax. Sections were cut at 5μ on a rotary microtome, and mounted on slides (without the use of albumen). To obtain a certain degree of quantitative comparison between tissues at differing stages of gestation, or alternatively between different placental types, the sections were bulk processed so that the same solutions in adequate quantity were used for all tissues at the same time. Following staining by the methods detailed in (1) to (3) above the sections were dehydrated through alcohol (or acetone where so stated in 3), cleared in xylol and mounted in D.P.X. No counterstaining was used at any time, to eliminate interference with the localization of stained tissue components, or with the detection of small quantities thereof. Quantitation was assessed visually and classified on an arbitrary basis from + to ++++.

(4) Frozen on "Drikold" (I.C.I.) at -73°C immediately following collection. Wherever possible this material was sectioned immediately, and certainly no specimen was stored for more than 7 days before sectioning. Storage, where necessary was carried out in sealed

containers in a deep-freeze cabinet at -30°C.

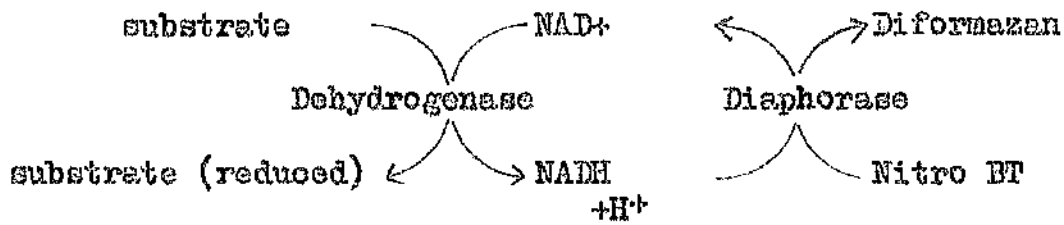
Frozen sections of these specimens were cut at 14μ in a cryostat maintained at -30°C, quickly air-dried on cover-slips, and incubated within 24 hours, for a variety of enzyme activities listed in Table 2. Control sections were incubated for each method in the medium lacking substrate. Lipid was also stained using Sudan black B in propylene glycol as recommended by Chiffelle and Putt (1951).

Following staining by these methods the sections were washed and mounted in either Hydramount (Gurr) or Farrants medium (B.D.H.). Enzyme activity (or lipid concentration) was again assessed visually and arbitrarily classified + to ++++.

For semi-quantitative assessment of enzyme activity as observed at different stages of gestation, or in different types of placenta, sections were incubated, en masse, in one lot of incubation medium. In addition, to eliminate the possibility of staining in non-active sites due to enzyme diffusion from other sections, sections of each specimen were incubated separately in separate containers. Enzyme diffusion was further minimised by the addition to the media of polyvinyl-pyrrolidone, 7.5%. Thus the medium used for the dehydrogenases (carbohydrate) and described in Table 2 as "Modified from Pearse (1960)" consisted of substrate, phosphate buffer pH 7.4,

NAD (or NADP for isocitrate, glucose-6-phosphate and 6-phospho-gluconate dehydrogenases), Nitro BT, sodium cyanide to inhibit the cytochrome system, and polyvinyl-pyrrolidone. For succinate dehydrogenase only substrate, buffer, Nitro BT and polyvinyl pyrrolidone were used, this enzyme being FAD dependent.

For the detection of any dehydrogenase linked to NAD or NADP as hydrogen acceptor, the presence in the tissues of the appropriate diaphorase is necessary, the mechanism of the reaction being shown below:-



Thus the localization of diaphorases acting on the cofactors (i.e. NAD and NADP) was also investigated in all tissues examined, to eliminate the possibility that the absence of a particular enzyme activity as demonstrated histochemically was due to a concomitant absence of the appropriate diaphorase.

TABLE 2

Histochemical methods used with frozen sections

Enzyme	Reaction catalyzed	Substrate	Buffer	Author ref. for method
Hydrolytic enzymes				
(1) acid phosphatase	$R\text{-}O\text{-}PO_3H_2 + H_2O \rightleftharpoons R\text{-}OH + H_3PO_4$	Sodium α-naphthyl phosphate	Veronal-acetate pH 5	Pearse (1960)
(2) non-specific esterase	$R\text{-}COOR^1 + H_2O \rightleftharpoons R\text{-}COOH + R^1OH$	Naphthol-AS-acetate	Phosphate pH 7.4	Gomori (1952)
(A,B,C-Pearse (1960)		α-naphthyl acetate	Phosphate pH 7.4	Pearse (1960)
		5-bromo-indoxyl acetate	Tris-HCl pH 6.0	Holt (1958)
For inhibition of B esterase, pre-incubation in E 600, 10^{-5}M, for 1 hour at 37°C was used. For inhibition of A esterase para-chloromercuribenzoate, 10^{-4}M was used as a pre- incubation, and in the incubating medium. Esterase activity after inhibition was detected with Naphthol-AS-acetate, or 5-bromo-indoxyl acetate as substrates.				
(3) β-glucuronidase	R-glucuronide + $H_2O \rightleftharpoons$ R-OH + glucuronic acid	Naphthol-AS Bl-β-D-glucuronide	Acetate pH 5.2	Hayashi, Nakajima and Fishman (1964)

TABLE 2 (continued)

Enzyme	Reaction catalyzed	Substrate	Buffer	Author ref. for method
Alkaline phosphatases				
(4) non-specific alkaline phosphatase	$R\text{-}O\text{-}PO_3H_2 + H_2O \rightleftharpoons R\text{-}OH + H_3PO_4$	Sodium α-naphthyl phosphate	Tris pH 9	Pearse (1960)
(5) adenosine monophosphatase	Adenosine monophosphate $+ H_2O \rightleftharpoons$ adenosine $+ H_3PO_4$	Adenosine monophosphate	Tris-maleate pH 7.2	Novikoff and Goldfischer (1961)
(6) adenosine triphosphatase	Adenosine triphosphate + $H_2O \rightleftharpoons$ adenosine diphosphate + H_3PO_4	Adenosine triphosphate	Tris-maleate pH 7.2	"
(7) inosine triphosphatase	inosine triphosphate + $H_2O \rightleftharpoons$ inosine diphosphate $+ H_3PO_4$	Inosine triphosphate	Tris-maleate pH 7.2	"
(8) thiamine pyrophosphatase	thiamine pyrophosphate + $H_2O \rightleftharpoons$ thiamine + pyrophosphate	Thiamine pyrophosphate	Tris-maleate pH 7.2	"

TABLE 2 (continued)

Enzyme	Reaction catalyzed	Substrate	Buffer	Author ref. for method
(9) Uridine diphosphatase	Uridine diphosphate $+H_2O \rightleftharpoons$ uridine monophosphate $+H_3PO_4$	Uridine diphosphate	Tris-maleate pH 7.2	Novikoff and Goldfischer (1961)
(10) Fructose-6-phosphatase	Fructose-6-phosphate $+H_2O \rightleftharpoons$ fructose $+ H_3PO_4$	Fructose-6-phosphate	Tris-maleate pH 7.2	"
(11) Fructose-1:6-diphosphatase	Fructose-1:6-diphosphate $+H_2O \rightleftharpoons$ fructose-6-phosphate $+H_3PO_4$	Fructose-1:6-diphosphate	Tris-maleate pH 7.2	"
(12) β-glycerophosphatase (control for 5 to 11 above)	$R-O-PO_3H_2+H_2O \rightleftharpoons$ $R-OH + H_3PO_4$	Sodium β-glycerophosphate	Tris-maleate pH 7.2	"
(13) Glucose-6-phosphatase	Glucose-6-phosphate $+ H_2O \rightleftharpoons$ Glucose$+H_3PO_4$	Glucose-6-phosphate	Tris-maleate pH 6.7	Wachstein and Meisel (1957)
(14) β-glycerophosphatase (control for 13)	$R-O-PO_3H_2+H_2O \rightleftharpoons$ $ROH+H_3PO_4$	Sodium β-glycerophosphate	Tris-maleate pH 6.7	"

TABLE 2 (continued)

Enzyme	Reaction catalyzed	Substrate	Buffer	Author ref. for method
Carbohydrate dehydrogenases				
(15) α-glycerophosphate dehydrogenase(αGP)	α-glycerophosphate ⇌ dehydroxyacetone phosphate	α-glycerophosphate	Phosphate pH 7.4	Modified from Pearse (1960)
(16) β-hydroxy-butyrate dehydrogenase(βOH)	β-hydroxy-butyrate ⇌ aceto-acetate	β-hydroxy-butyrate	"	"
(17) glucose-6-phosphate dehydrogenase(G-6-P)	glucose-6-phosphate → 6-phospho-gluconate	Glucose-6-phosphate	"	"
(18) 6-phospho-gluconate dehydrogenase (6-PG)	6-phospho-gluconate → D-ribulose-5-phosphate	6-phospho-gluconate	"	"
(19) lactic dehydrogenase (LDH)	lactate ⇌ pyruvate	lactate	"	"
(20) isocitric dehydrogenase (IDH)	isocitrate ⇌ cis-aconitate	Isocitrate	"	"
(21) succinic dehydrogenase (SDH)	succinate ⇌ fumarate	Succinate	"	Pearse (1960)
(22) malic dehydrogenase (MDH)	malate ⇌ oxaloacetate	Malate	"	Modified from Pearse (1960)

TABLE 2 (continued)

Enzyme	Reaction catalyzed	Substrate	Buffer	Author ref. for method
(23) glutamate dehydrogenase (GDH)	glutamate $\rightleftharpoons$ α-keto-glutamate	Glutamate	Phosphate pH 7.4	Modified from Pearse (1960)
(24) alcohol dehydrogenase (ADH)	alcohol $\rightleftharpoons$ acetaldehyde	Ethanol	"	"
(25) furfuryl alcohol dehydrogenase (FDH)	furfuryl alcohol $\rightleftharpoons$ furfuraldehyde	Furfuryl alcohol	"	"
(26) sorbitol dehydrogenase (Sorb DH)	sorbitol $\rightleftharpoons$ fructose	Sorbitol	"	"
Diaphorases				
(27) NAD diaphorase	NADH + Nitro BT $\rightarrow$ NAD+ + Diformazan	NADH (reduced nicotinamide adenine dinucleotide)	"	"
(28) NADP diaphorase	NADPH + Nitro BT $\rightarrow$ NADP + Diformazan	NADPH (reduced nicotinamide adenine dinucleotide phosphate)	"	"

TABLE 2 (continued)

Enzyme	Reaction catalyzed	Substrate	Buffer	Author ref. for method
Steroid dehydrogenases	Reaction tables and formulae for these steroids are given in the discussion. (Text figs. 9 - 15).			
(29) α3αhydroxy-steroid dehydrogenase	Dehydrogenation	3α-hydroxy-5α-androstan-17-one	Phosphate pH 7.4	Modified from Pearse (1960)
(30) β 3α-hydroxy-steroid dehydrogenase	"	aetio-cholanolone	"	"
(31) Δ^5-3β-hydroxy-steroid dehydrogenase	"	(1) dehydro epiandrosterone (DHA)	"	"
		(2) pregnaneolone	"	"
		(3) 17α-hydroxy-pregneolone	"	"
(32) α-3β-hydroxy-steroid dehydrogenase	"	3 β-hydroxy-5α-androstan-17-one	"	"
(33) β-3β-hydroxy-steroid dehydrogenase	"	3 β-hydroxy-5β-androstan-17-one	"	"
(34) 6β-hydroxy-steroid dehydrogenase	"	6β-hydroxy-oestrone	"	"

TABLE 2 (continued)

Enzyme	Reaction catalyzed	Substrate	Buffer	Author ref. for method
(35) 11β-hydroxy-steroid dehydrogenase	Dehydrogenation	(1) cortisol	Phosphate pH 7.4	Modified from Pearse (1960)
		(2) 11β-hydroxy-androstenedione	"	"
(36) 16β-hydroxy-steroid dehydrogenase	"	(1) 16β-hydroxy-androst-4-ene-3-one	"	"
		(2) 16β-hydroxy-oestrone	"	"
(37) 17β-hydroxy-steroid dehydrogenase	"	(1) testosterone	"	"
		(2) oestradiol	"	"
(38) 20β-hydroxy-steroid dehydrogenase	"	20β-hydroxy-progesterone	"	"

RESULTS.

RESULTS

Implantation.

The morphology of implantation in the rat and rabbit has been described in detail by many authors, in the rat, Duval (1891), Krehbiel (1937), Bridgman (1948a,b), Amoroso (1952); in the rabbit, Duval (1891), Amoroso (1952), and Boving (1959). Therefore a brief summary only will be given here for the purposes of orientation, both spatial and temporal, with respect to the histochemical changes observed.

Rat

In the rat, the first change observed in the uterus, is the enlargement of the antimesometrial stromal cells (Fig. 1) which commences at 5 days (for details of timing see "Material and Methods"). These cells line up parallel to the uterine lumen, and accumulate basophilia. They form the primary decidua which enlarges and spreads laterally and mesometrially on either side of the uterine lumen over the period from 5 to 6 days.

This period also marks the first attachment of the embryo, which consists of an outer shell of trophoblast and a few cells forming an inner cell mass (Fig. 2) to the uterus, which occurs at $5\frac{1}{2}$ days (Fig. 3) in the region of the antimesometrial shelf. This attachment is effected by the abembryonic trophoblast which enlarges to form the abembryonic giant cells. That the attachment is not as tenuous as was previously believed is seen in Fig. 3 where portions of a

trophoblastic cell have adhered to the maternal tissue, although the embryo has shrunk away from it during processing.

Processes of the giant cells extend antimesometrially, deep to the uterine epithelium (Fig. 4) which they remove from its basement membrane into the uterine lumen where it degenerates and is absorbed for embryonic nutrition. By some as yet unknown process the disappearance of the epithelium extends, in an orderly fashion, from antimesometrial to the embryo to mesometrial to it, and this disappearance is followed, 12 hours later, by a degeneration of the basement membrane which again commences antimesometrially and spreads towards the mesometrium (Fig. 5).

External to the epithelium, at this stage, the primary decidual cells, with the exception of a few immediately adjacent to the embryo—implantation zone, are enlarging further, and being converted into the typical, large uni-, or bi-nucleate cells of the secondary decidua. (Figs. 6, 7). External to the decidual region the uterine stroma shows a generalised increase in cellularity (Fig. 6). With further development the secondary decidua enlarges laterally, and, to a certain extent, mesometrially (Fig. 8), and compresses the stroma laterally into a narrow zone just internal to the muscle layer, which is known as the "fibrinoid capsule", and which lies immediately interior to the region through which splitting to re-form the uterine

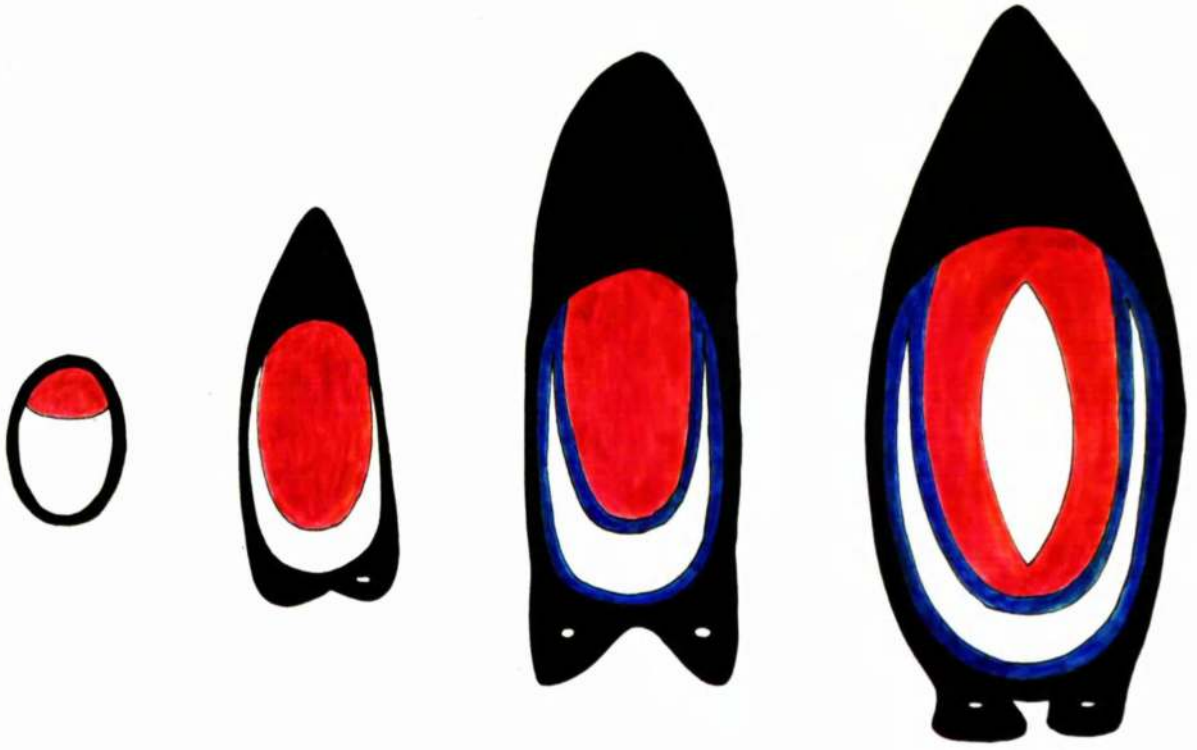
lumen will occur. At about 7 to 7½ days the cells immediately mesometrial and lateral to the embryo start to appear vacuolated (Fig. 9) in paraffin sections stained by routine histological methods. This vacuolation which is due to glycogen in the cells extends laterally and mesometrially on either side of the embryo as the "glycogen wings" (Fig. 10), which in later development will form the decidua basalis.

While these changes are taking place external to the embryo, and uterine lumen, marked alterations are occurring in the embryo itself, leading to the formation of the primary germ layers, and of the yolk-sac placenta, and commencing the development of the chorio-allantoic placenta.

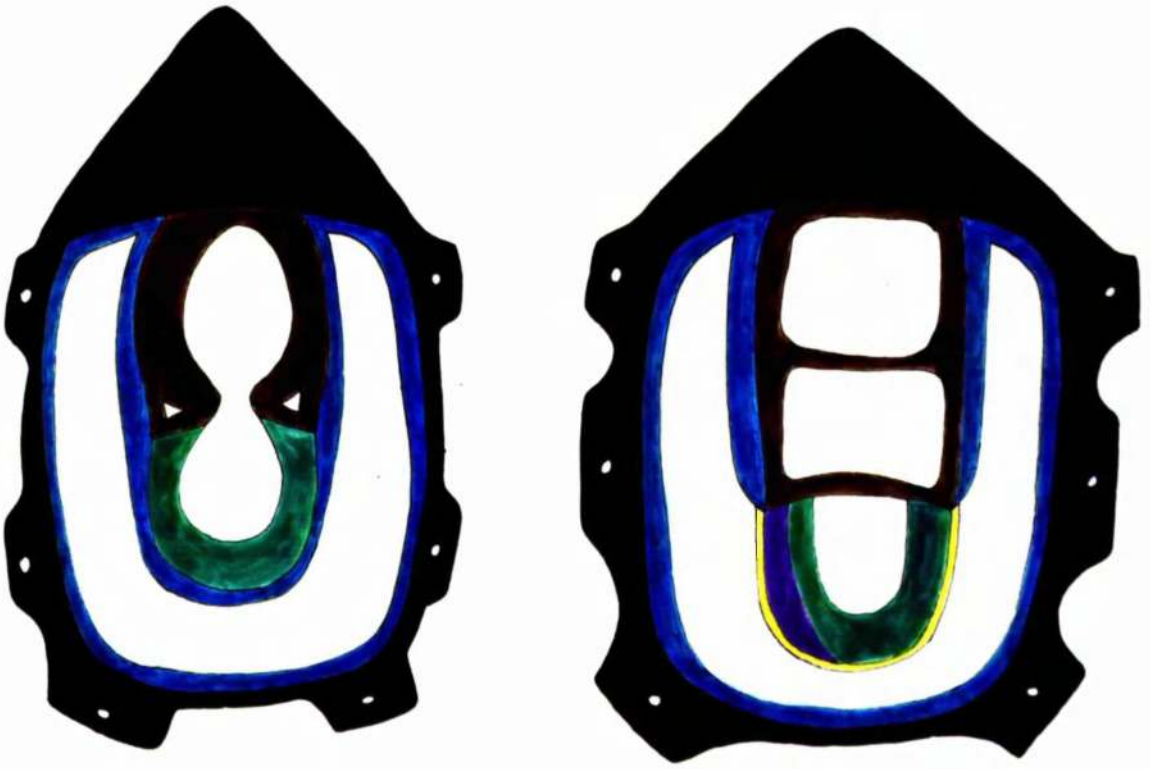
The changes which take place in the embryo are summarised in text - fig. 1. Initially the embryo has the form of an inner cell mass surrounded by an outer layer of trophoblast. The development of the antimesometrial abembryonic giant cells has been detailed previously. Mesometrially the trophoblast enlarges into the ecto-placental cone which projects into the uterine lumen mesometrial to the embryo, and to the sides of which the maternal tissues become closely applied, particularly after degeneration of the uterine epithelium (Fig. 9). With further development the ectoplacental cone enlarges and becomes broader-based, while the remainder of the luminal epithelium degenerates, and the lumen becomes obliterated. Thus,

Text-fig. 1. The development of the rat embryo from $5\frac{1}{2}$ to $9\frac{1}{2}$ days, showing the expansion of the trophoblast (black) and differentiation of the abembryonic and lateral giant cells, and of the ectoplacental cone (upper end of egg cylinder). The increase in size of the inner cell mass (red), its cavitation, and differentiation into ectoderm (green) and extra-embryonic mesoderm (brown) is seen, and the development of the extra-embryonic coelom (cavities in the folds at the junction of the brown and green areas at $8\frac{1}{2}$ days) by fusion of the folds, and subsequent coalescence of the cavities contained therein can be appreciated. The appearance of the visceral (round the inner cell mass) and parietal (lining the inner aspect of the trophoblast) areas of endoderm (blue) is shown, and the subsequent flattening of the visceral layer related to the embryo to form the embryonic endoderm (yellow). The appearance of the primitive streak (purple) in the posterior end of the embryo between $8\frac{1}{2}$ and $9\frac{1}{2}$ days is shown.

(The figures beneath the diagrams in this text-figure show the embryonic age in days).



5½ 6½ 7 7½



8½ 9½

at the end of the implantation stages examined, the ectoplacental cone has the form of a blunt spearhead pushing into the mesometrial maternal tissues (Fig. 8). By this stage giant cells have developed lateral to the embryo, where they represent the lateral trophoblast, and lateral to the ectoplacental cone (Fig. 11).

The inner cell mass enlarges, and on its surface the visceral endoderm (Fig. 9) differentiates. The endoderm quickly spreads round the inner aspect of the trophoblast to form the flattened parietal layer (Fig. 12) which is separated from the trophoblast, and later the trophoblastic giant cells by a basement membrane - Reichert's membrane (Fig. 13). Following this the inner cell mass cavitates, and the ectoderm differentiates in the antimesometrial half, internal to the visceral endoderm (Text fig. 1), the remainder of the inner cell mass forming the extra-embryonic mesoderm. In the junction between this and the ectoderm, at $8\frac{1}{2}$ days, there appears a cavity - the extra-embryonic coelom- and the mesodermal chorio-amniotic folds thus formed approach each other and fuse. The extra-embryonic coelom becomes continuous, by breakdown of the tissue separating its components, and the egg cylinder, as it is now termed, presents three cavities. The ectoplacental cavity lies beneath the ectoplacental cone, the extra-embryonic coelom antimesometrial to that separated from it by the "lamina" (Duval), and the amniotic cavity antimesometrial to the coelom,

from which it is separated by the amnion (Fig. 14). As will be observed, the relatively early inversion of the germ layers which takes place in this species leads to the formation of a yolk sac cavity almost enclosing the embryo.

The further development of the embryo considered in this section consists of the development of the allantois, as a mesodermal outgrowth from the posterior end of the embryo which invaginates the lamina and presses it against the base of the ectoplacental cone with which it fuses (Fig. 11), thus beginning the development of the chorio-allantoic placenta. Also at the posterior end of the embryo the primitive streak appears, and buds off mesoderm round the embryo on either side of the midline between the ectoderm and the endoderm. The latter differentiates, between $8\frac{1}{2}$ and $9\frac{1}{2}$ days into the columnar yolk-sac endoderm mesometrial to the embryo, and a flattened layer of endoderm related to the embryo itself.

Rabbit

In the strain of rabbits used in this investigation (New Zealand Albino) blastocysts can first be detected macroscopically, on inspection of the exterior of the uterus, between 5 and 6 days post coitum, as swellings of the antimesometrial side of the uterine horn. At this stage the blastocyst consists of a single outer layer of trophoblastic cells, and a few flattened endodermal cells interiorly at the embryonic pole, which usually lies at the mesometrial side of

the uterus. Around the blastocyst are found the inner mucolemma - homogenous and hyaline - and the outer gliolemma - which appears in this material to be an accumulation of uterine secretion, and is fibrillar following fixation (Fig. 15). The uterus shows some flattening of the antimesometrial mucosal folds, with hypertrophy of those at the mesometrial side destined to form the future placental, and paraplacental folds.

Between 5 and 7 days the mucolemma becomes considerably thinned as the blastocyst expands, and the gliolemma becomes less obvious (Fig. 16). In the abembryonic hemisphere of the blastocyst, enlargement of some areas of trophoblastic cells occur to give rise to the "trophoblastic knobs", which will later invade the maternal tissues. The endoderm spreads round the inner aspect of the trophoblast converting the uni-laminar omphalopleure into a bilaminar one. Mesometrially the primitive streak appears (Fig. 17) and begins to bud off mesoderm between the ectoderm and the embryonic endoderm. The antimesometrial mucosa of the uterus becomes flattened against the muscular wall, while further hypertrophy of the mesometrial mucosal folds occurs.

Implantation begins antimesometrially in the rabbit, at 7 days and 12 hours in this strain. It is characterised by disappearance of the lemmas, and invasion of the uterine epithelium by trophoblastic processes which arise from the trophoblastic "knobs", and insinuate

themselves between the epithelial cells (Fig. 18) usually directly over a sub-epithelial capillary. Initially very narrow, these processes rapidly spread out laterally, both in the plane of the epithelium, and beneath it. Thus a primitive, non-vascular yolk-sac placenta is formed (Fig. 19). During this period conversion of the antimesometrial epithelium into a symplasma (Fig. 19) occurs, and this is followed by a generalised degeneration of all of the antimesometrial epithelium except that lining the deepest parts of the uterine glands. The invading trophoblast comes into contact with maternal blood and differentiates giant cells which appear in the submucous connective tissue at about 9 days post coitum (Fig. 20). The further changes which take place in this region later in pregnancy leading to the formation of the definitive yolk-sac placenta are dealt with in the section on "Comparative Placentation".

Mesometrially, at 7 days and 12 hours, the lemmas are still present, and separate the embryonic structures, which have not progressed far beyond the appearance at 7 days, from the uterine mucosa of the hypertrophied placental folds. In these the endothelium surrounding the maternal blood vessels is becoming thickened and hypertrophied - the commencement of decidua formation (Fig. 21). The lemmas disappear, in relation to the mesometrial side of the blastocyst, between 7 days, 12 hours and 7 days, 22 hours at which time the ectoderm comes into contact with the uterine epithelium of

the placental folds (Fig. 22). It is of interest to note that that part of the ectoderm in contact with these folds, on either side of the midline, becomes thickened, while, at this stage at least, the part destined to form the ectodermal embryonic structures, which lies in the midline, is not thickened except posteriorly in the region of the primitive streak. Thus the ectoderm on either side of the midline may now be referred to as trophectoderm. On its inner surface lies mesoderm derived from the primitive streak, and this mesoderm extends laterally, between the trophectoderm and the (mesometrially thickened) endoderm, whose cells contain a few acidophil inclusions, as far as the edge of the placental folds. Blood vessels are not yet seen. The epithelium with which the trophectodemm is in contact still appears cellular in character.

During the remainder of gestation the embryo - whose development will not be studied further in this thesis - develops in the midline between the placental folds, while the chorio-allantoic placenta develops from the contact between them and the trophectoderm. On the surface of the trophectoderm syncytio-trophoblast appears (Fig. 23) and this fuses with the maternal epithelium, which has become converted into a symplasma, the boundaries between the two disappearing. This is later followed by removal, by some unknown mechanism, of the maternal epithelium, so that the syncytio-trophoblast comes into contact initially with the maternal connective tissue (Fig. 25) then

with the endothelium, and finally with the blood stream itself. While the syncytio-trophoblast has been invading the symplasma of the apices of the uterine glands of the placental folds the epithelium in the deeper parts of the glands has been undergoing a similar symplasmic change (Fig. 24). With further development this symplasma degenerates, the products probably being absorbed by the syncytio-trophoblast for embryonic nutrition. Changes have also been taking place in the developing decidua around the maternal blood vessels (Fig. 24), characterised by multiplication and enlargement of the cells, and vacuolation which is due to the accumulation of glycogen (see next section).

The other feature of histological note over this period of development of the rabbit is the accumulation of a fibrillar, acidophilic material (? yolk) in the cavity of the blastocyst, which commences at 7 days and 22 hours, reaches a maximum at 8 days and 16 hours, and then slowly decreases. To it, from about 8 days and 16 hours on is added a material whose staining reactions are similar to those of the maternal red blood cells, although I have never observed actual red cells in the yolk. In the endoderm lining the inner surface of the placental region, too, similarly stained inclusions (Fig. 26) are added to the acidophil material previously described. Vascularisation of the endoderm has also taken place, commencing at about 8 days post coitum.

RAT

Glycogen.

Embryo

At 5 days, prior to implantation occasional granules of glycogen are found confined to the trophoblast (Fig. 27).

As implantation takes place, however, the quantity of glycogen increases, particularly in the abembryonic giant cells (Fig. 28), although the increase soon affects the inner cell mass also.

By 6 days (Fig. 29) the ectoplacental cone has appeared, and, like the trophoblast from which it is derived, contains some glycogen. Some granules are also seen in the visceral endoderm, which is in the process of differentiation, although the inner cell mass has now become negative. Later in the 6th day the parietal endoderm begins to differentiate, and exhibits a similar staining reaction to the visceral layer.

The expansion of the ectoplacental cone throughout the remainder of the implantation period is accompanied by a gradual accumulation of glycogen, initially distributed throughout the substance of the cone (Fig. 29) but later particularly in an area confined to the central part, (Fig. 30) and separated at $9\frac{1}{2}$ days from the extra-embryonic mesoderm by a glycogen negative area.

At about 7½ to 8 days the lateral trophoblast becomes less obvious, and lateral giant cells appear (see Text fig. 1) at about 8½ days, containing some glycogen, like the abembryonic group. A third group of giant cells, which will form the definitive giant cells of the rat placenta appears round the ecto-placental cone particularly at its base towards 8½ days of gestation. These also contain some glycogen, which, like the lateral and abembryonic giant cells, they lose by 9½ days.

As the inner cell mass differentiates into the ectoderm and extra-embryonic mesoderm glycogen begins to re-accumulate, particularly in the mesodermal part, where it reaches a maximum at 8½ days (Fig. 30), when quite intense staining is observed in the ectoderm also. Also present, in the cavity of the embryo is a "secretion", containing some glycogen whose other histochemical characteristics will be described later. Although the embryonic and parietal endoderm at this stage still contains some glycogen the yolk-sac endoderm has lost its content. Between 8½ and 9 days the glycogen content of all the tissues derived from the inner cell mass disappears, with the exception of the parietal endoderm where some staining persists. Some glycogen appears in the yolk-sac cavity contents at 9½ days (Fig. 33).

Epithelium

Throughout the period of pregnancy examined the alterations

in glycogen content were quite marked, and mirrored those of the underlying decidua or stroma. While this would suggest that diffusion during the dimedone procedure had occurred, staining of sections by the PAS method without prior incubation showed an identical distribution of glycogen, suggesting that the picture observed was a true one.

At 5 days, prior to implantation, (Fig. 27) no glycogen is found in the epithelium, nor does any appear until 6 days, by which time decidual development is advancing. A granular PAS-positive diastase-labile reaction is observed at 5½ days in the epithelial basement membrane, however, particularly mesometrially, and is presumably due to glycogen. At 6 days the reaction in the epithelium is moderately intense mesometrial and lateral to the embryo and decreases as it is traced antimesometrially (Fig. 29). Some PAS-positive secretion, mainly glycogen, is present in the lumen mesometrial to the embryo.

Increase in glycogen content in the epithelium occurs over 6½ and 7 days, during which time the epithelium in immediate relation to the embryo degenerates. Secretion is still present in the uterine lumen but its content of glycogen seems to decrease somewhat. Degeneration of the epithelial basement membrane also occurs.

This degeneration extends at 7½ days to affect most of the mesometrial epithelium, and as a result the lumen becomes filled

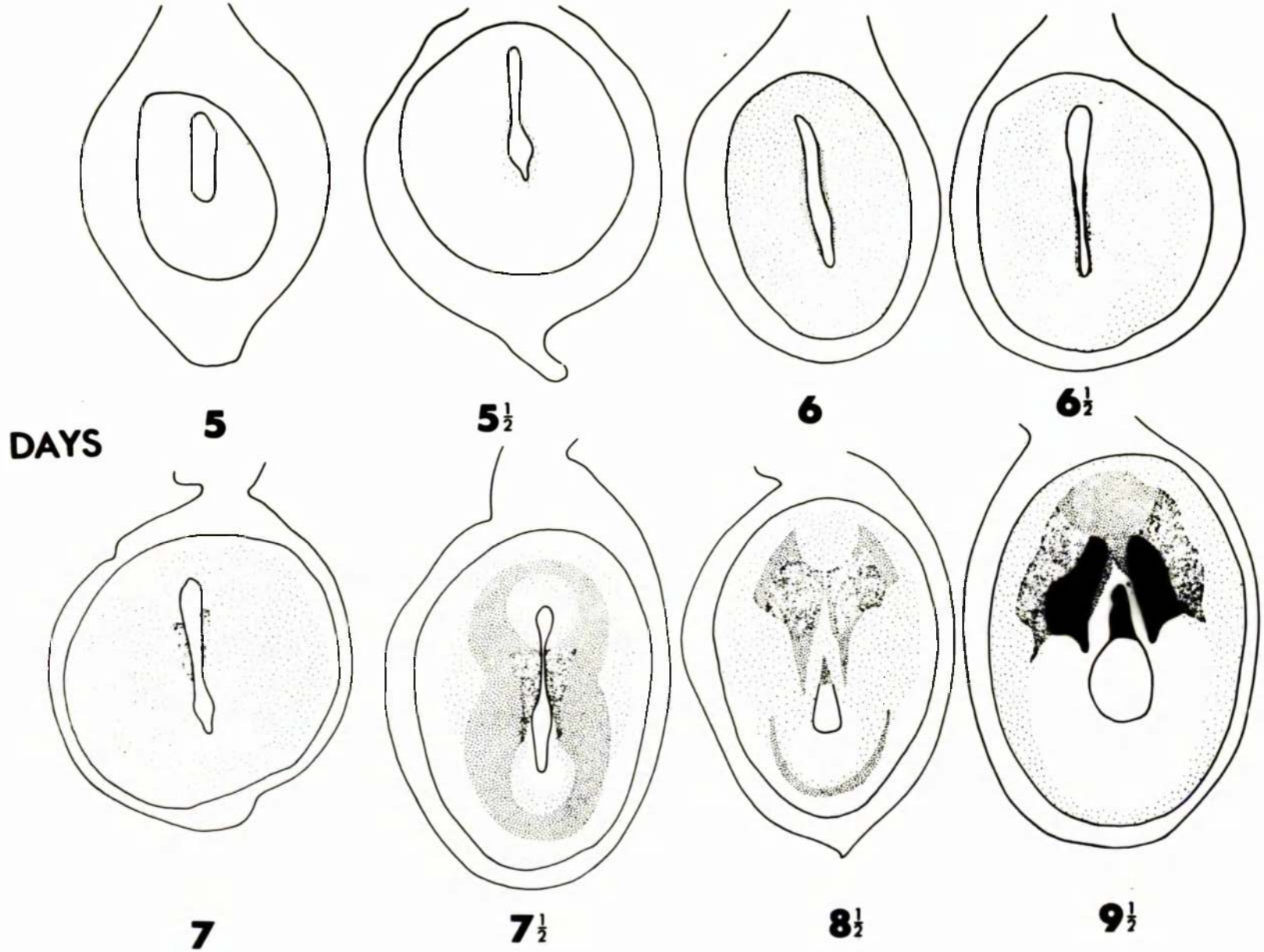
with PAS-positive material containing some glycogen. Where the embryo has implanted fairly far mesometrially, the antimesometrial epithelium may still be intact, and contains a variable quantity of glycogen. Secretion is still seen in the lumen - PAS-positive, diastase-fast, and containing some glycogen. At this stage the residual uterine luminal epithelium is intact and exhibits a similar appearance, but degeneration soon affects it also, and by 9 days its basement membrane is no longer continuous, and the lumen is filled with debris, which shows a similar staining reaction to the secretion described above. At 9½ days little trace remains.

Decidua and stroma

The changes in glycogen concentration which occur over the period examined are detailed below, and summarised in Text - fig. 2.

At 5 days, no glycogen is found in decidua or stroma, (Fig. 27) but immediately prior to implantation a few granules become detectable in a few cells beneath the antimesometrial epithelial basement membrane, i.e. in the primary decidua. Glycogen accumulates in this site as implantation occurs (Fig. 28), and as the transformation from primary to secondary decidua occurs, but by 6 days an even greater quantity is found in the decidua mesometrial and lateral to the embryo, the amount 'tailing off' as the decidua is traced anti-mesometrially. A few granules appear in the remainder of the stroma at this stage.

Text-fig. 2. The distribution of glycogen in the decidua and stroma at various stages during the implantation period in the rat. The intensity of shading is proportional to the glycogen concentration.


DAYS
5
5½
6
6½
7
7½
8½
9½

The most obvious change which occurs over the next 24 hours is a considerable accumulation of glycogen in the stroma cells lateral and antimesometrial to the area destined to form the glycogen 'wings' (Fig. 31). To my knowledge this appearance, which will persist although in altering distribution until the end of the stages examined, has not been described previously, possibly due to lack of sensitivity of the methods used. Initially the concentration is slightly more marked antimesometrially, and increases as the stroma is traced peripherally from immediately adjacent to the decidua. Early in the period the decidua shows the same appearance as at 6 days with quite marked quantities mesometrial and lateral to the embryo, but the 'tailing off' is more obvious, and while the mesometrial part with further development accumulates even more glycogen, and the first traces of the glycogen wings appear, the antimesometrial decidua loses it altogether by late 7 days. A decrease in quantity, but not to such a degree, also occurs around the mesometrial part of the lumen.

Thus over 7⅓ and 8⅓ days, with further lateral extension of the decidua, which shows only an occasional cell containing glycogen, the area of positive staining in the stroma becomes condensed laterally, and antimesometrially. Further accumulation occurs in the glycogen wings, and mesometrial to the embryo, but lateral to the embryo the staining decreases and is lost all together. The laterally

condensed capsule of positively staining stroma is separated from the glycogen 'wings' by areas of slightly less intense reaction radiating out from its ends, and from the decidua by an area of low intensity. Concurrent with the accumulation of glycogen in the 'wings' more becomes obvious around the residual uterine lumen mesometrially, a process which will continue until the end of the stages examined.

Over the rest of this period extension of the negative decidua laterally leads to further compression of the stroma into the area of the so-called 'fibrinoid' capsule, which reacts intensely at 9 days, but has become completely negative by 9½ days. Accumulation of glycogen also occurs in the glycogen wings until by 9½ days they form, in sections, an almost solid block of bright red (Fig. 32). Apart from the glycogen around the residual uterine lumen noted above, the remainder of the decidua and stroma is negative by 9½ days.

Muscle layers. Little change occurs in the glycogen content of the muscle layers of the uterus over the period of pregnancy examined, which remains moderately positive throughout.

Diastase-resistant PAS-positive substances

Embryo

At 5 days the inner cell mass, and trophoblast cells exhibit a moderate staining reaction, with occasional granules in the latter.

As the trophoblast differentiates into the abembryonic giant cells, inclusions appear in them also, and in the ectoplacental cone when it appears, and in both sites the number of these increases as the epithelia with which they are in contact degenerate (Fig. 34). Later in development similar inclusions appear in the lateral giant cells, and those round the base of the ectoplacental cone (Figs. 35, 36). Similar inclusions are seen in the cells of the lateral part of the ectoplacental cone at 9 to 9½ days, but not in the central part.

Throughout the period of development examined the inner cell mass and its derivatives, except the visceral endoderm, show a moderate cytoplasmic reaction. The visceral endoderm, however, develops a more intensely stained cytoplasm, and a strongly positive brush border and basement membrane (Fig. 34), although the brush border staining is lost at 9½ days where the embryonic endoderm differentiates. From 7½ to 9½ days the parietal and visceral (but not embryonic) endoderms show abundant cytoplasmic inclusions (Fig. 37) and, in the later part of the period similarly staining material is observed in the cavity of the yolk sac also.

The differentiation of the ectoderm, while not accompanied by any change in cytoplasmic staining by this method, shows a short-lived increased staining on the inner edge of the cells at 7 days

(Fig. 34) which has disappeared by 7½ days.

Immediately following the differentiation of the parietal endoderm, the lateral trophoblast becomes thinned out prior to appearance of the lateral giant cells. It is at this time that the epithelial basement membrane, which is gradually disappearing, becomes replaced, between the trophoblast and the parietal endoderm, by a thickening PAS-positive homogenous membrane - Reichert's membrane.

Epithelium

Throughout the period of pregnancy examined the diastase-fast PAS-positive reaction of the epithelium did not alter, until degeneration occurred, and was characterised by a moderate positivity, more intense towards the free edge of the cell. Degeneration of the epithelium is accompanied by the accumulation, in the lumen, of numerous PAS-positive droplets and granules, some of which seem to be absorbed by the cells of the ectoplacental cone.

Decidua and stroma

Little change occurs in the diastase-fast PAS-positive reaction of the decidua or stroma cells throughout the period examined and they remain moderately positive, with a more intense reaction in the connective tissue between the cells.

Muscle layers

These exhibit an unchanging moderate reaction with this staining method, more intense in the connective tissue, throughout the period examined.

Metrial gland cells

These were occasionally observed, at 9½ days in the stroma of the future decidua basalis, just mesometrial to the apex of the ectoplacental cone, and exhibited the typical binucleate appearance, with diastase-resistant granules in the cytoplasm.

Acid mucopolysaccharides

Embryo

Acid mucopolysaccharides were detected in the embryo in two sites only, firstly in the cavity of the yolk sac (Fig. 38) where they appeared at 6 days, when increased growth of the inner cell mass, whose cells they bathed, was occurring. Staining, in the form of dark blue granules, was observed in this site until the end of the period examined. The second location in which they were detected was in the cavity formed in the inner cell mass, where they appeared as soon as cavitation commenced (Fig. 38), became more apparent when the chorio-amniotic folds occurred (Fig. 39) and thereafter decreased in concentration, although remaining detectable in the ectoplacental, extra-embryonic, and amniotic cavities.

Epithelium and stroma

Throughout the period examined, no obvious acid mucopolysaccharide material was detected in the epithelium of the uterine glands, or cavity. Some was present, however, in the uterine secretion.

In the stroma, a moderately intense reaction throughout was observed initially (Fig. 40). As the decidua, and later the glycogen wings, developed and compressed the stroma laterally and mesometrially, and into the "fibrinoid" capsule antimesometrially, the staining for acid mucopolysaccharides became concentrated in the mesometrial part of the stroma, but not in the "capsule" (Fig. 41).

Decidua

The primary decidua showed loss of acid mucopolysaccharide staining compared to the stroma (Fig. 40). When the secondary decidua formed, and expanded laterally, a similar lack of staining was observed (Fig. 41).

RAT

RNA

Embryo

RNA is present in the trophoblast from the beginning of the implantation period, and reaches a maximum at 6½ to 7 days (Fig. 42). Thereafter its concentration decreases, except in the abembryonic giant cells, where the level of staining is maintained. A similar concentration is seen in the lateral giant cells when they differentiate. Initially the ectoplacental cone shows more staining than the trophoblast, but this quickly reverts to the level seen in the giant cells and remains there thereafter.

A similar higher level is seen in the inner cell mass (Fig. 42) and this concentration is maintained until it differentiates into the various areas of endoderm, ectoderm, and mesoderm. When this occurs the ectodermal concentration remains high, less staining is seen in the mesoderm, and less still in the parietal endoderm (Fig. 43). Initially the visceral endoderm shows fairly intense staining, but when the brush border differentiates at 8 days, the cytoplasmic staining decreases considerably, although to a lesser degree in the actual border itself. Further differentiation of the endoderm into the yolk-sac and the embryonic endoderm is not accompanied by any alteration in RNA concentration (Fig. 44).

Extra-embryonic tissues

The appearance of the primary decidua in the process of

implantation of the rat embryo is accompanied by the accumulation of RNA in the stroma cells from which it is derived (Fig. 45). By 6½ days, the conversion of primary to secondary decidua occurs, and the latter also contains RNA (Fig. 43) although in lesser amounts, whose concentration remains constant until the end of the implantation period, when it falls off to a certain extent.

Lateral to the secondary decidua, and antimesometrially, the stroma cells also contain RNA, higher in concentration than in the decidua. Antimesometrially, as the stroma becomes compressed laterally, the concentration begins to fall off, particularly from 8 days onwards; mesometrially, however, the concentration increases initially in both the region destined to form the decidua basalis and the glycogen wings, but later falls off in intensity in the glycogen wing region from 8 days onwards.

The uterine epithelium shows a faint staining for RNA until it degenerates. Antimesometrially the degeneration is accompanied by the appearance in the abembryonic giant cells of some ribonuclease resistant, chrome alum-gallocyanine positive inclusions (Fig. 43).

Lipid

Embryo

Initially the implanting rat embryo contains some lipid in the trophoblast (Fig. 46) and, when it becomes obvious, the inner cell mass. Later lipid is found in their derivatives - the ectoplacental

cone (Fig. 47) in which it reaches a maximum at 8 to 8½ days when the latter is exposed to the uterine lumen, and then falls off in concentration; the giant cells (Fig. 47) which contain some lipid at all times; the visceral endoderm, in which it reaches a ++ concentration by 9½ days in the yolk sac endoderm although the embryonic and parietal endoderm remain negative; and, in trace amounts, in the ectoderm and mesoderm.

Extra embryonic tissues

Lipid is found mainly in the epithelium lining the uterine cavity, where its concentration increases antimesometrial, and mesometrial to the embryo, prior to epithelial degeneration, which occurs at 7 days antimesometrial to the embryo, 8 days mesometrial to the embryo, and 8½ days mesometrial to that.

Outside the epithelium, in the implantation zone (Fig. 48), quite marked quantities of lipid occur, from 6½ days onwards, and reaching a maximum at 7½ days, falling off thereafter.

Small quantities of lipid are also found in the primary and secondary deciduas (Fig. 48) where its changes parallel that in the implantation zone, although the concentration is considerably less at all times.

Hydrolytic enzymes

Acid Phosphatase

Embryo

Acid phosphatase activity is observed initially in the trophe-

blast at 5 days (Fig. 49) where its activity increases up to 6½ days and then decreases, a similar lessening of activity being observed in the ectoplacental cone, from ++ at 6½ days to + at 9½ days. No decrease in activity is observed in the abembryonic, or lateral, giant cells, whose activity remains moderately high throughout (Fig. 50).

At 6 days (Fig. 51) some acid phosphatase activity appears in the inner cell mass, and this persists later in certain of its derivatives, particularly the visceral endoderm. When this differentiates into yolk-sac and embryonic endoderms, however, the latter loses its activity (Fig. 52). The parietal endoderm shows moderate activity throughout, and some staining is seen in the developing ectoderm (Fig. 52) and mesoderm.

Extra-embryonic tissues

Acid phosphatase is seen particularly in the uterine epithelium (Fig. 49) where its activity shows a decreasing gradient from mesometrial to antimesometrial. The degeneration of the epithelium later in the implantation period is preceded by an increase in enzyme activity. (Compare the epithelium antimesometrial to the embryo in Figs. 49 and 51).

Decidua formation is associated with increasing levels of acid phosphatase activity. Initially the enzyme is found in moderate quantity in the primary decidua (Fig. 49) but, as the conversion to

secondary decidua occurs, and the latter spreads mesometrially (Fig. 53), the enzyme activity increases, particularly mesometrial to and around the embryo, less activity being visible in the anti-mesometrial region. With further decidual development, further increase in enzyme activity around the mesometrial end of the embryo occurs, and this is associated with spreading of the staining laterally and mesometrially into the region of the developing glycogen wings (Fig. 54). Antimesometrially the enzyme activity gradually increases towards the end of the implantation period (Fig. 54).

Throughout the stage of gestation examined, some acid phosphatase activity is observed in the stroma, where it decreases in quantity as the stroma is traced laterally.

Trace activity is found throughout in the muscle layers.

Non-specific esterase

Embryo

The distribution of staining for this enzyme in embryonic tissues was substantially the same as for acid phosphatase, but the enzyme concentration was considerably less (Fig. 55). Both A and B type esterase activity (predominantly A) was detected, but no C esterase in any site.

Extra-embryonic tissues

Here again the distribution of staining was the same, but less in activity (Fig. 56) with the one exception that no build up of enzyme activity in the glycogen wings was observed (Fig. 57).

Alkaline phosphatases

Non-specific alkaline phosphatase (Fig. 58 A-D)

The distribution of this enzyme was examined at pH 9, and at pH 7.2, the latter acting as a control for the specific phosphatases. No difference in distribution was observed, and the following description applies to the results at both levels of pH.

Enzyme activity is observed over $3\frac{1}{2}$ - $4\frac{1}{2}$ days in the epithelium lining the uterine lumen and glands, traces in the adjacent stroma and quite a marked positive reaction in endothelium. More intense staining is seen in the primary decidua at 5 days, which increases with increase in size of the decidua at $5\frac{1}{2}$ days. However, at 6 days almost the whole of the stroma including the decidua becomes quite markedly positive, particularly antimesometrially. Between 6 and $7\frac{1}{2}$ days the staining in decidua and stroma increases to its maximum and spreads to the region round the residual uterine lumen, but the implantation zone immediately outside the embryo becomes less positive. The staining in the decidua falls off as it is traced laterally to its junction with the stroma. Thereafter the reaction continues to fall off laterally to negative just internal to the muscle layers, which remain almost negative throughout the period of pregnancy examined, as do the glycogen wings. The endothelium seems to lose its staining reaction (except peripherally) at about $6\frac{1}{2}$ days.

From 7½ - 9½ days, a gradual fall off in the intensity of staining, particularly antimesometrially, occurs, beginning in the centre of the decidua and spreading laterally. The mesometrial stroma and region of the "fibrinoid capsule" are less affected. Some positive staining appears in the centre of the ectoplacental cone, but the rest of the embryo remains negative.

Adenosine-5'-monophosphatase (Fig. 59 A-D)

The distribution of this enzyme shows marked differences from that of non-specific alkaline phosphatase.

At 3½ days the epithelium of glands and uterine lumen show some activity supra-nuclearly, and traces are present in the adjacent stroma. The muscle layers exhibit a moderate degree of activity which they will maintain throughout. The endothelium lining the blood vessels never shows a positive reaction with this substrate, in this species.

The changes in distribution of the staining in the antimesometrial decidua (primary and secondary) parallel these observed with β-glycero-phosphate, but the reaction is much more intense in a larger area of the decidua, and does not spread to the region round the residual uterine lumen. The sudden increase in stromal staining between 5½ and 6 days is again present, and involves the glycogen wing region. Into this, at 7½ days the very intense decidual staining spreads, and further increase is seen in this region between 7½ and 9½ days, while a diminution in intensity of staining, as was

observed with non-specific alkaline phosphatase, occurs anti-mesometrially, commencing at 8½ days. Some activity is again seen in the centre of the ecto-placental cone at 9½ days. The reaction of the implantation zone is less than that of the surrounding tissues throughout the period of pregnancy examined.

Adenosine-5'-triphosphatase (Fig. 60 A-D)

This enzyme alone shows definite changes between 3½ and 4½ days. At 3½ days it is present in the apices of the epithelial cells of the uterine lumen and glands, with traces in the adjacent stroma, strongly positive blood vessel endothelium, and moderately positive muscle layers and vascular smooth muscle. By 4½ days, however, although the remainder of the tissue shows the same reaction, the stroma in the antimesometrial region becomes more positive within a triangle whose boundaries extend antimesometrially and laterally from either side of the mid point of the uterine lumen. With further development the stroma becomes gradually more positive, without the abrupt transition seen between 5½ and 6 days with AMPase and βG-Pase. Accumulation of enzyme occurs in primary and secondary decidua where the intensity of reaction again falls off as the decidua is traced laterally. Staining occurs in the region round the residual uterine lumen, and the reaction is intermediate in intensity between that seen with AMP and βG-P as substrates. The reaction of the glycogen wings is intermediate

between that of the decidua, from which they are separated by a less positive area, and the mesometrial stroma. The endothelium lining the sinusoids, however, gives a very intense reaction as does that lining the peripheral blood vessels and the maternal blood vessels supplying the ectoplacental cone. No obvious falling off in intensity of staining is observed later in the period examined, but the reaction of the decidual cells lessens between a more intense region immediately surrounding the embryo centrally, and the "fibrinoid capsule" peripherally. Apart from some reaction in the ectoplacental cone and in the brush border of the visceral endoderm at 9½ days little activity is again seen in the embryo.

Inosine triphosphatase

The distribution and alterations in the intensity of the reactions for this enzyme almost exactly paralleled those of ATPase, with one important exception. At 4½ days, where the concentration of ATPase is found to be most pronounced antimesometrially, the staining for ITPase is found to be more concentrated all round the uterine lumen, falling off as it is traced laterally.

Thiamine pyrophosphatase (Fig. 61 A-D)

At 3½ days of pregnancy, staining for this enzyme is found in the Golgi zone of the epithelial cells lining the uterine lumen and glands, to a lesser degree in the surrounding stroma and particularly

in the endothelium of the blood vessels. A moderate reaction is observed in the smooth muscle also.

Little change occurs at 4½ days, but the development of the decidua is accompanied by gradual accumulation of enzyme, except in the implantation zone, up to 7½ to 8½ days. The reaction in the decidua is more marked centrally, and affects the region around the residual uterine lumen, but not the glycogen wings. As it is traced laterally the reaction fades out across decidua and stroma to negative internal to the muscle layers. The mesometrial part of the stroma accumulates a moderate degree of activity and the glycogen wings less. At its maximum the activity of enzyme in the decidua is slightly less than that observed with a βG-Pase, and unlike the latter the blood vessel endothelium remains positive. Between 7½ and 9½ days this reaction becomes very marked in the endothelium lining the sinusoids and maternal blood vessels supplying the cone and lateral to the ectoplacental cone, while that in the decidua becomes less, with the result that the blood vessels there also become more obvious.

Several changes of interest affect the embryo over the period from 7½ to 9½ days. When the visceral endoderm differentiates staining becomes obvious in the supra-nuclear region of the cells, but the embryonic part of this endoderm flattens and the staining there ceases to be obvious. Thus, at 9½ days the two different

types of visceral endoderm are clearly distinguished on this basis. The abembryonic giant cells also show quite a marked degree of staining with this enzyme, at 9½ days, as does the central part of the ectoplacental cone.

Uridine diphosphatase (Fig. 62 A-D)

At 3½ days some activity is present in the epithelium (supranuclear region) of lumen and glands, with less in the adjacent stroma. The endothelium shows an intense reaction, while the muscle layers and smooth muscle walls of the blood vessels are moderately stained.

Between 4½ and 7½ days the changes are similar to those seen with AMP as substrate (apart from the blood vessel staining) but the reaction tends to spread more towards the mesometrial part of the uterine lumen.

By 8½ days the reaction in the decidua and glycogen wings increases to a maximum. Thereafter an abrupt fall off in intensity occurs, affecting all areas. The mesometrial blood vessels retain their reaction throughout this period, but the lining of the sinusoids in the glycogen wings loses most of its reaction. At 9½ days some staining is seen in the free border of the embryonic endoderm, and in the ectoplacental cone.

β-Glycerophosphatase at pH 6.7 (Fig. 63 A)
(Control for glucose-6-phosphatase)

The distribution of staining with this enzyme is the same at this pH as at pH 7.2, but the intensity is very much less. The pattern of staining is quite different from that observed with glucose-6-phosphate as substrate.

Glucose-6-phosphatase (Fig. 63 B)

Less reaction for this enzyme is seen than with any other of the substrates used.

Trace activity is present in the epithelium lining the uterine lumen and glands up to 4½ days, the rest of the section being negative. At 5½ days some staining becomes visible in the primary decidua, and with the appearance of the secondary decidua this becomes more intense. The reaction, which is not seen in the implantation zone, spreads towards the mesometrial region by 7½ days, and increases steadily in the decidua up to the end of the period examined. By 8½ days staining is present in the glycogen wings, greater in intensity there than in the decidua, an area of lesser activity being interposed. No fall off in staining is seen in the decidua up to 9½ days, and that in the glycogen wings becomes more intense. No staining of blood vessels is ever seen with this substrate.

Fructose-6-phosphatase and fructose-1:6-diphosphatase

The distribution of staining by this method using fructose-6-phosphate, and fructose-1:6-diphosphate as substrates did not show any qualitative differences from that seen with β-glycerophosphate as substrate. The intensity of staining was slightly less with fructose-6-phosphate, however, and considerably less with fructose-1:6-diphosphate. Thus no evidence of specific enzymes hydrolysing these substrates was obtained, all activity that was present appearing to be due to non-specific alkaline phosphatase.

Dehydrogenases - carbohydrate

Embryonic tissues

The enzyme localisations observed divided the enzymes into 4 groups.

1. concerned with lipid metabolism -αGP and βOH- whose localisation corresponded well with that of the lipid.

2. concerned with ribose production (possibly for RNA synthesis) - G-6-P and 6-PG - whose distribution corresponded well to that of RNA.

3. concerned with glycolysis - LDH, IDH, SDH, MDH - or with reversal of glycolysis - ME - whose distributions have relationships to the glycogen concentration which were constant for the group, but varied depending on the area under examination.

4. GDH - concerned with breakdown of protein and its entry into the citric acid cycle, or with divergence of α-keto-gluterate from the cycle into protein synthesis.

ADH - concerned with alcohol degradation.

FDH - which may be concerned with RNA degradation.

The distribution of these enzymes did not correspond with any of the major metabolites examined.

Group 1

βOH (but not αGP) is found in the trophoblast, and both enzymes accumulate in the ectoplacental cone and giant cells at 9½ to 10½ days, particularly in the central part of the cone. The yolk sac endoderm shows only βOH at 9½ to 10½ days, but traces of both enzymes are seen in the embryonic endoderm (Fig. 64).

Group 2

G-6-P activity appears in the trophoblast and abembryonic giant cells at 7 days and accumulates in the latter to +++ activity, but decreases in the trophoblast as it disappears. When the lateral giant cells appear at 9½ days, however, they quickly accumulate large quantities of the enzyme and exhibit a similar staining reaction to the abembryonic group. Slight activity is seen in the ectoplacental cone at 7 days and increases rapidly, particularly centrally. Both layers of endoderm show + activity

from 7 (visceral) or 9½ (parietal) days onwards (Fig. 65) and when the visceral layer differentiates into yolk sac and embryonic endoderm, the latter accumulates further activity. Ectoderm, mesoderm, and amnion all show + activity as did the inner cell mass from which they are derived (Fig. 66).

Similar, but less intense, staining is found with 6-PG.

Table 3

Group 3

The results observed in embryonic tissues with these enzymes are summarised in Table 3.

All except ME are found in the trophoblast (Fig. 67) where they gradually decrease in intensity as it thins out and disappears. In the tissues to which it gives rise, however, the abembryonic (Fig. 68) and lateral giant cells, and the ectoplacental cone - activity increases further (to maximal or near-maximal levels) and, towards 10½ days, ME activity also appears. In the ectoplacental cone at 9½ and 10½ days, the enzyme activity is greater centrally than peripherally (Fig. 69).

The inner cell mass initially shows similar, but lesser enzyme activities as the trophoblast. Thereafter LDH and IDH decrease, although SDH and MDH increase to their maxima at 7 days, after which SDH maintains that level while MDH falls off in

TABLE 3

A-anterior intestinal portal. P-posterior intestinal portal. Emb. Meso. - embryonic mesoderm

		$5\frac{1}{2}$	$6\frac{1}{2}$	7	$7\frac{1}{2}$	8	$8\frac{1}{2}$	$9\frac{1}{2}$	$10\frac{1}{2}$
Tropho-blast	LDH	++++	++	++	+				
	IDH	+	+	Tr	Tr				
	SDH	+	++	++	Tr				
	MDH	++	++	++	+				
	ME	-	-	-	-				
Ecto-placental cone	LDH		++	++	++	++	++	+L +++C	+L +++C
	IDH		-	-	-	++	++	+L +++C	+L +++C
	SDH		++	++	+++	+++	+++	++L +++C	++L +++C
	MDH		+	++	++	++	++	+L +++C	+L ++C
	ME		-	-	-	-	Tr	TrL ++C	TrL +++C
Giant Cells (Abembryonic)	LDH			+	++	++	++	++++	+++
	IDH			-	Tr	Tr	Tr	++	++++
	SDH			++	+++	+++	+++	+++	+++
	MDH			+++	+	+	+	+++	++++
	ME			-	-	-	-	++	+++
Giant Cells (Lateral)	LDH							+++	++
	IDH							+++	++
	SDH							+++	+++
	MDH							++++	++++
	ME							+	++

TABLE 3 (contd.)

	5½	6½	7	7½	8	8½	9½	10½
Endoderm (Parietal)								
ME								+
MEM								‡
MES								‡
MEL								‡
MET								‡
Endoderm (Embryonic)								
ME								+
MEM								Tr
MES								+
MEL								Tr
MET								Tr
Endoderm (Visceral or Yolk Sac)								
ME								+
MEM								+
MES								‡
MEL								‡
MET								‡
Inner Cell Mass								
ME								
MEM								
MES								
MEL								
MET								

(3+ A and P)
(1 " ")
(2+ " ")
(3+ " ")
(Tr " ")

TABLE 3 (contd.)

		5½	6½	7	7½	8	8½	9½	10½	
Ectoderm	LDH					++	+++	Tr	Tr	
	IDH					Tr	Tr	Tr	Tr	
	SDH					++	+++	+	Tr	
	MDH					+	++	Tr	Tr	
	ME					-	-	Tr	Tr	
Mesoderm	LDH					-	++	+	+	(Emb. Meso. +)
	IDH					Tr	+	+	+	(Emb. Meso. -)
	SDH					+++	+++	+	+	(" " Tr)
	MDH					+	++	++	++	(" " Tr)
	ME					-	-	+	Tr	(" " -)
Amnion	LDH							+	+	
	IDH							Tr	Tr	
	SDH							+	+	
	MDH							+	+	
	ME							-	-	

activity. When the visceral and parietal layers of endoderm differentiate the enzymes appear in them also, their levels remaining static (visceral layer) or accumulating slowly (parietal layer - Fig. 70). Interesting changes are observed when the visceral layer differentiates into yolk-sac and embryonic endoderm. All enzyme activities decrease in the embryonic region and two (LDH, SDH) in the yolk-sac region also. In the anterior and posterior intestinal portals of the embryo, however, marked concentrations of LDH, MDH, IDH, and SDH appear (Fig. 71) unlike the remaining embryonic endoderm (+ or Tr activity). In both sites ME appears.

In the remaining tissues derived from the inner cell mass - ectoderm and mesoderm - LDH, IDH, SDH and MDH activities increase to their maxima at about 8½ days (Fig. 70). Thereafter their activity falls off and ME appears. At 10½ days the embryonic mesoderm shows less activity than the extra-embryonic mesoderm with all enzymes, except posteriorly in the region of the primitive streak (Fig. 72).

The amnion shows Tr to + staining with all enzymes of this group except ME.

Group 4

GDH - This enzyme only appears at 10½ days in Tr amounts in the parietal endoderm and ectoderm and + amounts in the mesoderm, abembryonic and lateral giant cells, and centre of the ectoplacental cone.

ADH - At 9½ days this enzyme is found in trace amounts in both yolk sac and parietal endoderm (but not embryonic endoderm), in the ectoderm and mesoderm, and in the giant cells. More activity (+) occurs in the centre of the ectoplacental cone. Activity in the ectoderm, mesoderm, and giant cells increases to + at 10½ days, accompanied by appearance of the enzyme in the embryonic endoderm also, but the yolk sac and parietal endoderm reaction does not alter.

FDH - This enzyme is absent from the trophoblast, but is seen in its derivatives at 9½ and 10½ days where it accumulates to ++ levels (centre of ectoplacental cone only) (Fig. 73). Initially the inner cell mass is negative, but some activity accumulates (again to ++ levels) in the yolk sac, and parietal and embryonic endoderm over the same period. Ectoderm and mesoderm show + activity at 10½ days.

Diaphorases

With the exception of the implantation zone over 7½ to 8½ days, and the most lateral part of the stroma, in both cases with NADPH, no tissue was found in this study which did not contain sufficient quantities of NAD or NADP diaphorase to give a coloration with Nitro BT.

However, the glycogen wings, in the latter part of the period examined showed less activity for NADP diaphorase than might have been expected in this relatively active area.

Extra-embryonic tissues

Group 1

αGP and βOH activities are observed in the uterine epithelium immediately prior to degeneration appearing fairly intensely at the mesometrial end of the lumen (Fig. 74), less so antimesometrial to that, and then gradually disappearing as the epithelium degenerates.

Trace activity is observed with both enzymes in the secondary decidua from $7\frac{1}{2}$ (βOH) or $9\frac{1}{2}$ (αGP) days onwards.

Group 2

G-6-P and 6-PG are enzymes of the pentose shunt pathway, which may be linked to RNA production. The former, but not the latter is markedly present in the primary decidua at $5\frac{1}{2}$ days, and both enzymes appear at $6\frac{1}{2}$ days in the secondary decidua where they persist in trace concentrations until 8 days (6-PG) or the end of the period examined (G-6-P). No activity is observed in the antimesometrial stroma; however the mesometrial stroma, excluding the glycogen wings, shows quite intense activity (particularly centrally) from $6\frac{1}{2}$ days (Fig. 75) till the end of the period examined. Here again, the activity is greater with G-6-P than 6-PG and falls off in intensity from 8 days onwards. No activity is observed in the glycogen wings with either enzyme, and the luminal epithelium shows trace activity with G-6-P at the mesometrial end until epithelial degeneration occurs.

Group 3

The enzymes of this group are localised heavily in the uterine epithelium, in which they reach maximal intensity of staining shortly before degeneration when they rapidly decrease in amount. A zone of intense staining is also observed in the secondary decidua (Figs. 76A,B) in which the enzymes reach their maxima over the period 7-8½ days, and then decline in activity. Lateral to the decidua, the stroma shows intense coloration which decreases as it is traced further laterally, and here again some falling off in intensity is observed towards 10½ days, more particularly anti-mesometrially than mesometrially in the future decidua basalis.

The period of decreasing activity described above, is accompanied by an accumulation of enzyme activity in the glycogen wing region which is seen as persistence of staining there, whilst that in the stroma, from which it is differentiating, tails off. As in the secondary decidua LDH (Fig. 77) and SDH staining is more intense than IDH and MDH, however, a reversal of that situation is seen in the primary decidua; in which the maximal staining (++) observed with this group of enzymes is found with IDH while LDH, SDH, and MDH only reach + intensity.

ME is found only in the secondary decidua where it reaches + activity at 7½ to 8 days and then tails off.

Group 4

GDH activity is observed only occasionally and, in the tissues excluding the embryo, reaches trace levels first in the uterine epithelium at the mesometrial end just prior to degeneration and then in the secondary decidua towards 10½ days.

ADH activity (Tr) is observed in all areas of the uterine epithelium, but particularly mesometrially, for 24 hours preceding degeneration. In the secondary decidua it reaches trace levels of activity briefly at 7½ days.

FDH is distributed similarly to ADH but persists in trace amounts from the beginning of the period examined to the time of degeneration (epithelium) or to the end of the period (secondary decidua). In the glycogen wings it appears from 8½ to 10½ days in increasing intensity. Its distribution in the stroma, however, differs from that of the other enzymes examined. It is present in trace amounts throughout the period examined, but from 8½ days on, is found only anti-mesometrially, the mesometrial region (except the glycogen wings) being negative. Trace activity is also observed with this enzyme in the primary decidua.

Throughout the period examined, the maternal blood vessels show ++ activity with LDH and NADH, + activity with MDH and NADPH, trace activity with ME, G-6-P, 6-PG, αGP, SDH, IDH, and FDH, and

negative with ADH, GDH and βOH. Both muscle layers are + active with LDH, NADH, and MDH, trace active with NADPH, FDH, and SDH, and negative with the remaining enzymes examined.

RABBIT

Glycogen

Embryo

Glycogen is not seen in the rabbit trophoblast or embryo up until early on the 8th day post coitum, when it appears in the mesoderm being budded off from the primitive streak. The intensity of staining for glycogen remains the same in this mesoderm (although increasing in the intra-embryonic mesoderm of the developing embryo (Fig. 80) until 9 days when it begins to fall off in relation to the placental folds (Fig. 80).

Immediately following the appearance of glycogen in the mesoderm it is seen in the ectoderm and endoderm also (Fig. 78). In the ectoderm, and later the cytotrophoblast it increases to a maximum around late 8 days (Fig. 79) when the concentration begins to fall off and disappears completely by 9 days. At 8 days, when the syncytiotrophoblast differentiates, glycogen is briefly seen in trace amounts in its cytoplasm. It is quickly lost, however, and is not present 6 to 8 hours later.

In the endoderm, glycogen appears both in the future visceral layer, related to the developing embryo, and in the "parietal" layer

opposed to the inner surface of the trophoblast. In the former it persists in unchanging quantity till the 9th day, when glycogen ceases to be visible in the layer related to the placental folds, although increasing in quantity in the intra-embryonic endoderm (Fig. 80). In the "parietal" endoderm it remains constant in quantity up to the end of the period examined, (Fig. 81). The fall off of glycogen concentration seen in the visceral endoderm coincides with a marked loss of glycogen from the yolk-sac where it accumulated suddenly at early 8 days (Fig. 78), in considerable quantity, and gradually decreased thereafter.

Epithelium and stroma

At 6 days, glycogen was observed in small quantities, in the subepithelial stroma antimesometrially, but not mesometrially, and in the deep parts of the antimesometrial glands (Fig. 82).

Thereafter glycogen appears in the uterine secretions, and in the mesometrial stroma also, particularly in the placental and paraplacental folds, and to some extent in the epithelium itself, particularly in the paraplacental folds, and depp parts of the uterine glands (Fig. 83). It reaches a maximum at early 8 days, when antimesometrial attachment of the embryo is occurring, and thereafter falls off in concentration, more particularly antimesometrially, and in the placental folds, than in the paraplacental folds. The fall off affects both epithelium and stroma, and by the

time that the mesometrial attachment is beginning the epithelium shows no glycogen staining.

The degeneration of the antimesometrial epithelium, and later the paraplacental epithelium, is accompanied by considerable production of glycogen (Fig. 81), presumably for histiotrophic nutrition of the embryo. Degeneration of the deepar parts of the mesometrial glands also produces some glycogen (Fig. 84).

Decidua

In the rabbit, the development of the decidua is accompanied by the accumulation of considerable quantities of glycogen in the cells surrounding the blood vessels (Fig. 84) which commences early on the 8th day and increases thereafter (up to the 20th day - see section on Comparative Placentation).

Muscle layers

The quantity of glycogen present in these increased slightly from 6 to 8 days, and remained constant thereafter.

Diastase-resistant, PAS-positive substances.

Embryo

Throughout the period examined, the cells of the embryo showed a faint pink staining, with this method, which was diffuse, and did not change in intensity throughout. Cytoplasmic inclusions were seen at various stages, however.

None were observed until 7 days, when the embryo was still contained in an intensely staining inner mucolemma, and a granular, less well stained, outer gliolemma (Fig. 85). At this stage a few intra-cytoplasmic granules were observed in the cells around the primitive streak (Fig. 85), and in the trophoblastic knobs (Fig. 86).

When the embryonic coverings disintegrate and the invasion of the maternal tissue commences (Fig. 87) the trophoblast maintains the same granular appearance, which does not disappear till early 8 days. No inclusions are ever observed in the cyto- or syncytio-trophoblast when they differentiate.

Up to early 8 days the endoderm exhibits a faint, pink, diffuse staining. Thereafter, however, the visceral layer develops a brush border, and accumulates intra-cellular droplets of various sizes (Fig. 90), similar in staining to the contents of the yolk-sac, which appear, histochemically speaking, at early 8 days, and do not show much increase with this staining. Their physical appearance, however, changes from granular to fibrillar at late 8 days. No inclusions are observed, over this period, in the intra-embryonic endoderm.

Epithelium

Up to the time of disintegration this shows a faint pink staining reaction, with a fine brush border.

At 7 days, the mesometrial epithelium shows some granules of muco-protein, particularly in the deeper parts of the glands, and in their lumina (Fig. 85), and this appearance increases, particularly on either side of the paraplacental furrow up until early 8 days, after which there is a disappearance of granular staining in these sites, although a thin layer of what appears to be uterine secretion persists for some time on the outside of the blastocyst.

When the antimesometrial epithelium degenerates it forms a denser pink, non-granular coagulum patchily related to the lateral, and antimesometrial walls of the blastocyst. In contrast (Fig. 89), the "degeneration" of the deeper parts of the subplacental glands produces granules of muco-protein in the deepest area, above which is a faint pink syncytium.

Decidua

These cells exhibit a faint pink, homogenous staining throughout the period from their appearance to the end of the implantation process.

Muscle layers

A similar faint pink staining, unchanging throughout the period examined, is observed here also.

Acid mucopolysaccharides

Embryo

No acid mucopolysaccharide staining was observed in the embryonic tissues of the developing rabbit blastocyst. Both of its outer coverings, however, gave a positive reaction, the inner mucolemma being intensely positive, particularly immediately prior to its removal and thereafter (Fig. 91). The gliolemma shows a less intensely positive reaction. After the disappearance of these two coats, a covering of uterine secretion remains on unattached parts of the blastocyst wall for a varying period, and this too is positive for acid mucopolysaccharides (Fig. 91).

Epithelium and stroma

Prior to loss of the coats of the blastocyst the epithelium shows little staining reaction and scanty secretion is observed in the glands.

After the antimesometrial loss of the mucolemma, however, considerable secretion accumulates in the glands, both antimesometrially (Fig. 92) and mesometrially (Fig. 91), which gives an intense positive reaction. Antimesometrially masses of this secretion appear to become trapped between the invading trophoblast and the epithelium (Fig. 93) which as it becomes symplasmic, appears to cease secretion. The deep parts of the glands, which remain

cellular, continue to produce a secretion, however, and this becomes trapped beneath the trophoblast, and forms "pockets" of intensely reacting material (Fig. 94). Similar material is found in the deepest parts of the placental fold glands (Fig. 95), beneath the area which becomes symplasmic.

From the time of antimesometrial implantation onwards the degree of secretion of the uterine glands, as judged histochemically, becomes less.

RNA

Embryo

From 5 to late 7 days the rabbit embryo shows only small quantities of RNA in the cells of the blastocyst.

Differentiation of the trophoblastic knobs, however, is accompanied by accumulation of RNA in their cytoplasm (Fig. 96), to a moderate degree, which lessens as they invade the maternal epithelium. As the invasion widens, the trophoblast on either side of it fuses to the maternal epithelium, and accumulates RNA in the cytoplasm of its hypertrophying cells (Fig. 97), to a degree greater than that seen in the actual invasive process. This process of fusion and RNA accumulation continues over the entire antimesometrial half of the blastocyst.

Mesometrially, RNA accumulates in the neural plate, particularly in the edge thereof facing the maternal tissue (Fig. 98) and later

in development, the thickening of the trophectoderm facing the placental folds is accompanied by a similar RNA increase and distribution. When the syncytiotrophoblast differentiates from the cytotrophoblast RNA is seen in the cytoplasm of both where it gradually accumulates up to the end of the implantation period (Fig. 100).

In the other embryonic tissues, small amounts of RNA are seen in the extra-, and intra-embryonic mesoderms, but traces only in the intra-embryonic endoderm (Fig. 99), and none in the extra-embryonic endoderm. Some RNA accumulates, however, in the developing neural tube, and ectoderm (Fig. 99).

Epithelium and stroma

Only small quantities of RNA are seen at any time in the epithelium. Symplasma formation is accompanied by decrease in the quantity observed, to a greater degree antimesometrially, than mesometrially in the deeper parts of the subplacental glands where some RNA can still be detected histochemically after symplasma formation (Fig. 99).

In the stroma, RNA staining is negligible until early 8 days, when the commencement of decidua formation is accompanied by increase in RNA content of the stromal cells (Fig. 99).

Decidua

The development of the decidua is accompanied by gradual increase in the RNA content of its cells (Fig. 99) until near the end of the implantation period when the content appears to decrease again, consequent upon the vacuolation of the cells brought about by their increasing glycogen content.

Lipid

Lipid was not found to be a conspicuous feature of the rabbit implantation specimens.

Traces accumulated in the embryonic endoderm and in the yolk-sac contents towards the end of the period examined.

A faint staining was also observed supra-nuclearly in the uterine epithelium, until degeneration occurred.

Hydrolytic enzymes

Embryo

Until 7 days of gestation, only small quantities of acid phosphatase and non-specific esterase are observed in the trophoblast (Fig. 101). Later on the 7th day, however, the development of trophoblastic knobs is associated with increase in enzyme activity, which persists when they invade the maternal epithelium (Fig. 102). Mesometrially, at this stage, no alteration in the trophoblastic staining reaction has occurred, and the mucolemma is still present.

Early on the 8th day post coitum, the mesometrial mucolemma disappears, and the trophoblast comes into contact with the maternal epithelium. When this occurs increase in hydrolase activity appears in the trophoblast, more marked in the regions in contact with the epithelium than in between these regions, the differences being more marked with acid phosphatase than with esterase (Fig. 103 A,B).

Later in the 8th day, syncytiotrophoblastic differentiation is associated with further accumulation of hydrolase activity, (Fig. 104), particularly in the syncytiotrophoblast, but to some degree in the cytotrophoblast also. Fusion of the syncytiotrophoblast to the mesometrial epithelium, and the disappearance of the latter, is associated with further increase in enzyme activity, so that the differentiation between the syncytio-, and cytotrophoblast becomes more marked (Fig. 105).

At this stage the trophoblast antimesometrially has fused with the epithelial symplasma, and both are undergoing degeneration (Fig. 106).

Initially (Fig. 102) the embryonic endoderm shows only traces of hydrolase activity, but this rapidly increases (Figs. 103, 104) both mesometrially and antimesometrially (Fig. 106), this increase

being associated with the appearance of traces of acid phosphatase activity in the yolk sac contents.

Hydrolase activity is also seen in the intra-embryonic endoderm, the notochord, and the neural tube, and appears in both extra- and intra-embryonic mesoderms in trace amounts.

Epithelium and stroma

Three regions of epithelium and subjacent stroma can be considered.

(1) antimesometrially, moderate hydrolase activity is observed until implantation commences (Fig. 102) when a rapid increase in epithelial enzyme content occurs. This coincides temporally with the conversion of the epithelium into a symplasma, and is followed by further increase as degeneration occurs (Fig. 106). The deeper parts of the uterine glands, which remain cellular do not show the same increase in enzyme content (Fig. 107). A slight increase in stromal staining over the period examined is observed.

(2) The epithelium of the paraplacental folds and glands shows an early increase in activity when mesometrial implantation begins, and the higher level of enzyme is maintained until degeneration occurs towards 9 days. A similar change in the stroma is observed.

(3) mesometrially the epithelium of the placental folds and glands shows increase in enzyme activity from early 8 days, i.e. the time

of mesometrial implantation onwards (Fig. 104). Degeneration is accompanied by further increase in activity, which affects the surface epithelium more than the glands (Fig. 108A, B). In the glands, the symplasmic degeneration of the middle and deeper portions is accompanied by increased hydrolase activity but the deepest portions show less marked changes (Fig. 108 B). Stromal changes in the placental folds consist of an increase in cellularity accompanied by a moderate increase in enzyme activity.

Decidua

The differentiation of decidual cells in the rabbit is accompanied by the gradual accumulation of hydrolytic enzymes (Fig. 109, 110).

Muscle layers

Traces of acid phosphatase and esterase were found in these throughout the implantation period, and did not alter in concentration.

All esterase activity observed proved, on inhibitor studies to be organophosphate sensitive, that is B type esterase.

Alkaline phosphatases

The patterns of staining observed for non-specific, and specific alkaline phosphatases were almost identical, and the following descriptions apply to both. In most sites the staining was most intense with inosine triphosphate as substrate, and then

with adenosine triphosphate, adenosine monophosphate, or uridine diphosphate, thiamine pyrophosphate, and β-glycero-phosphate at pH 7.2 in that order. The reaction with sodium α-naphthyl phosphate at pH 9 was equal in intensity to that apparent with adenosine triphosphate. Where necessary the differences from the general staining pattern are indicated below.

Embryo

Phosphatase activity decreases in the trophoblast, from a very intense reaction at 5 days (Fig. 111) to almost negative at 7 days, although some activity persists in the trophoblast knobs particularly with inosine triphosphate or uridine diphosphate as substrate. This activity increases as the invasive trophoblast penetrates the maternal epithelium (Fig. 112) and further increase appears to take place as the trophoblast fuses with the epithelium between the invasion areas and both degenerate.

Mesometrially the trophoblast never loses its activity completely, except with sodium α-naphthyl phosphate, although the staining reaches trace levels for a short period in the area immediately related to the placental folds. However syncytiotrophoblastic differentiation is accompanied by increase in enzyme activity in its cytoplasm (Fig. 113), and this becomes very intense, with all substrates except β-glycerophosphate and thiamine pyrophosphate, as the syncytiotrophoblast

thickens (Fig. 114). Some activity is also seen in the cyto-trophoblast, with inosine triphosphate as substrate, between 8 and early 9 days (Fig. 114).

Phosphatase activity is also seen in the embryonic mesoderm, but only from mid 8 days onwards with adenosine monophosphate (Fig. 115) or inosine triphosphate as substrate initially, although at 9 days post coitum some activity appears with adenosine triphosphate and uridine diphosphate also, while activity with the two previous substrates decreases somewhat. The activity is greater in the somatopleuric layer of mesoderm, rather than in the splanchnopleuric layer, and, at 9 days some activity is seen in the intra-embryonic mesoderm also (only with adenosine monophosphate). The endoderm shows some activity mesometrially with inosine triphosphatase (+) and thiamine pyrophosphatase (Tr) for a short period on day 8, which coincides with an increase in inosine triphosphatase and adenosine triphosphatase activity of the yolk sac contents, as judged histochemically.

Epithelium and stroma

The same three areas of epithelium and subjacent stroma as were considered in describing hydrolase activity may be examined here:- (1) antimesometrially, from 5 days to 7 days the epithelium shows a slight cytoplasmic staining, with rather more activity concentrated

in a brush border with all substrates except thiamine pyrophosphate, the activity of adenosine triphosphatase (Fig. 116) and inosine triphosphatase being particularly marked. When trophoblastic invasion of the epithelium begins an abrupt increase in enzyme activity is seen with all substrates (Fig. 117). This continues until degeneration occurs, when very marked activity becomes visible in the degenerating tissue, with less in the underlying bases of the glands, (Fig. 118). Little change occurs in the staining reaction of the antimesometrial stroma throughout the implantation period.

(2) in the paraplacental region a similar abrupt increase in enzyme activities occurs late on the 7th day, and the higher level is maintained until the commencement of mesometrial implantation, when the enzyme activity gradually decreases. The decrease in activity continues until degeneration of the epithelium occurs late on the 9th day.

Apart from a generalised moderate increase in enzyme activity, no alteration of the distribution of staining for alkaline phosphatases occurs in the paraplacental stroma.

(3) mesometrially, the epithelium of the placental folds shows a more gradual increase in enzyme activity from 7 days to the period immediately preceding mesometrial implantation, becoming more marked

with the disappearance of the mesometrial part of the mucolemma. This increase affects chiefly non-specific alkaline phosphatase at pH 9. Over the period late 7 to early 8 days, however, the activity of thiamine pyrophosphatase (found in the Golgi apparatus) and uridine diphosphatase increases quite markedly and then falls off again, and the Golgi apparatus becomes prominent in the epithelium of the placental folds and glands (Fig. 119). Early on the 8th day increase in specific phosphatase activity becomes evident in the deep parts of the sub-placental glands, and continues thereafter. The increased activity of non-specific alkaline phosphatase noted above is seen until degeneration of the mesometrial epithelium occurs, with formation of the chorio-allantoic placenta.

The hypertrophy of the placental folds observed during the implantation period, is accompanied by steady increase in alkaline phosphatase activity of the stroma, with all substrates used, except adenosine monophosphate, whose staining reached a peak at early 8 days and then declined (Fig. 120 A-D).

Decidua

Phosphatase activity steadily increased, in the developing decidual cells, up to mid 8 days and then declined rapidly, more particularly in the outer zone of each decidual area, than in the zone

immediately surrounding the central blood vessel. At peak activity the substrates utilised in descending order of staining were inosine triphosphate and adenosine triphosphate; adenosine monophosphate (Fig. 120, A-D), uridine diphosphate and thiamine pyrophosphate; and sodium α-naphthyl phosphate at pH 9. No activity with β-glycerophosphate at pH 7.2 was observed in the decidua at any stage.

Glucose-6-phosphatase

No evidence of a specific enzyme splitting this substrate was found in any of the tissues related to the implanting rabbit embryo.

Dehydrogenases (carbohydrate)

Embryo

Between 5 and late 7 days, the activity of lactic and malic dehydrogenases in the trophoblast alter, the former increasing to a peak at 7 days and thereafter decreasing, more mesometrially than in the trophoblast knobs antimesometrially, while the latter shows a steady increase in both sites. Little alteration occurs in the remaining dehydrogenases examined in the trophoblast over this period.

The trophoblast knobs show dehydrogenase activity with glucose-6-phosphate, lactate, malate, isocitrate, succinate, glutamate, and furfuryl alcohol, and the staining pattern does not change when they

invade the maternal epithelium (Fig. 121). Beyond this period the ab.imesometrial trophoblast fuses to the maternal epithelium and its staining reaction becomes indistinguishable from that of the epithelium. Mesometrially, however, the activity of all dehydrogenases examined increases, more in the regions of trophoblast not in contact with the maternal epithelium (Fig. 122) than in areas of contact, except with glucose-6-phosphate, and 6-phosphogluconate as substrates, when the reverse situation obtains. The establishment of complete contact quickly eliminates these differences, however, and the development of syncytiotrophoblast is accompanied by the accumulation of further enzyme activity with all substrates except α-glycerophosphate and β-hydroxybutyrate. Staining is more marked in the syncytiotrophoblast, than in the cytotrophoblast (Fig. 123), and with further development of the placenta the differences are accentuated (Fig. 124), particularly with isocitric, glucose-6-phosphate, and lactate dehydrogenases (less so with the remainder, Fig. 125). The accentuation is due partly to increase in enzyme activity in the syncytiotrophoblast, and partly to decrease in activity in the cytotrophoblast.

From the time of its appearance, the endoderm shows dehydrogenase activity (Fig. 122) initially with all substrates except glucose-6-phosphate and 6-phospho-gluconate, and later with these two also.

The activity with all substrates increases over the period examined (Figs. 124, 125).

A similar increase is seen in the mesoderm as it develops with isocitrate, succinate and malate (Figs. 124, 125) dehydrogenases. The activity with the remaining substrates remains unaltered at trace to + levels, except with α glycerophosphate dehydrogenase, and β-hydroxy butyric dehydrogenases, which do not appear in the mesoderm.

Epithelium and stroma

(1) Antimesometrially dehydrogenase activity with all substrates remains fairly constant in the epithelium until trophoblastic invasion (Fig. 121). Thereafter activity increases with all substrates except glucose-6-phosphate and 6-phospho-gluconate, the increase being particularly marked with lactate, isocitrate, malate, succinate and furfuryl alcohol; and less so with α-glycero-phosphate, β-hydroxy butyrate, and glutamate (Fig. 126) and continuing until degeneration of the epithelium occurs, less activity is seen in the surviving deep parts of the uterine glands.

In the stroma activity is seen with all substrates except α-glycerophosphate and glutamate at 5 days and increases from late 7 to early 8 days (except β-hydroxy butyrate, glucose-6-phosphate, and 6-phosphogluconate, which disappear at this stage). Thereafter, however, the stromal dehydrogenase activity disappears.

(2) In the paraplacental epithelium the following staining patterns are seen. Glucose-6-phosphate and 6-phospho-gluconate, dehydrogenases reach a peak of activity at late 8 days and then fall off in activity; isocitric dehydrogenase, increases from late 7 days, the level of activity having been unaltering before that, furfuryl dehydrogenase increases from late 8 days, and the remainder do not alter their levels of activity. Prior to these alterations, the preferential order of substrate utilisation is malate, furfuryl alcohol and lactate, isocitrate and α-glycero-phosphate, succinate and glucose-6-phosphate, and , poorly, β-hydroxy-butyrate, 6-phospho-gluconate, and glutamate.

The stroma again shows dehydrogenase activity with all substrates except α-glycero-phosphate and β-hydroxy-butyrate and the concentration changes little, except for a short-lived increase with all substrates at the time of the first mesometrial attachment of the trophoblast.

(3) The mesometrial epithelium exhibits certain definite changes of staining reaction over the period prior to its fusion with the trophoblast. The reaction for glucose-6-phosphate, 6-phospho-gluconate, isocitrate, and furfuryl alcohol dehydrogenases decreases from 5 to 7 days, then increases again up to the time of trophoblastic attachment (Fig. 127 A-B). Activity with the remaining dehydrogenase

substrates does not appreciably alter in this site over the period of implantation, nor is any definite alteration in staining pattern observed in the epithelium lining the glands of the placental folds, except when symplasma formation occurs late in the period examined, and accompanied by increase in all enzyme activities (Fig. 128 A,B).

As in previously described areas of stroma, the cells of the mesometrial region show dehydrogenase activity, in unaltering concentration, with all substrates except α-glycero-phosphate, and β-hydroxy-butyrate.

Decidua

Glutamate, α-glycero-phosphate, and β-hydroxy butyrate dehydrogenases are only found in trace amounts in rabbit decidua, and do not change appreciably in concentration during its development. However, the remaining substrates are oxidised to a greater degree, particularly isocitrate, and malate, and slightly less, glucose-6-phosphate, lactate, succinate, and furfuryl alcohol, and all of these reach a peak at late 8 days (Fig. 129) and then decrease slightly.

Comparative Placentation

The morphology of the placental types, whose histochemistry will be described below, is well known (see Mossman, 1937, and Amoroso, 1952 for reviews). However to facilitate the description of the distribution of enzymes and other substances to be given, a brief review of the morphology of the placenta and associated structures of the various species examined, will be presented.

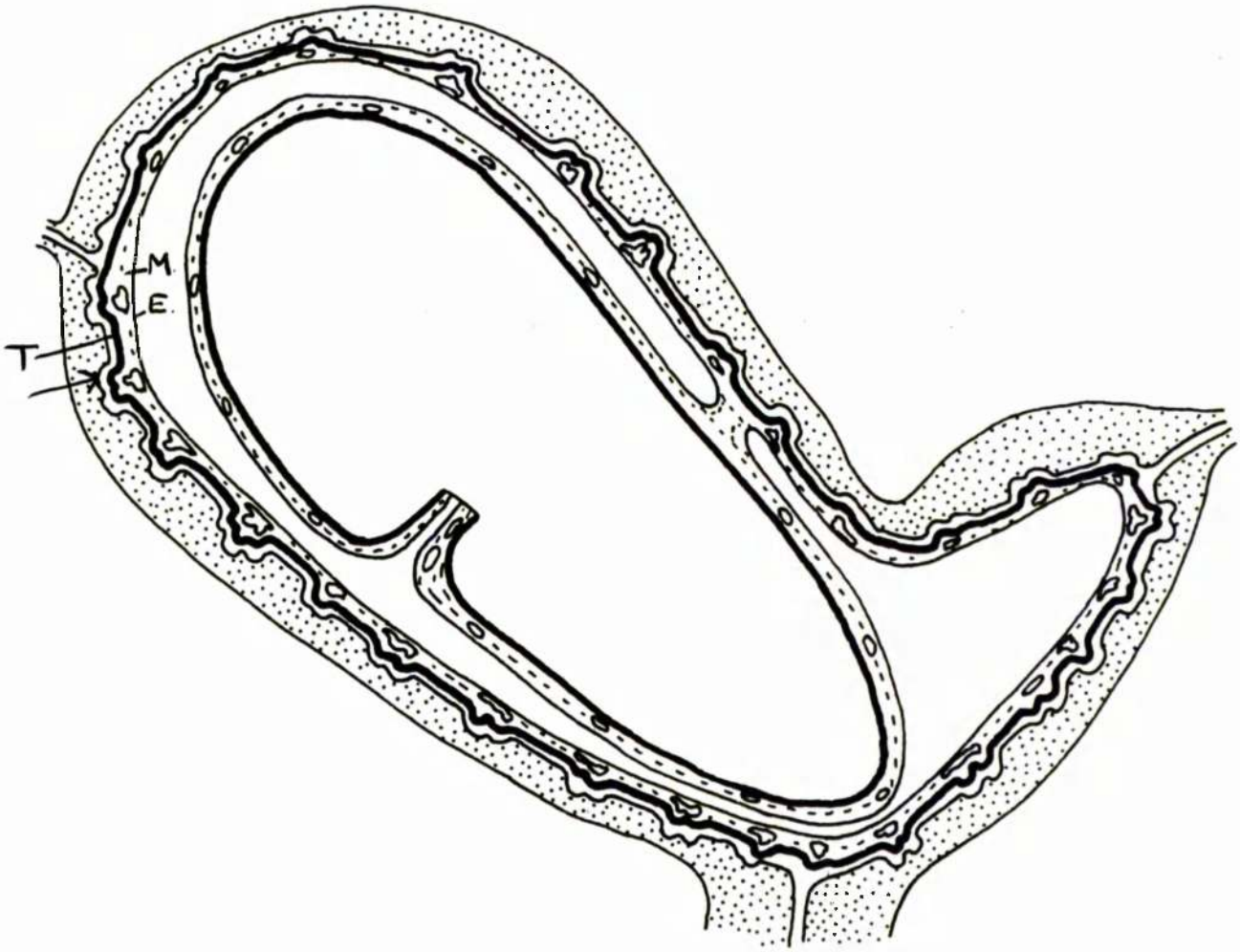
(A) Epithelio-chorial placenta - horse (Figs. 130-132) (Text fig. 3)

The foetal tissues only of this type were available.

The horse placenta is diffuse, villous (with short branches) and epithelio-chorial, the villi lying with their trophoblastic covering in close relationship with the thinned out maternal epithelium of the uterus, which separates them from the maternal capillaries, and being bathed by copious quantities of maternal glandular secretion.

The basophilic trophoblastic covering of the villi is very thinned out, in comparison with the columnar cells of the chorionic plate, which have a poorly defined brush border, and contain some inclusions. It lies on a poorly differentiated basement membrane which separates it from the vascular, loose connective tissue stroma of the villi, many of whose capillaries indent and thin

Text-fig. 3. Diagram of the term horse placenta (after Mossman, 1937) showing the trophoblast (T.) apposed to the uterine epithelium (arrowed) and lined on its inner aspect by allantoic mesoderm (M.), and endoderm (E.).


M
E
T

out the trophoblastic covering, and may appear intra-epithelial.

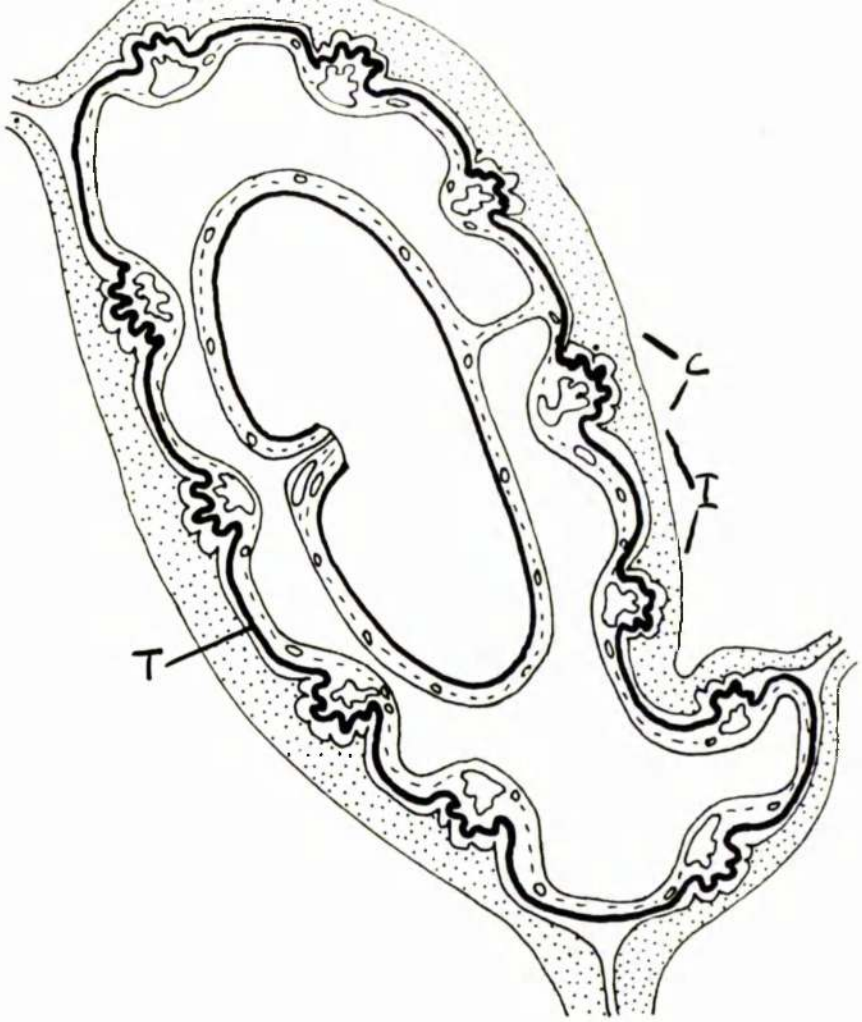
The connective tissue beneath the chorionic plate contains the allantoic blood vessels, and is rather denser in structure. On its inner surface lie the cuboido-columnar, pale staining, cells of the allantoic endoderm.

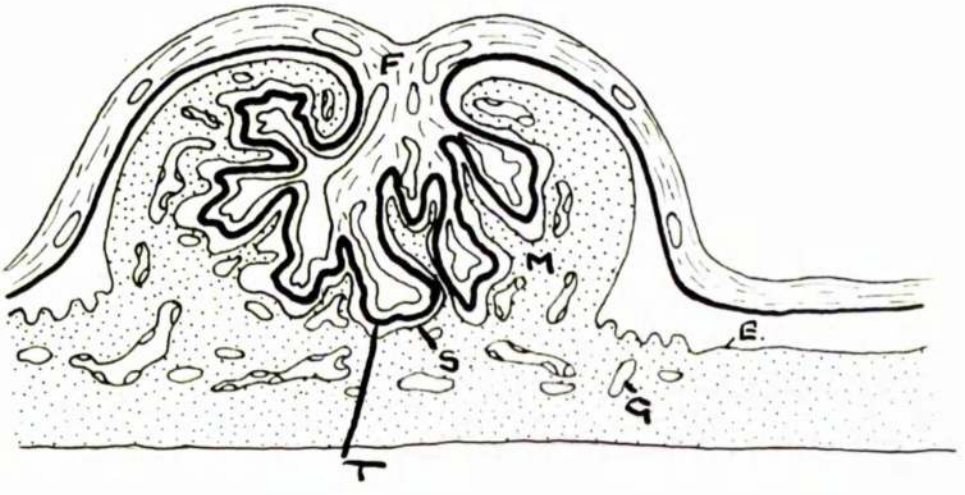
(B) Syndesmo-chorial - sheep (Fig. 133-136, Text. fig. 4)

The placenta of the sheep is cotyledonary, villous (long-branched), and syndesmo-chorial, although the intercotyledonary areas present an epithelio-chorial relationship.

In the cotyledons, villi of foetal vascularised loose connective tissue, covered by a layer of cellular trophoblast, interdigitate with columns of maternal connective tissue, rather denser in appearance and highly vascular, which are covered by a layer of syncytial tissue, containing clumps of nuclei of various numbers. At the bases of the villi, the trophoblast cells become columnar, and present a brush border and varying numbers of inclusions. Here the foetal stroma is rather denser in appearance, and contains the allantoic blood vessels, and it is lined, on its inner (foetal) surface with the squamous allantoic endoderm. The base of the cotyledon rests on the inner muscle layer of the uterus, and is separated from it by a layer of dense connective tissue. On either side of the cotyledon, the mucosa is packed with dilated,

Text-fig. 4. Diagramof the sheep placenta (after Mossman, 1937) showing cytotrophoblast (T.), syncytiotrophoblast (S.), foetal (F.) and maternal (M.) stroma, uterine glands (G.) and the uterine epithelium (E.). C is the cotyledon, I the intercotyledonary area.


C
I
T


F
M
E.
S
LG
T

tortuous, uterine glands, which open into the cavity of the uterus. The lining epithelium of the uterus in simple columnar, and, with its underlying dense connective tissue presents ridges which interdigitate with folds of the chorion.

Scattered throughout the trophoblast lining the foetal villi, and the intercotyledonary chorion, are numerous binucleate giant cells, the diplokaryocytes. These are less numerous on the sides of the villi, compared to the apices, and intervillous area of the chorionic plate. Their number does not change throughout the period of pregnancy examined in this study (34 days to term).

Towards the later part of pregnancy considerable quantities of blood accumulate between the bases of the villi, whose branching has become increasingly complex. The appearance of these haematomata is accompanied by the accumulation of large numbers of inclusions in the trophoblastic cells of the chorionic plate.

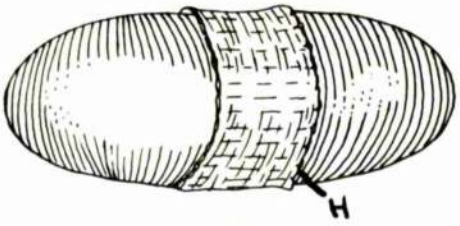
Endothelio-chorial - cat, dog, ferret (Figs. 137-145, Text. fig.5)

The early stages of development of this placental type were studied in the dog, and the definitive placenta and related structures in the dog, cat, and ferret.

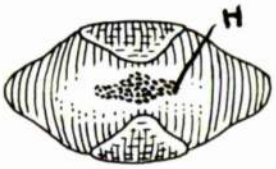
Trophoblastic invasion of the maternal tissues is accompanied by symplasmic change in the superficial part of the glands of the uterine mucosa (Figs. 137, 138). Deep to this region the middle part of the glands dilate and become filled with secretion, while

Text-fig. 5. Diagrams of the carnivore placenta (after Mossman, 1937) showing the circumferential placental band of the cat and dog (A), with the marginal haematoma (H), the zonary placenta of the ferret (B) with the central antimesometrial haematoma (H). C is a cross section of the pregnant cat or dog uterus showing the relations of the foetal membranes, and D a similar section of the pregnant ferret uterus.

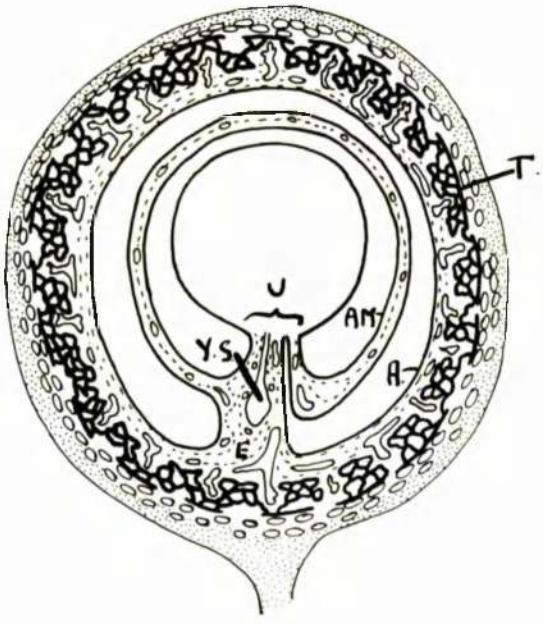
H - haematoma
T - trophoblast
Y.S. - yolk sac
A - allantoic endoderm
E - exocoelom
U - umbilical cord
AM. - amnion



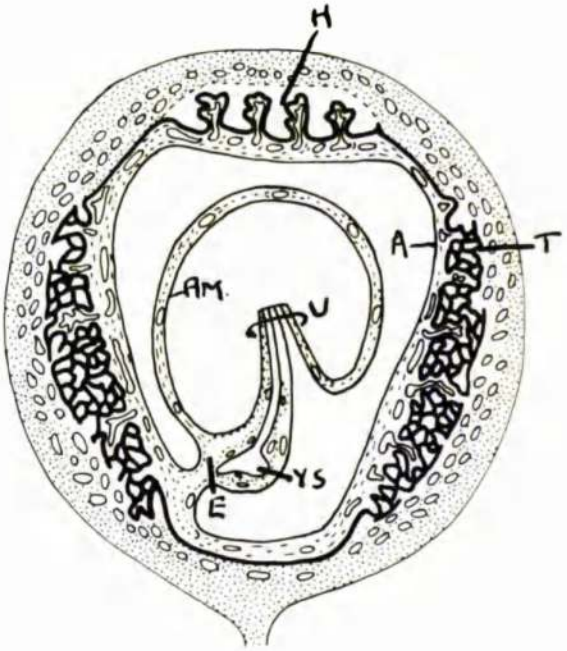
A



B



C



D

the deepest parts remain contracted. When the superficial parts of the glands become plugged by trophoblast, the dilatation becomes very pronounced, and the maternal tissue becomes reduced to thin interglandular septae of connective tissue, covered by a cuboidal to columnar epithelium. The trophoblastic invasion, penetrates to the luminal edge of this region, removes the superficial glandular symplasma, and the connective tissue, but spares the endothelium and blood vessels, thus forming the definitive endothelio-chorial labyrinth. Initially the trophoblast is cellular, but as it invades syncytiotrophoblast differentiates (Fig. 139) over all but the tips of the penetrating villi, and, in the definitive placenta little cellular trophoblast is found, except in that site.

Decidualization is not marked in the dog, although some enlargement of the stromal cells occurs in the superficial glandular region between the invading trophoblastic villi (Fig. 139).

With minor differences, the morphology of the definitive chorio-allantoic placenta in the specimens of cat, term dog, and mid-pregnancy ferret examined (Fig. 140 A-C) is the same. Each can be divided into the labyrinth, the junctional zone, the spongy zone, and the haematoma region, the latter lying on either

side of the placental band (Text fig. 5) in the cat and dog, and antimesometrially in the ferret.

The histology of the labyrinth is well shown in Figs. 141 A-C. In these, the syncytiotrophoblast covers the outer aspect of the trophoblastic tubules (cat) or lobules (dog, ferret) and lies between the foetal stroma, and the interstitial matrix of the maternal tissue. Beneath this lies the maternal endothelium, which is thin in the cat and dog, but markedly thickened in the ferret. All three species show intra-epithelial capillaries in the syncytiotrophoblast, although these were not observed in mid-pregnancy in the cat. The other noticeable feature in the maternal tissues is the large, often binucleate decidual giant cells seen only in the cat. In the labyrinth of the cat occasional cytotrophoblastic cells were seen at mid-pregnancy, but had disappeared by term.

Cytotrophoblast forms the most conspicuous feature of the junctional zone, however, where it covers the tips of the foetal villi where they contact the maternal tissues, either the dilated parts of the uterine glands, or degenerating tissue - histiotrophe - therein (Fig. 142). This cellular trophoblast, whose cells exhibit a brush border (in the ferret) and some inclusions, overlies a vascular, loose connective tissue foetal stroma. In

contact with the outside of the trophoblast is uterine glandular secretion, some cell debris, and leucocytes, and passing up, between the tips of the foetal villi, into the labyrinth, are maternal blood vessels, surrounded by transition stages from the stromal connective tissue cell of the spongy layer to the typical decidual giant cell (Fig. 142).

This figure also shows the dilated glands of the spongy zone (not so dilated in the ferret - Fig. 143). This zone is derived from the bases of the uterine glands which become dilated with uterine secretion during placental development, and whose columnar epithelium persists unchanged adjacent to the muscle layer, although it becomes degenerate as it is traced towards the labyrinth. In the ferret the cells lining the upper parts of the glands become hypertrophied, and the nuclei become very large (Fig. 143).

The structure of the haematoma region in the three placentae is basically the same, and consists of a mass of blood, or blood breakdown products trapped between a layer of maternal epithelium and a layer of columnar cellular trophoblast whose cells exhibit a brush border and numerous cytoplasmic inclusions of the same staining reaction as the enclosed material. In the cat, attachment

is seen between the chorion, and masses of densely staining symplasmic material (Fig. 144) lying beneath or between the maternal epithelial cells. Similar tissue is found at frequent intervals attached as rounded syncytial masses to the outer aspect of the trophoblast unattached to maternal tissues, and occasionally under the uterine epithelium unattached to the chorion. The significance of these will be discussed later.

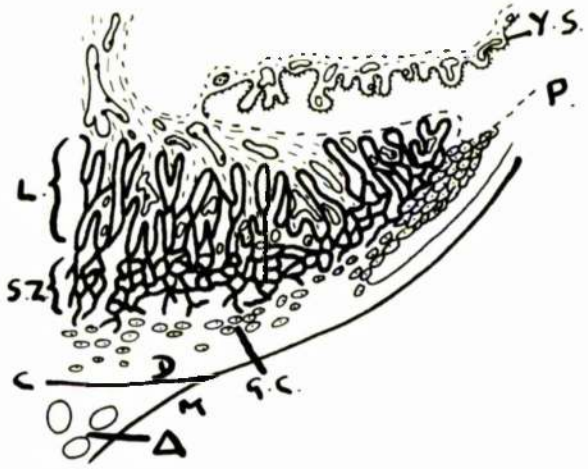
For a period of pregnancy in the carnivores prior to that examined in the cat and ferret, the yolk-sac forms a chorio-vitelline placenta, which, although later separated from the chorion by the exocoelom (Text fig. 5) remains functional until term, at least in the cat. In the dog, the yolk sac endoderm is cuboidal, and basophil, and rests on a very vascular mesoderm, the outer layer of which is flattened into a "serosa"; the ferret presents a similar picture except that the cells are more columnar, and their apical cytoplasm is vacuolated (Fig. 145 A). A similar appearance, but with less obvious vacuolation is seen in the cat yolk sac in mid-pregnancy, but by term (Fig. 145 B) the nuclei have become apical in the cells, and acidophil secretion containing some cell debris is visible in the lumen.

Haemochorial - rat, rabbit, guinea-pig, human (Figs. 146-157 Text figs. 6,7)

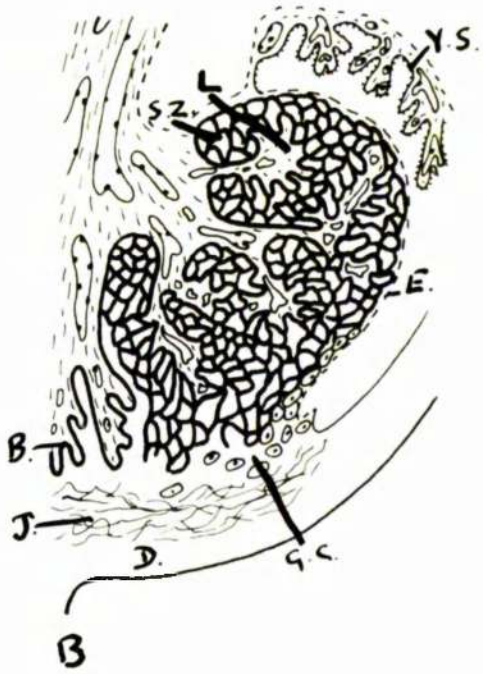
Of the haemochorial placentae examined, those of the rat, rabbit, and guinea-pig are labyrinthine, with a functional yolk-sac persisting

Text-fig. 6. A - diagram of the rat placenta (after Mossman, 1937) showing the relations of the labyrinth (L.), spongy zone (S.Z.), trophoblast giant cells (G.C.), and decidua basalis (D.). The mesometrial triangle with its blood vessels (△) is also shown between the inner circular (C.) and outer longitudinal (M.) muscle layers. Y.S. is the yolk sac endoderm, and P. the parietal layer which degenerates after 14½ days.

Text-fig. 7. B - a similar diagram of the guinea-pig placenta showing the same structures. The subplacenta (S.) and junctional zone (J.) are also shown, and the placental endoderm (E.).



A



B

until term, and may be considered separately from the human one.

In the placentae of the rat and guinea pig, (Fig. 146) the labyrinth (in which the foetal and maternal blood streams are separated by syncytial trophoblast, minimal discontinuous foetal mesenchyme and foetal endothelium) is related on its outer aspect (rat), or outer aspect and between the lobules (guinea-pig) to a spongy zone consisting of basophil cytotrophoblast (rat) or syncytiotrophoblast (guinea-pig), in which maternal blood circulates, and which is in turn related basally to a layer of giant cells. External to this is the decidua basalis (rat) with an intervening junctional zone of necrotic tissue (guinea-pig). The decidua basalis consists of vacuolated connective tissue cells (rat) or enlarged, non-vacuolated, rather basophil cells which accumulate around the mesometrial glands, and external to the junctional zone (guinea-pig). During the course of gestation in both species, the spongy zone, and the decidua basalis become progressively reduced in thickness, as the labyrinth enlarges. Cytotrophoblast is not seen, except for a few clumps of cells at 14½ and 17½ days in the rat, in the labyrinth at the stages of pregnancy examined. In the rat placental spongy zone, a second type of cell appears, maximal at 17½ days in this material, (Fig. 147) which appears vacuolated, with only a thin rim of cytoplasm, and frequently 2 nuclei. These tend to spill over through the inner circular muscle layer; into the mesometrial triangle.

By term they have almost disappeared from both sites. No similar cells were seen in the guinea-pig.

A functional yolk-sac placenta is present in both species until term. In the rat it consists of two layers - a parietal flattened layer of cells, related externally to a homogenous membrane, Reichert's membrane which separates it from the trophoblastic giant cells and degenerating decidua capsularis (Fig. 148). At 17½ days this decidua disappears, and Reichert's membrane ruptures, exposing the cavity of the yolk sac to that of the uterine lumen. The other layer is the visceral layer, composed of a non-villous area anti-mesometrially and a villous area mesometrially, the cells being columnar in shape, with a brush border and apical vacuolation, lying on a basement membrane, on the inner surface of which is a fine layer of vascular mesoderm. Towards term pockets of yolk sac, and parietal endoderm become invaginated into the surface of the chorio-allantoic placenta, forming "endodermal sinuses" (Fig. 149).

The visceral layer of the guinea-pig yolk sac is similar to that of the rat (the parietal layer never developing in this species) but the cells do not exhibit such a marked brush border, at least in the earlier part of pregnancy when they are exposed to the degenerating decidua capsularis. At this stage the apical surface of the cell projects above the level of the epithelial surface, giving the

appearance of "pegs", projecting into the yolk-sac cavity (Fig. 150). In the guinea-pig the attachment of the yolk-sac to the placenta is more extensive than in the rat, and extends as a pseudo-stratified columnar epithelium round onto the under surface of the placenta (Fig. 146 B). In this species again, when the decidua capsularis degenerates, the visceral layer of endoderm is exposed to the uterine lumen. No "endodermal sinuses" were observed in the guinea-pig.

Three final specialisations of placenta or related tissues should be mentioned in these species:-

(1) in the guinea-pig, in the centre of the base of the placenta, collections of oval masses of trophoblast occur. This appears mainly syncytial at the early stage of pregnancy examined, and is related internally to foetal mesenchyme, and externally to the junctional zone. These collections form the "sub placenta" (Fig. 151) which is largely necrotic by term.

(2) in the rat, binucleate cells, containing basophil granules appear from 10½ days onwards, mesometrial to the developing placenta, amd increase im number, particularly in the mesometrial triangle, where they accumulate around blood vessels. These "metrial gland cells" increase in number up to 18½ days of gestation (Fig. 152), after which they become less evident, until at term they have practically disappeared.

(3) also in the rat, trophoblastic cells appear to grow up the inner aspect of the lining of the maternal blood vessels supplying the developing placenta, and form a discontinuous lining to the vessels, the endo-vascular plasmodium (Fig. 152). This again practically disappears by term.

In the rabbit, the labyrinth is formed as a series of trophoblastic tubules, running from the foetal to the maternal surface of the placenta, which consists of two cotyledons placed one on either side of the mesometrial inter-cotyledonary groove. These tubules, which are closed at the foetal end, and open at the maternal end, are lined by trophoblast (chiefly syncytial in the stages examined here) which separates the foetal endothelium, and some mesenchyme, from the maternal blood (Fig. 153). As pregnancy advances these tubules become steadily more complex, and the trophoblast becomes much thinned out, but never disappears completely, a haemo-chorial condition always existing.

Deep to the ends of the tubules, masses of multinucleate decidual cells are found, and between them the degenerating (Fig.154) symplasma of the ends of the uterine glands, which disappears by 17 days. Later in development the deeper part of this intermediate region forms the separation zone, and considerable quantities of degenerating tissue, and lenococytes are found in it.

Deeper still in the placenta are found the regions of uninucleate, vacuolated decidual cells, surrounding maternal blood vessels with thickened endothelium, and transition stages between these and the multinucleate cells are seen in the deep part of the intermediate zone. A similar process of decidualization, but without multinucleate cell formation is seen in the remnants of the para-placental folds, whose epithelium, like that in the antimesometrial region regenerates over the period 13 to 14 days.

Between the placental cotyledons, and on either side thereof the trophoblast extends as a single layer of cells, from the outer aspect of which multinucleate cellular masses are budding off. The region on either side of the placenta is known as the trophoblastic fringe (Fig. 155).

In the earliest placental stage examined (13 days) the non-vascular parietal endoderm was still present as a degenerating flattened cellular membrane, related externally to the regenerating antimesometrial epithelium, deep to which the obplacental trophoblast giant cells are increasing in size and number (Fig. 156). Immediately thereafter, the parietal endoderm disappears, and the visceral layer becomes exposed to the uterine lumen. This visceral layer extends round the embryo from the trophoblastic fringe on either side, and is composed of a single layer of columnar, moderately basophil cells with a brush border, lying on a basement membrane and related internally to vascularised mesoderm (Fig. 155).

Unlike the labyrinthine placentae described above the human placenta is villous, and consists of:-

(a) anchoring villi, which extend from the chorionic plate to the maternal tissues, through the full thickness of the placenta. These contain a core of vascular foetal mesoderm, and are covered on the outside by a layer of cytotrophoblast, earlier in pregnancy, and an outer layer of syncytiotrophoblast which persists until term. Their tips show columns of cytotrophoblast (Fig. 157) which expand and cover the basal plate completely, separating the foetal tissue from the enlarged maternal decidual cells. Later in pregnancy these cell columns disappear.

(b) free, or absorbing villi, which hang freely in the maternal blood. Their structure is basically similar to that of the anchoring villi, but they do not have their cytotrophoblast cell columns. In them, also, as pregnancy advances, the cytotrophoblast to a large extent disappears.

Deep to the trophoblastic basal shell the ends of the uterine glands become filled with secretion earlier in pregnancy, but later disappear. Between them, the stromal cells enlarge, become polyhedral and basophil, and form the decidua.

In the non-placental chorion, five layers may be distinguished:

(1) the cuboidal, or flattened amnion.

(2) a layer of loose amniotic mesenchyme, more or less fused with

(3) a layer of chorionic mesenchyme

(4) a layer of trophoblast, irregular cells

(5) a layer of flattened, compressed, decidual cells.

Beneath these, remnants of the uterine glands are occasionally seen.

In the later placental specimen areas of fibrin are found between the villi.

At 1.5 cm some sections of yolk-sac were obtained. This consists of a layer of columnar endodermal cells, lying on a basement membrane, which separates them from a layer of vascular loose mesenchyme.

Glycogen

Epithelio-chorial placenta (Fig. 158)

Glycogen is found in the horse placenta in two sites only:-

(a) the allantois which is fairly heavily stained

(b) the allantoic blood vessel walls, where the muscle layer contains small quantities.

Syndesmo-chorial placenta (Fig. 159)

Little change was observed in the distribution and concentration of glycogen in the sheep placenta over the period examined, except in the diplokaryocytes, which exhibited some staining at 2.5 cm, but none thereafter.

Intense reactions for glycogen were found in

(1) the uterine secretion

(2) the allantoic endoderm

(3) certain of the cells of the trophoblastic arcades, where the reaction increased with age and varied between negative and 3+.

Less glycogen was observed around the blood vessels in the maternal stroma, less still in the allantoic blood vessels, and traces in the foetal stroma. All other tissues were negative.

Endothelio-chorial (Fig. 160-162)

The results for glycogen distribution in this placental type are summarised in Table 4.

Haemo-chorial (Figs. 163-165)

The distribution of glycogen in the placenta and accessory structures of the rat and guinea-pig is shown in Table 5, and of the rabbit in Table 6. In the human placenta at 7.5 cm glycogen was found in small concentrations in the foetal stroma, the decidua, and the maternal muscle layers.

Diastase-resistant PAS-positive material

All tissues showed a faint pink cellular staining, with slightly stronger basement membranes, and these reactions will not be described further. The results detailed below will be concerned with cellular inclusions, thick basement membranes where they are present, and any other features of interest.

TABLE 4

Glycogen distribution in the carnivore placenta

	days	Yolk sac		Allantoic		Foetal stroma	Trophoblast			Uterine secretion	Maternal epithelium		Musc
		endoderm	mesoderm	endoderm	blood vessels		placental	haematoma *	abembryonic		haematoma region	spongy zone	
DOG	24	Not seen	Not seen	-	-	-	-	-	-	Tr	-	-	+
	32	-	-	-	-	-	-	-	-	+	-	-	+
	45	Tr	-	-	+	-	-	+	-	+	-	-	Tr
	60	Not seen	Not seen	-	++	-	-	+	+	Tr	-	-	Not seen
CAT		6.5cm ++	+	+++	+	+	+ near cavity Tr base (syncytial layer	- +++ layer of connect. tissue beneath	-	-	++	++	-
		12.5cm ++ + secretion in cavity	Tr	+++	++	+	Tr	- + layer beneath	-	-	++	++	-
FERRET	22	+++	+	++++	++ + vessels in labyrinth	-	-	+++	-	Tr	-	-	-

*"haematoma" includes that of the ferret, the "green border" of the dog placenta, and the "brown border" of the cat placenta.

TABLE 5

Glycogen distribution in the placenta of the rat, and guinea pig

	Days	Decidua basalis	Metrial gland cells	Spongy zone trophoblast	Spongy zone vacuolated cells	Giant cells	Labyrinth	Allantoic blood vessels	Parietal endoderm	Visceral endoderm	Muscle
RAT	14½	+++		-	++	Tr	Faint tr	-	- ++ layer just external to Reichert's membrane	-	Tr
	18½	+	+	-	+++	Tr	++	+	Degenerate	++	Tr
	20½	+	Not seen	-	occasional +++ cell	-	++	++		+++	Tr
			Junctional zone						Placental endoderm		
GUINEA PIG	20	-	++	-		-	occasional ++ vessels in deeper placenta	-	+	+	+
	63	+ 3+ subplacental decidua	++	-		-	+ vessels as above	+	++ Some +++ degenerating cells outside visceral endoderm	++	+

TABLE 6

Glycogen distribution in the rabbit placenta

Days	Decidua	Multinucleate decidual cells	Parietal endoderm	Visceral Endoderm	Allantoic blood vessels	Labyrinth	Epithelium-antimesc-metrial	Giant cells	Muscle
13	++++	+++++	+	Tr	+	Tr deep	+	-	+
17	++++++	++++	Degener-ated	Tr	+	-	Tr	-	+
20	++++++	++++++	"	Tr	+	-	Tr	-	+
	Decidual remnants								
Term		++++	"	Tr	+	-	-	-	Tr

Epithelio-chorial placenta

This method stained the uterine secretion previously noted variably diffuse or intense pink, a marked brush border was seen on the trophoblast, and occasional dense pink inclusions occurred in the cytotrophoblast of the chorionic plate.

Syndesmo-chorial placenta

In the sheep placenta, an intense reaction is seen in the maternal stroma (Fig. 166) which increases around the small blood vessels near the trophoblastic arcades. The reaction of foetal stroma remains less throughout the period examined.

The diplokaryocytes lose staining in the cotyledon from quite intense reaction at 2.5 cm, but are not altered in the chorion outside it.

Also outside the cotyledon, an intensely reacting secretion occurs in the uterine lumen (Fig. 167) trapped between the chorion, and the uterine epithelium which exhibits a brush border on the surface, but not in the glands.

The remaining tissues show only a slight reaction, increasing slightly in the syncytiotrophoblast as pregnancy proceeds, and in the trophoblast of the arcades, in which some intra-cellular inclusions appear towards the later part of the period examined.

Endothelio-chorial

The most marked feature of the endothelio-chorial placenta was the presence, in all types examined, of a very intensely reacting membrane - the interstitial matrix, between the foetal and maternal tissues (Fig. 168). With the exception of the ferret, in which little difference was observed, the trophoblast outside this membrane generally reacted more strongly than the maternal endothelium inside. Similarly reacting tissue was found in the spongy zone, between the glands, and surrounding differentiating decidual cells in the cat.

Brush borders were observed in the uterine epithelium, and in the trophoblast of the brown border of the cat, whose cells also contained some positive inclusions. Similarly stained masses were observed in between the epithelium and the trophoblast in this site, and were mixed with intensely stained uterine secretion. Similar secretion occurred in all species examined, and cell inclusions occurred in the trophoblast cells of all in the haematoma or "border" region (Fig. 169).

In the yolk sac of the ferret and cat (Fig. 170) but not dog, positively stained secretion occurred, but similar material was observed in the yolk sac endoderm of the cat only (Fig. 170).

The symplasma masses occurring in relation to the paraplacental chorion of the cat stained faintly.

Haemo-chorial

In the rat intense reactions are observed in Reichert's membrane, external to the parietal endoderm; in the form of inclusions which decrease and disappear by 18½ days in the sub-placental giant cells; and in the uterine secretion at 17½ to 18½ days, when it comes to bathe the visceral endoderm, with its brush border, after rupture of the structures forming the decidua capsularis. Prior to this time the inverted yolk-sac contained trace secretion only, and few droplets were present in the visceral endodermal cells. After rupture, however, the number of droplets increases dramatically (Fig. 171 A). The other site in the rat in which reacting material is seen is in the metrial gland cells, whose granules are intensely positive (Fig. 171 B).

In the guinea-pig, the uterine secretion again gives the most intense reaction, particularly at term. A marked reaction is also seen in the junctional zone, and in the giant cells in the form of inclusions. The placental and visceral endoderms both exhibit a positive brush, with some inclusions.

The only sites of intense reaction in the rabbit are the tissues of the separation zone, the glandular symplasma, and the lamellae of what appears to be connective tissue which are laid down between the multinucleate decidual cells and the advancing

trophoblast later in the period examined (Fig. 172). Some reaction is also seen in the uterine luminal contents, both outside the parietal endoderm, and in the cavity of the yolk sac between it and the visceral layer. Brush borders are observed markedly in the visceral endoderm, whose cells accumulate positive inclusions up to term, and less well developed in the antimesometrial epithelium when it regenerates.

In the human placenta (Fig. 173) intense reactions are seen:-

(a) in the secretion of the uterine glands

(b) in the layer of fibrin laid down between the maternal tissue and the invading trophoblast, and

(c) in the areas of fibrinoid degeneration in the placenta.

A brush border is observed on the outer edge of the syncytiotrophoblast.

Acid mucopolysaccharides

Material staining intensely with the dialysed iron (DI) method, and metachromatically (?) with toluidine blue, was identified as acid mucopolysaccharide and is described below. Also described are sites of intense dialysed iron staining, although whether they represent acid mucopolysaccharide, or other substances, is questionable.

Epithelio-chorial placenta

In the horse placenta no γ metachromasia was observed.

Uterine secretion adherent to the exterior of the villi showed intense DI staining.

Syndesmo-chorial placenta

Marked γ metachromasia was observed in the foetal stroma of the sheep placenta at 15 cm which diminished thereafter. Slight staining was observed in diplokaryocytes at all stages, and in certain cells of the maternal uterine epithelium. These sites also showed considerable DI staining (Fig. 174) which was also found in the intercotyledonary chorionic brush border, and stroma, and in the uterine secretion. Later in development the reaction of the diplokaryocytes in the foetal villi decreases, those in the chorion being unaffected. Mast cells are present at all stages in the maternal muscle layers.

Endothelio-chorial placenta

The only site in which γ metachromasia was observed in this placental type was the uterine secretion in all species examined, that of the cat reacting most intensely, while the reaction of that of the dog and ferret was much less.

All uterine secretions reacted intensely with DI (Fig. 175).

Haemo-chorial placenta

Only the rat showed γ metachromasia, in the granules of the metrial gland cells early in pregnancy, the reaction decreasing later.

DI staining was seen in the rat metrial gland cell granules at all stages (Fig. 176) in the uterine secretion of the rabbit early in pregnancy, (Fig. 177) and in the maternal connective tissue between the decidual masses in the rabbit at the same stage. Human uterine secretion reacted intensely with dialysed iron, and gave a β metachromasia with toluidine blue.

The results of the further investigation of materials stained by dialysed iron, or metachromatically with toluidine blue are summarised in Tables 7 and 8.

RNA

Epithelio-chorial placenta

The horse placenta lacks large quantities of RNA, and traces only are found in the trophoblast and allantoic endoderm.

Syndesmo-chorial placenta

Early in the period examined, the cytotrophoblast and syncytiotrophoblast of the sheep placenta show moderate quantities of RNA (Fig. 178), although the

TABLE 7

Staining reactions of acid mucopolysaccharide materials

		Azur A pH 1.5	Azur A pH 4.5	Alcian Blue	Dialyzed iron	Aldehyde fuchsin	Toluidine blue pH 4.6	Tol blue after RNAse	Dialized iron after RNAse
SHEEP	15cm Foetal Stroma	-	+P	++	+++	++	+++R	+++R	+++
	Diplokaryocytes	-	-	Tr	++	++	+R	-	++
	Uterine secretion	-	-	-	+++	++++	-	-	+++
FERRET	Uterine secretion	-	-	+	++++	++++	-	-	++++
CAT	Uterine secretion	++B	++P	++	++++	++++	+++R	+++R	++++
DOG	Uterine secretion	++B mid-gestation	+P	++	++++	++++	+R mid-gestation	+R mid-gestation	++++
RAT	Metrial gland cell granules	-	++P	++	++++	++++	-	-	++++
HUMAN	Uterine secretion	-	-	+++	++++	+++	3+P	3+P	++++
RABBIT	Uterine secretion	-	-	-	++++	++++	-	-	++++

B-orthochromasia P-metachromasia (Azur A), β metachromasia (Tol.Blue), R - γ metachromasia (Tol.Blue)

TABLE 8

Effect of methylation on staining reactions of acid mucopolysaccharides

		Azur A pH 1.5	Azur A pH 4.5	Alcian Blue	Dialyzed iron
SHEEP	Foetal stroma	-	-	+	+++
	Diplokaryocytes	-	-	-	++
	Uterine secretion	-	-	-	+++
FERRET	Uterine secretion	-	-	+	+++
CAT	Uterine secretion	+B	TR P	++	+++
DOG	Uterine secretion	+B	TR P	++	+++
RAT	Metrial gland cell granules	-	-	Tr	+++
RABBIT	Uterine secretion	-	-	-	+++

diplokaryocytes are less positive. The syncytial staining decreases towards term, but the cytotrophoblastic staining persists.

Both maternal and foetal stromal cells show traces of RNA in their cytoplasm, and similar quantities are seen in the chorionic epithelium outside the cotyledon, in the maternal epithelium, and initially in the gland epithelium. Later in gestation the gland epithelium shows increased RNA, which decreases again just before term.

Endothelio-chorial placenta

In both cat and dog the trophoblast earlier in pregnancy shows greater quantities of RNA. In the cat the basal cytotrophoblast shows more staining than the syncytial layer (Fig. 179), while in the dog the reverse is the case, but both exhibit only trace amounts in the trophoblast of the "green" or "brown border". Towards term the level of staining for RNA in all trophoblastic areas decreases to trace, similar to that seen in the ferret syncytiotrophoblast, although here again the basal cytotrophoblast is more positive.

In all three species, small amounts of RNA are seen in the epithelium of the maternal glands of the spongy zone

and haematoma region, although the surface epithelium of the latter shows less staining.

Some RNA is seen in the endoderm of the yolk sac in all species examined.

The thickened endothelium of the ferret placenta shows marked RNA content (Fig. 180).

Haemo-chorial placenta

In both the rat and the guinea-pig the spongy zone trophoblast shows considerable RNA content (Figs. 181, 182), while, later in pregnancy at least, that of the labyrinth appears less positive. In the rabbit also, the marked staining for RNA in the earlier trophoblast seems to decrease as pregnancy advances. Whether this lesser degree of staining is due to decreased RNA content, or to thinning of the labyrinthine trophoblast as pregnancy advances is doubtful, but it seems likely that, in the rat at least, the second explanation is the case, as, at 14½ days, masses of trophoblast are found in the labyrinth exhibiting as intense a reaction for RNA as that in the spongy zone. In the rat and the rabbit the trophoblast giant cells contain RNA, in contrast to those of the guinea-pig.

All three species show RNA in the visceral yolk sac, the decidua and the uterine epithelium. In the rat, RNA

is quite copious in the endovascular plasmodium at 17½ and 18½ days; in the rabbit, in the layer of decidual cells immediately adjacent to the vessel wall; and in the guinea-pig, in the sub-placenta.

In the human placenta RNA is found in both layers of trophoblast, and the decidua, and less in the foetal stroma. Some staining is also seen in the yolk-sac endoderm.

Hydrolytic enzymes

Epithelio-chorial placenta (Fig. 183 A-C)

The horse placenta shows considerable activity with acid phosphatase, non-specific esterase (A, B and C type) and β-glucuronidase, in the trophoblast of the chorionic plate, and of the villi to a lesser degree, and in the allantoic endoderm. Non-specific esterase (B type only) is seen in the allantoic mesenchyme also.

Syndesmo-chorial placenta (Fig. 184)

Acid phosphatase

The staining reaction for this enzyme increases in the sheep cotyledon up to 15 cm and thereafter remains fairly constant until term, when some fall off is observed.

The reaction is intense in the trophoblast of the chorion on the surface of the cotyledon (Fig. 184 A), and

in intercotyledonary areas, but deep in the cotyledon cytotrophoblastic staining is less intense than that of the syncytiotrophoblast (Fig. 184 B) at all times.

The maternal stroma is markedly positive, in the sub-epithelial condensation of connective tissue, and in the cotyledon, particularly towards the base of the foetal villi, where rings of activity surround the blood vessels (Fig. 184 C). Much less activity is seen in the foetal stroma.

Degenerating uterine epithelium (Fig. 184 A) early in pregnancy, and intact epithelium thereafter, gives as moderate positive reaction, that of the glands being rather less.

No specific staining of diplokaryocyte was seen.

Non-specific esterase

The staining pattern for this enzyme was similar to that for acid phosphatase, except that peaks of activity in the cotyledon were observed at 15 cm and at term, the increase in activity being due to increase in trophoblastic staining.

Staining was observed in the cells in the substance of the connective tissue at the base of the cotyledon, (unstained with acid phosphatase) which increased to a

maximum at 15 cm and then decreased (Fig. 185).

Most of the esterase activity observed was of the B type, but some A type was seen in the chorionic trophoblast, the villous trophoblast, the cells of the cotyledonary connective tissue, and the maternal epithelium. C esterase was observed in the trophoblast of the chorion and villi only.

β-glucuronidase (Fig. 186)

This enzyme appears mainly in the maternal stroma both within the cotyledon, where its activity increases as the septae of maternal tissue are traced towards the bases of the foetal villi, and in the intercotyledonary area. The activity increases to a maximum at 25 cm. and then decreases gradually towards term.

Some activity is observed in the foetal stroma also, and, later in pregnancy in the trophoblast of the arcades, and of the extra-cotyledonary chorion.

Endothelio-chorial placenta

The distributions of acid phosphatase, non-specific esterase, and β-glucuronidase are detailed in Tables 9, 10, and 11, and illustrated in Figures 187 to 194.

Haemo-chorial placenta

The distributions of acid phosphatase, non-specific esterase, and β-glucuronidase in the rat, rabbit, and

TABLE 9

Distribution of acid phosphatase in the endothelio-chorial placenta

	Cat		Dog				Ferret
	7cm.	12.5cm.	24 Days	32 Days	45 Days	60 Days	20 Days
Trophoblast							
Placental Syn.	++++	++++			++	+	+++
Cyt.			++	+			
Basal Cyt.	++	++			++	++	++
Haematoma	++	++	++	++	+	+	++
Non Placental	++	++	++	+++	+++	+++	+++
Stroma							
Foetal	+	+	TR	TR	-	TR	TR
Maternal	++	++	+	+	+	+	TR
Maternal Epithelium							
Spongy Zone	+	+	+++ Deep +Superficial	+++ Deep +Superficial	+++ Deep +Superficial	++ Superficial (Deep layer missing	++ Deep +superficial
Haematoma	+	+	++	++	++	Absent	++
Non Placental	+++ Glands	+ Surface	++	++	++	Absent	++

TABLE 9 (Continued)

Distribution of acid phosphatase in the endothelio-chorial placenta

	Cat		Dog				Ferret
	7cm.	12.5cm.	24 Days	32 Days	45 Days	60 Days	20 Days
Decidua	TR	TR	-	+	+	Absent	
Histiotrophe	+++	+++					++
Yolk Sac							
Endoderm	+	+	TR	TR	-	Degenerated	+
Mesoderm	TR	TR	-	-	-	Degenerated	TR
Maternal Endothelium							++
Symplasma In Brown Border	+++	+++					

TABLE 10

Distribution of non-specific esterase in the endothelio-chorial placenta

	Cat		Dog				Ferret
	7cm.	12.5cm.	24 Days	32 Days	45 Days	60 Days	20 Days
Trophoblast							
Placental Syn.	++ (TR)	++			+++* (TR)	+++* (TR)	+++
Cyt.			+++ (TR)	+++			
Basal Cyt.	+	+			+++		++
Haematoma	++++* (+++)	++++* (++++)	++	++	++*	+++*	+++
Non Placental	++++ (+)	++++ (+)	++	++	++	++	+
Stroma							
Foetal	TR	TR	TR	TR	+	+	TR
Maternal	-	-	+	-	TR	TR	-
Maternal Epithelium							
Spongy Zone	++	++	+++	++	++*	+++*	++
Haematoma	++ (TR)	++ (+)	+++	++	++* (TR)	+++* (TR)	++
Non Placental	+++ (+)	+++ (+)	++	++	++	Absent	++

TABLE 10 (Continued)

Distribution of non-specific esterase in the endothelio-chorial placenta

	Cat		Dog				Ferret
	7cm.	12.5cm.	24 Days	32 Days	45 Days	60 Days	20 Days
Decidua	+	+	-	-	TR	Absent	
Histiotrophe	+++* (++++)	+++*					++*
Yolk Sac							
Endoderm	+++ (+)	+++	TR	+	+	Degenerate	+
Mesoderm	+	++	-	-	TR	Degenerate	TR
Maternal Endothelium							++
Symplasma In Brown Border	++	++					

Results indicate activity seen with Naphthol-AS-acetate. Significant amounts ofCesterase were found in sites marked withan asterisk.

Results in brackets thus (++) indicate indoxyl acetate esterase.

TABLE II

Distribution of β-Glucuronidase in the Endothelio-chorial placenta

	Cat		Dog				Ferret
	7cm.	12.5cm.	24 Days	32 Days	45 Days	60 Days	20 Days
Trophoblast							
Placental Syn.	-	-			+	++	-
Cyt.			-	-			
Basal Cyt.	-	-			TR	+++	-
Haematoma	-	-	-	-	-	+	-
Non Placental	-	-	-	-	-	+++	-
Stroma							
Foetal	-	-	-	-	-	-	-
Maternal	+	+ (In trophoblast tubules)	-	+	TR		+ Basal
Maternal Epithelium							
Spongy Zone	-	-	TR	TR	+	Absent	-
Haematoma	-	-	+ (In uterine glands)	+ (In uterine glands)	+ (In uterine glands)	Absent	-

TABLE 11 (Continued)

Distribution of β-glucuronidase in the endothelio-chorial placenta

	Cat		Dog				Ferret
	7cm.	12.5cm	24 Days	32 Days	45 Days	60 Days	20 Days
Non Placental	-	-	+ (In uterine glands)	+ (In uterine glands)	+ (In uterine glands)	Absent	-
Decidua	-	-	-	-	-		
Histiotrophe	+++	+++					+++
Yolk Sac							
Endoderm	+	++	-	-	-	Degenerated	-
Mesoderm	-	+	-	-	-	Degenerated	-
Maternal Endothelium							-
Symplasma in Brown Border	-	-					

guinea-pig are shown in Tables 12, 13, 14 and illustrated in Figures 195 - 198.

In the human placenta, (Fig. 199) acid phosphatase and non-specific esterase occurred in both syncytial and cellular layers of the trophoblast although more particularly in syncytiotrophoblast, the activity decreasing slightly towards term. Activity was seen to a much lesser degree in the trophoblast of the decidua capsularis, however. Some staining was also observed in the decidual cells, in cells in the foetal stroma, and in the lining of the uterine glands, in which activity decreased towards term.

Traces of β-glucuronidase activity were observed in the syncytiotrophoblast of the human placenta throughout gestation.

Yolk sac "placenta"

Yolk sac "placenta" acid phosphatase, and non-specific esterase (Fig. 200 A) activities were seen in the endoderm of the yolk sac, and increased with increasing egg incubation times. No β-glucuronidase activity was seen.

In the yolk sac endoderm of Limia Maculata acid phosphatase, non-specific esterase (Fig. 200 B) and traces

TABLE 12

Distribution of acid phosphatase in the placentae and related structures in the rat, guinea pig and rabbit.

	Rat				Guinea Pig		Rabbit			
Days	14½	17½	18½	20½	20	60	13	17	20	Term
Labyrinth										
Trophoblast	+	TR	+	TR	TR	-	++++	+++	++	++
Spongy Zone										
Trophoblast	+	+	+	++	++	+				
Vacuolated Cells	-	-	TR	-						
Giant Cells	++	+++	+++	++	++	+				
Antimesometrical Decidua							+++	++++	TR	Absent
Basalis	+++	TR	TR	TR	+++	+++	- +cells lining blood vessels	- +cells lining blood vessels	- +cells lining blood vessels	- +cell lini bloo vess
Endoderm										
Visceral	+++	++++	++++	+++	+	++	++	++	+	+++
Parietal	+						TR			
Placental					+	+				
Metrial Gland Cells	++	++	++++	++++						
Muscle Stroma					+	++++				

TABLE 12 (Continued)

Distribution of acid phosphatase in the placentae and related structures in the rat, guinea pig and rabbit.

Days:	Rat				Guinea Pig		Rabbit			
	14½	17½	18½	20½	20	63	13	17	20	Term
Multinucleate Bodies								+++	+++	+++
Antimesometrial Epithelium	++	++	++	+	++	+++	+++	+++	+++	+++
Yolk Sac Contents							- (++++ masses outside P.E.)	++	+	-
Fibrinoid Capsule	+++	+++	++++	+++						
Decidua Capsularis					+					
Multinucleate Decidual Cells							+++	++	+	Absent
Endoderm Sinuses				++						
Junctional Zone					+++ masses	+++ masses				
Gland Symplasma							-	-		

TABLE 12 (Continued)

Distribution of acid phosphatase in the placentae and related structures in the rat, guinea pig and rabbit.

	Rat				Guinea Pig		Rabbit			
Days:	14½	17½	18½	20½	20	63	13	17	20	Term
Separation Zone									++	++
Endovascular Plasmodium	-	-	-	-						
Subplacenta					+	Degenerated				
Trophoblastic Fringe							++	+++	++	Absent

TABLE 13

Distribution of non-specific esterase in the placentae and related structures in the rat, guinea pig and rabbit.

	Rat				Guinea Pig		Rabbit			
Days:	14½	17½	18½	20½	20	63	13	17	20	Term
Labyrinth	+	+	++	+	+	+	+	+++	+++	+++
Spongy Zone	TR	++	++*	++*	+	+				
Trophoblast										
Vacuolated Cells	-	-	-	-						
Giant cells	TR +abembryonic	++*	++*	++*	TR	TR				
Antimesometrial	++						++	++	++	Absent
Decidua Basalis	+	++*	+	-	+	+	++ (TR)	++ (±)	+	+
Endoderm Visceral	+ (++++)	++* (++++)	+++* (++++)	+++ (++)	+ (TR)	++ (TR)	+ (TR)	++	+++	+++ (+++)
Paristal	+						TR			
Placental					+ (TR)	+ (TR)				
Metrial Gland Cells	+	++	++*	+++*						
Muscle Stroma					+	++ (++)				

TABLE 13 (Continued)

Distribution of non-specific esterase in the placentae and related structure in the rat, guinea pig and rabbit.

	Rat				Guinea Pig		Rabbit			
Days	14½	17½	18½	20½	20	63	13	17	20	Term
Multinucleate Bodies								++	++	++
Antimesometrial Epithelium	+	+	+	TR	++	++	++	++	++	+
Yolk sac Contents							-	-	-	-
Fibrinoid Capsule	++	++	++	+						
Decidua Capsularis	+				+					
Multinucleate Decidual Cells							++	+	+	Absent
Endoderm Sinuses				++ (TR)						
Junctional Zone					++ masses (+++)	++ masses (+++)				
Gland Symplasma							++++	+++		

TABLE 13 (Continued)

Distribution of non-specific esterase in the placentae and related structure in the rat, guinea pig and rabbit.

Days:	Rat 14½	Rat 17½	Rat 18½	Rat 20½	Guinea Pig 20	Guinea Pig Term	Rabbit 13	Rabbit 17	Rabbit 20	Rabbit Term
Separation Zone									++	++ (++)
Endovascular Plasmodium	+	+	+	Absent	-	Degenerated				
Supplacenta										
Trophoblastic Fringe							++	+++		Absent

Results given are those seen with α-napthyl acetate, or Napthol-AS-acetate as substrates.

Results in brackets thus (++) refer to indoxyl acetate as substrate.

Results marked with an asterisk indicate sites of C-esterase.

TABLE 14

Distribution of β-glucuronidase in the placentae and related structures in the rat, guinea pig and rabbit

	Rat				Guinea Pig		Rabbit			
Days:	14½	17½	18½	20½	20	63	13	17	20	Term
Labyrinth Trophoblast	-	TR	++	++ increases towards base	TR	-	-	-	-	+
Spongy Zone Trophoblast	TR	-	-	-	-	-	-			
Vacuolated Cells	-	+	TR	-						
Giant Cells	- +antimesometrial	-	-	-	-	-				
Antimesometrial							-	-	-	Absent
Decidua Basalis	++	++	+	-	++	+++	-	- +cells lining blood vessels	- +cells lining blood vessels	- +cells lining blood vessels
Endoderm Visceral	-	+	+ ++ degenerating	+	++	++++	-	+	++	++
Parietal	-	++ degenerating								

TABLE 14 (Continued)

Distribution of β-glucuronidase in the placentae and related structures in the rat, guinea pig and rabbit

Days:	Rat 14½	Rat 17½	Rat 18½	Rat 20½	Guinea Pig 20	Guinea Pig 63	Rabbit 13	Rabbit 17	Rabbit 20	Rabbit Term
Placental					+	++++				
Metrial Gland Cells	+++	+++	+++	+++						
Muscle Stroma					+	++++				
Multinucleate Bodies								-	-	-
Antimesometrial Epithelium	+	+	+	+	+++	+++	-	-	-	-
Yolk Sac Contents										
Fibrinoid Capsule	++	+++	+++	+++						
Decidua Capsularis					++					
Multinucleate Decidual Cells							-	-	-	Absent
Endoderm Sinuses				TR						
Junctional Zone					++	+++ Masses				

TABLE 14 (Continued)

Distribution of β-glucuronidase in the placentae and related structures in the rat, guinea pig and rabbit

Days:	Rat				Guinea Pig		Rabbit			
	14½	17½	18½	20½	20	63	13	17	20	Term
Gland Symplasma							+ occasional + cell near	+ occasional + cell near		
Separation Zone									-	-
Endovascular Plasmodium	-	-	-	-						
Subplacenta					-	Degenerated				
Trophoblastic Fringe							-	-		Absent

of β-glucuronidase activities were observed.

The human yolk sac endoderm exhibits quite marked activity with acid phosphatase, (Fig. 200 C) and traces with Naphthol-AS-acetate esterase. Traces of activity were observed with both enzymes in the mesoderm.

Alkaline phosphatases

In this section and hereafter the following contractions will be used for the enzymes studied:-

non-specific alkaline phosphatase (azo-dye method at pH 9)	-	N.S.
adenosine monophosphatase	-	AMPase
adenosine triphosphatase	-	ATPase
inosine triphosphatase	-	ITPase
thiamine pyrophosphatase	-	TPPase
uridine diphosphatase	-	UDPase
glucose-6-phosphatase	-	G-6-Pase
fructose-6-phosphatase	-	F-6-Pase
fructose - 1:6-diphosphatase	-	F-1:6-Pase

Epithelio-chorial placenta (Fig. 201)

In the horse N.S. is found in the trophoblast cells of the chorionic plate, in patchy distribution.

Intense ATPase, ITPase, and TPPase activity is found in a similar distribution, and UDPase to a much lesser degree. AMPase is seen in the brush border, both here and in that of the trophoblast of the villi, where brush border activity occurs with ATPase, ITPase and UDPase also.

All of the enzymes except N.S., are stained fairly intensely in the large blood vessels of the chorionic plate.

Syndesmo-chorial placenta (Figs. 202 - 206)

N.S. is seen in the sheep foetal placenta in the syncytiotrophoblast, where it increases to a maximum at 25 cm and then falls off, and in the cytotrophoblast where a similar pattern of staining is seen in the diplokaryocytes, with some background trophoblast staining which disappears by 40 cm. In the maternal placenta, activity occurs in the maternal stroma, particularly in a condensed band just exterior to the syncytiotrophoblast, and in the uterine surface epithelium, and here again activity is lost by 40 cm. No less occurs in the intense activity of the uterine glands, however, and it persists throughout.

Specific phosphatase activity is seen in the foetal placenta (syncytio-, and cytotrophoblast) with AMPase, and UDPase which accumulate throughout gestation, with ATPase

and ITPase, which remain steady, and with TPPase which decreases in activity. Activity with all enzymes accumulates in the diplokaryocytes till 15 to 25 cm and thereafter remains steady. As with N.S. no enzyme activity is seen in the diplokaryocytes in the extra-cotyledonary chorion. The latter exhibits brush border activity with all specific phosphatases towards the later part of gestation, and activity is also seen in the intra-epithelial capillaries found in the chorionic trophoblast with all except UDPase. Brush border activity was also seen in the trophoblast of the arcades, related to the haematoma formed in later pregnancy.

In the maternal placenta, activity accumulated in the stroma, particularly that lying beneath the syncytio-trophoblast, to a marked degree throughout pregnancy. The heaviest staining was seen with AMPase, then UDPase, ITPase, TPPase, and ATPase in that order of decreasing intensity. Specific phosphatase activity with all substrates was observed in the maternal surface and glandular epithelium also, greatest early in gestation and then decreasing markedly. Between the cotyledons, intra-epithelial capillaries were again observed with all substrates except UDP. Uterine glandular epithelium

showed Golgi staining with TPPase and UDPase.

Endothelio-chorial placenta (Figs. 207 - 211)

The distribution of N.S. in this placental type is detailed in Table 15.

Specific phosphatases showed considerable differences from N.S. in their distribution, greatest in the cat, less in the ferret, and least in the dog.

In the cat, the trophoblast of the placental lamellae showed + staining with ATPase, ITPase, and TPPase, ++ with UDPase, and +++ with AMPase, the increase being paralleled by a decrease in staining of the interstitial matrix. Basal cytotrophoblastic staining was seen with all substrates. A brush border was stained on the maternal epithelium in the brown border, and in the non-placental region, with all substrates, and similar staining of the epithelium of the spongy zone glands was seen with AMPase. This enzyme also stained the trophoblast and symplasma masses of the brown border, and (with ATPase and ITPase) the maternal stroma of the junctional zone, although not that of the lamellae. In the yolk-sac ATPase and ITPase stained a brush border on the endodermal cells at term. The mesoderm was stained with AMPase, and its blood vessels with ATPase, ITPase, TPPase and

TABLE 15

Distribution of non-specific alkaline phosphatase in the endotheliochorial placenta

		Cat		Dog				Ferret
		7cm.	12.5cm.	24 Days	32 Days	45 Days	60 Days	20 Days
Trophoblast								
Placental	Syn.	+	+			++	+++	+
	Cyt.			+	++			
Basal	Cyt.	-	-		-	-	-	-
Haematoma		TR	TR	+	-	-	-	-
Non Placental		TR	TR	TR	-	TR	-	-
Stroma								
Foetal		-	-	TR	-	TR	-	-
Maternal		++++ (Interstitial matrix)	++++ (Interstitial matrix).	++	+	TR	-	+ (Interstitial matrix).
Maternal Epithelium								
Spongy Zone		+	+	+++ deep TR sup	+++ deep TR sup.	+++	-	++ deep + sup.
Haematoma		++++ deep TR sup.	++++ deep TR sup.	++++	++	+	Absent	+

TABLE 15 (Continued)

Distribution of non-specific alkaline phosphatase in the endotheliochorial placenta

	Cat		Dog				Ferret
	7cm.	12.5cm.	24 Days	32 Days	45 Days	60 Days	20 Days
Non Placental	TR	TR	++++	+	TR	Absent	TR
Decidua	-	-	++	++	++	Absent	
Histiotrophe	-	-					+
Yolk Sac							
Endoderm	-	-	-	-	+	Degenerate	TR
Mesoderm	-	-	-	-	-	Degenerate	-
Blood Vessels							
Foetal	TR	TR	-	-	-	-	-
Maternal	++	++	++	++	+	-	++++ in spongy zone
Symplasma in Brown Border	++	++					
Maternal Endothelium							-

UDPase. In general staining was greater with ITPase than ATPase, but the pattern was similar.

The main differences seen in the ferret placenta occurred in the labyrinth, where the maternal endothelium was heavily stained with AMPase, less with UDPase, less still with ITPase and ATPase both of which demonstrated a brush border facing the vessel lumen, and not at all with TPPase. The interstitial matrix was stained by ITPase, ATPase, UDPase, AMPase, and TPPase in that order of decreasing intensity. Staining of the cytotrophoblast of the haematoma zone was seen with ITPase and ATPase, and UDPase and TPPase which demonstrated a brush border thereon. The basal cytotrophoblast of the labyrinth showed a marked brush border with AMPase, and was slightly stained with the remainder (except TDPase). Staining of the spongy zone glands, greater nearer the labyrinth than at their bases, was seen with AMPase, and UDPase.

In the dog, the most marked difference was in the staining of the maternal blood vessels, which was very intense immediately beneath the invading trophoblast with ITPase and ATPase, and less with UDPase. Increase

in staing was noted later in pregnancy with the same enzymes in the maternal blood vessels of the labyrinth, but decreased towards term. The basal parts of the spongy zone glands showed increased staining in the earlier specimens with all enzymes. Later specimens showed staining of the yolk-sac mesoderm.

Haemo-chorial placenta (Figs. 212 - 220)

In the placentae of the rat, rabbit and guinea-pig the distribution of N.S. is as detailed in Table 16.

Marked differences are seen when these results are compared with those utilizing specific substrates.

The rat placental labyrinth shows more activity than with N.S. later in pregnancy with AMPase, and UDPase. ATPase and ITPase, although increasing similarly, never attain the level of staining with N.S. TPPase shows little activity. Staining is seen with all substrates in the trophoblast of the spongy zone, the relative intensities being similar to those in the labyrinth, and again increasing to term. Marked activity was seen with ITPase, ATPase, AMPase, UDPase, and TPPase in that descending order in the antimesometrial, and subplacental giant cells. In the visceral endoderm a brush border was stained, activity increasing to 18½ days and then

TABLE 16

Distribution of non-specific alkaline phosphatase in the placenta of the rat, rabbit and guinea pig

	Rat				Guinea Pig		Rabbit			
Days:	14½	17½	18½	20½	20	63	13	17	20	Term
Labyrinth Trophoblast	++	++++	+++	+++	+++	+++	++++	++++	++++	+++
Spongy Zone Trophoblast	-	-	-	-	-	-				
Vacuolated Cells		-	-	-						
Giant Cells	++	++	+++	++++	-	-				
Antimesometrial							-	-	-	-
Decidua Basalis	-	-	-	-	TR	+++	- (+cells lining vessels)	- (+cells lining vessels)	-	-
Endoderm Visceral	+	+++	++	-	++	TR	TR	-	-	-
Parietal	-						-			
Placental					-	-				
Metrial Gland Cells	-	-	-	-						
Muscle Stroma					-	-				
Multinucleate Bodies								-	-	-

TABLE 16 (Continued)

Distribution of non-specific alkaline phosphatase in the placenta of the rat, rabbit and guinea pig

Days:	Rat 14½	17½	18½	20½	Guinea Pig 20	65	Rabbit 13	17	20	Term
Antimesometrial Epithelium	-	-	-	+	-	++++	-	-	-	-
Yolk Sac Contents	-	-	-	-	-	-	-	+ masses	+ masses	-
Fibrinoid Capsule	++	-	-	-						
Decidua Capsularis					-					
Multinucleate Decidual Cells							-	-		Absent
Endoderm Sinuses			+	-						
Junctional Zone					-	-				
Gland Symplasma							-	-		
Separation Zone									-	+
Endovascular Plasmodium			++	+						
Subplacenta					-	Degenerated				—
Trophoblast Fringe							TR	-		Absent

decreasing with ITPase, ATPase, UDPase, AMPase, and TPPase. AMPase, and less intensely UDPase were stained in the decidua basalis. The metrial gland cells showed marked activity with ATPase and ITPase, and some staining with the remaining substrates.

Less alteration in enzyme activity was seen in the rabbit placenta, the most striking feature being a diminution in all specific phosphatases, compared with N.S. in the labyrinth. Activity was seen in degenerating glandular symplasma, and later in the junctional zone, with ATPase and ITPase. In the decidua, staining occurred markedly in the lining of the blood vessels with all specific substrates, and decreased gradually towards term. The decidual cells showed ITPase and ATPase activity, and increase in these enzymes was observed in the cells developing into multinucleate decidual cells. All specific phosphatases examined except AMPase appeared in the brush border of the visceral endoderm at 17 days and increased thereafter to maximal activity at term. Antimesometrially a brush border was observed on the uterine epithelium (surface and glandular) with all specific substrates, staining increasing towards term, and being most intense

with ITPase, ATPase, less with TPPase, and UDPase, and less still, initially, with AMPase, although its activity also increases to a maximum at term. Similar staining was observed in the material accumulated in the yolk sac cavity. The obplacental giant cells show intense activity with all substrates except TPPase.

In the guinea-pig, the relative distribution of AMPase between the labyrinth and spongy zone trophoblast is fairly similar to that seen with N.S., although some AMPase activity occurs in the spongy zone. With the remaining substrates, however, the relative distribution is reversed, and the activity is greater in the spongy zone, and less in the labyrinth, with ITPase, ATPase, UDPase, and TPPase in that descending order. The giant cells show activity with AMPase only. A brush border was stained on the visceral endoderm, except with AMPase, but not on the layer applied to the placental surface. Phosphatase activity occurred with all substrates in the junctional zone, in the decidua basalis, and in the brush border of the antimesometrial epithelium.

In the human placenta, N.S. was seen in the syncytiotrophoblast, its activity being minimal early in

pregnancy, and increasing thereafter, but becoming patchy in distribution. Specific phosphatases showed more activity than N.S. early in pregnancy, with AMPase; UDPase; and ATPase, ITPase, and TPPase in that descending order, in the syncytiotrophoblast, particularly the brush border. AMPase and UDPase were observed in the cytotrophoblast also. In the chorion laeve AMPase and ITPase activities were seen in the amnion. The trophoblastic reactions were similar to those of the placental trophoblast. Traces of activity were seen in the decidual cells with all substrates. The epithelium lining the uterine glands showed AMPase, UDPase, and TPPase (the last named in the Golgi zone) in the earlier part of pregnancy.

Yolk-sac placenta

Traces of N.S. activity were seen in the yolk-sac endoderm of the chick and limia maculata.

Apart from some ITPase activity at 10 days of incubation no specific phosphatases were observed.

In the human yolk sac no N.S. activity was observed. Specific phosphatases were moderately active in the

endoderm, and faintly reactive in the mesoderm. The blood vessels were positive with AMPase, ATPase, ITPase, and UDPase.

Glucose-6-phosphatase, fructose-6-phosphatase, fructose-1:6-diphosphatase

No evidence for specific enzymes hydrolyzing any of the above substrates in any placental type was obtained. The staining in each case was identical with that apparent with β-glycerophosphate as substrate over a wide range of pH.

Dehydrogenases - carbohydrate

In this study the carbohydrate dehydrogenases examined were found to fall into 3 groups within which the distribution of enzymes was similar (with occasional exceptions which will be noted) and a fourth group containing 4 enzymes, whose distribution was very variable. The enzymes included in the groups were as follows:-

Group 1 α-glycerophosphate dehydrogenase (αGP) and β-hydroxy-butyrate dehydrogenase (βOH).

Group 2 lactate (LDH), isocitrate (IDH), succinate (SDH) and malate (MDH) dehydrogenases.

Group 3 Glucose-6-phosphate (G-6P) and 6-phospho-gluconate (6-PG) dehydrogenases.

Group 4 alcohol (ADH), furfuryl alcohol (FDH), glutamate (GDH) and sorbitol (sorb DH) dehydrogenases.

Epithelio-chorial placenta (Fig. 210 A-6)

In the horse placenta the activity of the enzymes of group 1 was low, and they appeared in the trophoblast, more particularly that of the chorionic plate, in the

foetal blood vessels, and in the allantoic endoderm, where more activity was seen with ∝ GP.

Group 2 enzymes were found in the same sites with greater activity than group 1, and here again the staining was most intense in the allantoic endoderm.

This situation was reversed in group 3, where, although the enzyme localisations were similar, the staining in the trophoblast was most intense.

In group 4, ADH and FDH were found in the trophoblast, and GDH also, staining for this enzyme being quite intense in the allantoic endoderm. Only traces of Sorb DH occurred in the trophoblast and the allantoic endoderm.

Diaphorase activity with NADH and NADPH was seen, in trophoblast more intense with NADPH; in the allantoic endoderm more intense with NADH; and in the foetal blood vessels with NADH only.

Syndesmo-chorial placenta (Fig. 222 - 225)

In the sheep placenta Group 1 enzymes occur in + quantities in the cytotrophoblast and extra-cotyledonary chorionic trophoblast earlier in pregnancy, but were not seen after 15 cm. ∝GP occurs in similar amounts in the glands epithelium of the uterus throughout gestation.

Intense activity of Group 2 enzymes is found in the cytotrophoblast of the foetal villi and in the chorionic trophoblast throughout gestation, and increases in the trophoblast cells of the arcades later in pregnancy. Between 2.5 cm and 15 cm enzyme activity increases in the epithelium of the uterine glands and surface epithelium, in the maternal stroma, and in the chorionic and allantoic mesenchymes. Thereafter staining remains constant in these sites until term. The allantoic endoderm shows ++activity throughout the period of pregnancy examined. Gradual increase in syncytiotrophoblastic activity occurs.

Group 3 enzymes occur chiefly in the cytotrophoblast, reaching a peak of activity between 15 and 25 cm and thereafter decreasing slightly. The syncytiotrophoblast never shows more than trace activity (with G-6-P), and similar levels of staining are seen in the foetal stroma and allantois. In the maternal tissues, the surface and glandular epithelia show increasing staining from 2.5 cm to term, the stroma shows trace activity, and the maternal capillaries,+++.

In Group 4 the activity of ADH increases in the chorionic trophoblast, and in the maternal epithelium

during gestation, and remains at trace levels in the cytotrophoblast. FDH increases in the same sites, and in the cytotrophoblast up to 25 cm and then decreases towards term, when trace activity appears in the syncytiotrophoblast. GDH increases in the cytotrophoblast during gestation, but decreases in all other tissues. Sorb DH increases in the chorionic trophoblast up to 15 cm and thereafter remains constant, but decreases in the cytotrophoblast from + levels. All other tissues show no activity with this group of enzymes.

At no stage of pregnancy was any histochemical specialization of the diplokaryocytes with these enzymes observed, staining therein being similar to that of the trophoblast in which they were present.

Endothelio-chorial placenta

Group 1 (Fig. 226 - 228)

Dog

Traces of Group 1 enzymes are found in the trophoblast throughout gestation. In the green border more activity is observed with βOH than with αGP, but the reverse is the case in the non-placental chorion. The yolk-sac endoderm shows βOH activity just prior to

separation of the chorio-vitelline placenta from the chorio-allantoic placenta by the exocoelom, but no activity is seen in the yolk-sac. In the maternal tissues, activity is seen with both enzymes, but particularly ∝GP, in the spongy zone glands, more in the dilated portion than in the basal contracted area, and in the glands related to the non-placental chorion.

Cat

Both enzymes of Group 1 occur in the trophoblast of the placental lamellae, and of the brown border but not in the non-placental chorion, the activity in the labyrinth being greater with βOH in the deeper parts. Traces of activity are seen in all areas of maternal epithelium, and in the maternal decidual giant cells, and the areas of histiotrophe show considerable staining. The yolk sac endoderm shows trace staining with both enzymes. All other areas are negative.

Ferret

The majority of the staining in the ferret placenta occurs with ∝GP in the maternal epithelium, particularly in the uterine glands where activity is quite intense. Traces are seen with βOH in the surface epithelium and histiotrophe, and with ∝GP in the trophoblast of the basal region and haematoma.

Group 2 (Figs. 229 - 233)

Dog

In the trophoblast intense activity is seen with LDH and MDH (less with SDH and IDH) in the trophoblast of the labyrinth, and slightly less in that of the green border and non-placental chorion, throughout gestation. The endoderm of the chorio-vitelline placenta shows strong LDH, less MDH, and trace IDH activity both before and after separation of the yolk-sac from the chorio-allantoic placenta, and a similar pattern of staining is seen in the allantois throughout the period examined. In the maternal tissues activity is seen with all enzymes in the glands of the spongy zone, more intense in the dilated portion than the contracted portion, this difference becoming very marked later in pregnancy. The non-placental epithelium, both surface and glandular, and that of the green border glands show marked LDH and MDH, and less SDH and IDH activities at all stages examined. The area of decidualisation in the maternal stroma shows increase of all enzymes of this group from trace to +activity.

Cat

In the cat trophoblast intense activity with all enzymes of this group, except SDH whose activity was consistently less, is seen in all areas of non-placental and brown border trophoblast, and in the basal cyto-trophoblast and deeper parts of the placental lamellae, the more superficial parts of which show less activity. The maternal epithelium shows considerable enzyme activity in all areas, staining in the brown border being more marked in the deeper parts at term. Under the brown border epithelium the stroma shows trace to + activity, but patchy areas of more intense staining, related to the tips of chorionic villi, and possibly lying in the symplasma regions, are seen. Transformation of maternal stroma into decidual giant cells, between the deepest parts of the placental lamellae, is associated with increase in enzyme activity in the stroma from trace or + to ++++, and the decidual giant cells themselves show +++ activity with IDH and LDH, ++ with MDH, group was seen in the histiotrophe. LDH and MDH show

group was seen in the histiotrophe. LDH and MDH show

intense activity in the yolk-sac endoderm (less with IDH and SDH), and the same enzymes, in much less concentration are seen in the mesoderm.

Ferret

LDH and MDH (++++) and IDH and SDH (+) activity are seen in the placental trophoblast, the basal cytotrophoblast, and that of the haematoma region. The maternal epithelium shows marked activity in all areas with LDH and MDH, some activity with SDH (particularly in the haematoma regions) and IDH activity in the spongy zone and haematoma regions. The thickened maternal endothelium shows++ activity with LDH and MDH, and intense activity with both enzymes (trace with SDH) is found in the yolk-sac endoderm. Traces of activity with LDH and MDH are seen in the yolk sac mesoderm.

Group 3 (Figs. 234 - 236)

Dog

Moderate amounts of Group 3 enzymes are observed in the labyrinthine trophoblast throughout pregnancy. That of the green border, and non-placental areas, initially shows quite intense activity, which is lost in mid-pregnancy, but re-accumulates near term. Some staining

occurs in the allantoic endoderm throughout. All other foetal tissues are negative. In the maternal tissues, very heavy activity is seen in all epithelial areas early, but decreases dramatically in mid-pregnancy, particularly in the basal contracted part of the spongy zone glands. Towards term activity re-accumulates, at least in the dilated portion of the spongy zone glands, which was the only maternal tissue present in the term specimen. Decidualisation is associated with increase in cellular activity from trace levels in the stroma to + in the decidua.

Cat

Activity of these enzymes remains intense in the abembryonic trophoblast, and deep parts of the labyrinth (the superficial parts showing less activity). In that of the brown border chorion activity increases towards term. The basal cytotrophoblast remains highly active throughout. In the allantois traces of staining are seen both at 7 cm and at 12.5 cm. The activity in the yolk-sac endoderm decreases towards term. In the maternal tissues marked activity is seen in the epithelium of the brown border, decreasing towards term and more intense in the deeper parts of the mucosal folds. Less activity is seen in the epithelium of the spongy zone gland, and less still

in the non-placental regions, and no alteration occurs here during the period of gestation examined. Decidualization is associated with increase in staining from trace in the maternal stroma to++++, and the giant cells themselves show++++ (7 cm) to +++(12.5 cm) activity. No activity is seen in the histiotrophe.

Ferret

Activity of G-6-P and 6-PG is seen particularly in the maternal epithelium of the glands underlying the placenta, where it increases towards the apices, in the paraplacental epithelium, and in that of the haematoma region. Some activity is also seen in the basal cytotrophoblast, and in the trophoblast of the haematoma. All other tissues are negative.

Group 4 (Figs. 237 - 239)

Dog

ADH is seen in the placental trophoblast (Trace), in the green border trophoblast (- at 24 days to++ at term) and in the epithelium of the uterine glands (+) earlier in pregnancy.

FDH occurs in the same sites, but in greater intensity in mid pregnancy, and in the allantois also.

GDH is seen only in the epithelium of the dilated portions of the spongy zone glands.

Sorb.DH increases in activity in the same site early in pregnancy, and thereafter remains constant at the ++ level. A similar increase is seen in the trophoblast of the green border chorion, and traces of activity occur in the placental trophoblast, allantoic endoderm, and maternal epithelium also.

Cat

ADH and FDH are seen in the placental trophoblast (FDH more than ADH) and trophoblast of the green border (ADH more than FDH), in the decidual giant cells (both +) and the histiotrophe (both ++). Traces of activity are seen with both enzymes in the maternal epithelium in all areas. FDH activity alone is present in the allantoic endoderm (+) and basal cytotrophoblast (++).

GDH is seen particularly in the histiotrophe, in areas of brown border trophoblast enclosing degenerating tissue, and in the maternal epithelium of the brown border, where the deeper parts of the mucosal folds show more activity. The yolk sac endoderm exhibits + activity, and

the remaining maternal tissues trace.

The distribution of Sorb.DH is similar to that of GDH, with the addition of staining in the chorionic trophoblast of the brown border.

Ferret

ADH is found only in the histiotrophe and basal cytotrophoblast.

FDH occurs in the same sites, but also in the maternal epithelium, particularly just below the basal cytotrophoblast, in the trophoblast of the labyrinth, and haematoma region, and in the yolk-sac endoderm.

GDH is seen in the basal cytotrophoblast, and in the maternal and foetal tissues of the haematoma.

Sorb.DH is found in the maternal epithelium, with increased activity in the spongy zone glands, in the placental and basal trophoblast, and traces in the yolk sac endoderm.

Haemo-chorial placenta

Group 1 (Figs. 240 - 242)

Rat

In this species, enzyme activity (more with GP than

βOH) is seen in the trophoblast of the labyrinth, but not the spongy zone, and in the giant cells, where activity decreases to 18½ days and then increases again. Activity equal with both enzymes (+) is observed in the yolk-sac endoderm, the decidua basalis, and the metrial gland cells and traces in the maternal epithelium. Increase in activity at term, with αGP only, occurs in the visceral layer of the endodermal sinuses, and αGP alone is observed in the endovascular plasmodium. Traces of both enzymes appear in the muscle layers.

Guinea-pig

Enzyme activity with both αGP and βOH is seen in the yolk-sac and placental endoderms, where they increase towards term; particularly in the maternal epithelium; and in the tissues of the junctional zone. The giant cells show trace activity at term with βOH, and similar staining is seen in both spongy zone and labyrinthine trophoblast. βOH alone is seen in the muscle layers.

Rabbit

Here again the main site of activity is the glandular symplasma earlier in gestation (separation zone later)

where trace to + activity with both enzymes is seen. The yolk-sac endoderm and placental trophoblast show βOH activity earlier in gestation, and both enzymes later, while the endothelium lining the decidual blood vessels shows αGP activity earlier, and both enzymes later. Traces of βOH are seen in the maternal epithelium, and antimesometrial giant cells. βOH occurs in the muscle layers.

Human

During gestation the activity of αGP and βOH increase to trace levels near term in the syncytiotrophoblast and decidua. The amnion shows + activity throughout, and a similar level of staining is seen in the uterine gland epithelium.

Group 2 (Figs. 243-250)

Rat

In the trophoblast, activity of all enzymes of this group increases steadily from + at 14½ days (MDH) to ++++ at term. A gradual, but lesser increase occurs in the spongy zone trophoblast later in pregnancy, and in the vacuolated cells of this region activity increases to 17½ days and then falls off. The giant cells remain highly

active throughout, and the decidua basalis slightly so, although the "fibrinoid capsule" increases its activity until 17½ days and then decreases again. A similar wave of activity is seen in the visceral endoderm with its peak of ++++ activity at 17½ days (MDH), falling to ++ at term, and in the metrial gland cells, where the peak is at 16½ days and the decrease is fairly abrupt. Activity in the visceral layer of the endodermal sinuses remains high (++++ MDH at term). Activity is seen in the endovascular plasmodium and decreases from 17½ days onwards. Traces of activity with all enzymes are seen throughout in the parietal endoderm until it degenerates, and in the muscle layers, and + activity in the uterine epithelium.

Guinea-pig

In this placenta the reaction for all enzymes of this group is greater in the syncytiotrophoblast of the spongy zone (++++ MDH) than that of the labyrinth (+++ MDH) and remains constant. That of the subplacenta is highly reactive also (+++ MDH). The reaction of the visceral endoderm decreases slightly during gestation to around

+++MDH at term, but increases to the same level in the placental layer. When present the decidua capsularis shows a moderate reaction with all enzymes of the group, but much more intense staining is seen in the decidua basalis with little reaction in the junctional zone. The placental giant cells show high activity at both stages examined (++++ MDH). In the antimesometrial uterine epithelium the reactivity of all enzymes remains constant at ++ (MDH), except SDH which increases to ++++ at term. The muscle layers show + activity at all times.

Rabbit

The rabbit trophoblast, and visceral endoderm remain consistently highly active with the enzymes of this group until 20 days, but thereaft fall off in activity to term, with the exception of LDH activity which remains high in the endoderm. The trophoblastic fringe maintains a constant high level of activity throughout, and lesser staining is seen in the multinucleate bodies to which it gives origin. A similar level of activity is seen in the antimesometrial giant cells and their overlying uterine

epithelium, and does not change during gestation. In the decidua, the lining of the maternal blood vessels shows a higher level of activity (++ MDH) than the overlying decidual cells (+), and these levels are maintained until decidual disappearance, except with SDH which tails off later in pregnancy. The transition between uninucleate and multinucleate decidual cells is accompanied by the accumulation of large amounts of all of the enzymes of this group, but staining decreases to a certain extent in the resulting cells. No activity is seen in the glandular symplasma, or the separation zone, and the muscle layers show + activity constantly throughout gestation.

Human

In the human placenta all enzymes of this group are seen in small quantities in both cyto- and syncytiotrophoblast initially. Thereafter activity increases in the syncytiotrophoblast throughout gestation, the increase with IDH and LDH being particularly marked. Similar changes, to a lesser degree, take place in the stroma also. In the non-placental chorion, activity is seen with all enzymes in small quantity in the amnion, greater in the

amniotic and chorionic mesenchymes, and greater still in the trophoblast. The underlying decidua shows quite marked activity, particularly with IDH and LDH. In the epithelium lining the uterine glands activity is seen with all enzymes throughout gestation, but particularly with SDH.

Group 3 (Figs. 251 - 254)

Rat

With the enzymes of this group, staining increases in the trophoblast to +++(G-6-P) at term, and to a similar level, but starting from + at 17½ days, in the visceral endoderm. The parietal endoderm is negative, and endodermal sinuses consistently +++ . In the spongy zone the level of staining does not change from + in the trophoblast, but the vacuolated cells accumulate activity to +++ at 17½ days and then decrease in staining reaction for G-6-P. Fall off in activity from 17½ days on is observed in the giant cells (from +++++ to +++ at term), and in the decidua basalis (from + to negative). In the endovascular plasmodium activity increases during gestation, and a few cells are still obvious at term with +++ staining. The metrial gland cells show +++++ with G-6-P at 18½ days

and thereafter decrease. No staining is seen in the "fibrinoid capsule" or antimesometrial epithelium.

Guinea-pig

The two main areas of trophoblast differ in this species at 20 days, the spongy zone showing ++ (G-6-P) and the labyrinth ++++ , but activity decreases in the labyrinth until by term both show ++ . The subplacental trophoblast stains + at 20 days. In the visceral endoderm, activity decreases from ++++ at 20 days to trace at term, but the placental endoderm remains static at + staining reaction. No change of intensity is seen in the giant cells (++), antimesometrial epithelium (+), or muscle layers (+), either, but the reaction of the cells of the decidua basalis decreases from ++++ at 20 days to ++ at term. The decidua capsularis shows + to ++ when present.

Rabbit

The rabbit trophoblast shows a steady decrease in G-6-P and 6-PG from ++++ (G-6-P) at 13 days to ++ at term. No change occurs in the trophoblastic fringe, however, which exhibits a + reaction throughout, in contrast to the ++ reaction of the multinucleate bodies to which it

gives origin. Decrease in enzyme activity is seen in the visceral endoderm also, from its (previously constant) ++++level at 20 days to +++ at term, and in the multinucleate decidual cells from +++ at 13 days to + in their remnants at term. In most other sites activity remains constant, at + - antimesometrial giant cells, the lining of the decidual blood vessels, and the muscle layers. One exception is seen, however, in the antimesometrial epithelium, which increases from ++ with G-6-P at 13 days to ++++ at term. The uninucleate decidual cells show no activity, nor is any abrupt rise seen when they change into multinucleate decidual cells. No activity is seen in the gland symplasma or separation zone.

Human

In the human placenta G-6-P and 6-PG are seen in the syncytiotrophoblast, where their activities increase during gestation, and, to a lesser degree in the cytotrophoblast. No activity is seen in the stroma, a result which must be interpreted with caution due to the concomitant absence of NADP diaphorase (necessary for the demonstration of these enzymes) from this site. In the chorion laeve, neither enzyme is seen to any significant

degree, in the amnion, or amniotic or chorionic mesenchyme. Quite marked staining is observed in the trophoblast, however, and in the underlying decidual cells, although those below the placenta are even more active particularly from 6.5cm onwards. The epithelium lining the uterine glands shows staining with both enzymes.

Group 4 (Figs. 255 - 258)

Rat

ADH is seen with + activity in the trophoblast of the labyrinth, in the decidua basalis, the metrial gland cells, and the visceral endoderm where its activity decreases towards term.

FDH occurs in the trophoblast late, in the visceral endoderm where its activity decreases during gestation, in the decidua basalis, the giant cells, the metrial gland cells, and the endovascular plasmodium, the last four all with + activity. A brief increase in activity to ++ is associated with degeneration of the parietal endoderm.

GDH is seen in the same sites as FDH, but activity decreases during gestation in all except the decidua basalis, which increases towards term. Associated with this increase, is a ++ staining of the uterine epithelium

regenerating at the sides and under the edge, of the placental disc.

Sorb. DH occurs in the visceral endoderm, where its activity decreases during gestation, in the parietal endoderm during its degeneration, and in the giant cells where activity increases from trace to +. Some + staining is also seen in the decidua basalis, metrial gland cells, and endovascular plasmodium.

Guinea-pig

ADH occurs chiefly in the junctional zone throughout pregnancy. Some activity is also seen in the giant cells (++), spongy zone trophoblast (+), visceral endoderm (++ at 20 days, - at term) decidua basalis (+), subplacenta (+), and paraplacental uterine epithelium (+).

FDH is seen in the same sites and concentrations as ADH, except the decidua basalis which is negative.

GDH appears only in the paraplacental epithelium (++++ at 20 days, + at term), in the junctional zone (++) and in increasing quantities in the visceral endoderm (++++ at term), but no activity is seen in the placental endoderm.

Sorb.DH is stained in the junctional zone (++), and the visceral endoderm, where its activity increases from + at 20 days to ++ at term, traces of activity only appear in the placental endoderm.

Rabbit

ADH only appears in significant amounts in the rabbit in cells gathered round the glandular symplasma, and later in the separation zone.

FDH appears in the same cells as ADH, but also in the visceral endoderm, and decidual blood vessels, in both of which sites its activity decreases from ++ at 13 days to + at term. This decrease is accompanied by an increase in activity in the antimesometrial giant cells, from negative at 13 days to ++ at 20 days, after which they disappear. Some + activity is also seen in the trophoblast, trophoblast fringe, and multinucleate decidual cells throughout.

GDH is seen again in the cells of the separation zone, and occurs in the decidua (+), and visceral endoderm (+).

Sorb.DH occurs in the separation zone cells also, and in the visceral endoderm (++ in mid-pregnancy),

trophoblastic fringe (+++ at 13 days, - by 17 days) and blood vessels of the decidua (+ at 13 days only).

Human

Only traces of ADH activity are seen in the human placenta, in the trophoblast of the chorion laeve late in gestation.

FDH activity appears chiefly in the amnion at term, although traces of activity are also seen in the amniotic, and chorionic mesenchymes, and trophoblast.

GDH activity is seen in the syncytiotrophoblast in trace amounts, and in the amnion.

Sorb.DH was not stained in the human placenta.

Yolk-sac placenta (Figs. 259 - 261)

Chick

Up until 5 days of incubation activity is seen in chick endoderm with NAD diaphorase, and lactic dehydrogenase only. At 10 days, however, activity increases markedly with αGP, and βOH. Moderate increase of LDH and NAD diaphorase also occurs. MDH, IDH, SDH, ADH, GDH appear in small quantity, and FDH rather more strongly.

Limia Maculata

The yolk sac endoderm in this species exhibits activity

with LDH and NAD diaphorase (++++), MDH (++), and FDH, G-6-P, αGP, βOH, and Sorb.DH (trace to +).

Human

Only a few sections of human yolk-sac were available, through the courtesy of a colleague. LDH, G-6-P, and SDH were stained and exhibit +, ++ and - activity respectively.

Chorio-allantoic membrane - chick (Fig. 262)

Activity of FDH, LDH, MDH, IDH, G-6-P, and αGP and the diaphorases is found in the allantoic endoderm in trace to + quantities. The mesoderm exhibits LDH, MDH, and diaphorase only. The ectodermal layer, applied to the inside of the shell membrane shows LDH, MDH, and traces of FDH activities, NAD diaphorase rather more strongly, and very marked activity with NADP diaphorase. The shell membrane shows no activities.

Dehydrogenases - steroid (Figs. 263 - 280)

The results of the investigation of the localization of these enzymes in various placentae are detailed in Table 17. Only those tissues which possessed HSD activity are tabulated, and changes in enzyme content

TABLE 17

Localization of steroid dehydrogenases (HSD) in the placentae of various species

Only the contracted form of the HSD is given - for full substrate names see "Maternal and Methods."

Tissues	α3α	β3α	Δ5 3β	Δ5 3β	Δ5 3β	α3β	β3β	6β	C-ol	11β	16β (and)	16β (oest)	oest	test	20β
Horse (term)															
Trophoblast	+	+	TR	-	TR	++	+++	TR	-	-	+++	++++	+	+	+++
Sheep (early) 2.5cm.															
Cytotrophoblast	-	-	-	-	-	TR	++	-	-	-	++	++	-	-	-
Syncytiotrophoblast	-	-	-	-	-	-	++	-	-	-	TR	++	-	-	-
Extra-cotyledonary trophoblast	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-
(mid) 15 cm.															
Cytotrophoblast	TR	TR	-	-	-	TR	+++	-	-	-	++	++	TR	-	-
Syncytiotrophoblast	-	-	-	-	-	-	+	-	-	-	TR	++	-	-	-
Extra-cotyledonary trophoblast	-	-	-	-	-	-	++	-	-	-	++	+++	-	-	-
Maternal epithelium	-	-	-	-	-	-	+	-	++	-	-	-	-	-	-
Foetal mesoderm	TR	TR	-	-	-	-	TR	-	-	-	++	+	-	-	-
Allantoic endoderm	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-

TABLE 17 (Continued)

	α3α	β3α	Δ3β	Δ3β	Δ3β	α3β	β3β	6β	C-ol	11β	16β (and)	16β (oest)	oest	test	20β
(term)															
Cytotrophoblast	TR	TR	-	-	-	TR	+++	-	-	-	+	+	-	-	-
Syncytiotrophoblast	-	-	-	-	-	-	+	-	-	-	TR	+	-	-	-
Extra-cotyledonary trophoblast	-	-	-	-	-	-	TR	-	-	-	TR	++	-	-	-
Maternal epithelium	-	-	-	-	-	-	+	-	++	-	-	-	-	-	-
Foetal mesoderm	-	-	-	-	-	-	-	-	-	-	TR	-	-	-	-
Ferret (20 Days)															
Thickened maternal endothelium	TR	TR	TR	-	TR	TR	TR	-	-	-	TR	TR	TR	+	-
Spongy zone epithelium	-	-	-	-	-	-	-	-	+	TR	-	-	-	-	-
Haematoma epithelium	-	-	-	-	-	-	-	-	-	-	TR	TR	+	-	-
Yolk sac endoderm	TR	TR	TR	-	TR	+	+	-	-	-	TR	TR	++	+	-
Yolk sac mesoderm	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Cat (mid) 7 cm.															
Syncytiotrophoblast	TR	TR	+	TR	++	TR	++++	TR	TR	TR	+++	TR	TR	TR	-
Basal cytotrophoblast	TR	TR	+	TR	++	TR	++++	TR	TR	TR	+++	+++	TR	TR	-
Yolk sac endoderm	-	-	-	-	-	-	-	-	-	-	-	TR	TR	+	-
Spongy zone epithelium	-	-	-	-	-	-	-	-	-	-	-	-	++	+	-
Brown border symplasma	-	-	-	-	-	-	++	-	-	-	+	-	-	-	-

TABLE 17 (Continued)

	α3α	β3α	Δ3β	Δ3β	Δ3β	α3β	β3β	6β	C-o1	11β	16β (and)	16β (oest)	oest	test	20β
Cat (term) 12.5cm.															
Syncytiotrophoblast	TR	TR	+	TR	+	TR	+++	TR	+	TR	++	-	TR	-	-
Basal Cytotrophoblast	TR	TR	+	TR	+	TR	+++	TR	+	TR	++	-	TR	-	-
Yolk sac endoderm	-	-	-	-	-	-	-	-	TR	-	++	++	+++	+++	-
Spongy zone epithelium	-	-	-	-	-	-	-	-	-	-	-	-	+++	++	-
Brown border symplasma	-	-	-	-	-	-	+++	-	-	-	+	-	-	-	-
Brown border epithelium	-	-	-	-	-	-	-	-	+++	+	-	-	-	-	-
Dog (early) 24 days															
Trophoblast	-	TR	TR	-	TR	-	+	-	-	-	TR	TR	-	TR	-
Spongy zone gland epithelium -															
Contracted	-	-	-	-	-	-	+	-	-	-	++	+++	-	-	-
Dilated	-	-	-	-	-	-	-	-	-	-	+	++	-	-	-

TABLE 17 (Continued)

	α3α	β3α	Δ3β	Δ3β	Δ3β	α3β	β3β	6β	C-ol	11β	16β (and)	16β (oest)	oest	test	20β
Dog (early mid) 32 days															
Trophoblast	TR	TR	TR	-	TR	TR	++	-	-	-	TR	TR	TR	TR	-
Spongy zone gland epithelium -															
Contracted	TR	TR	-	-	-	-	+	-	-	-	++	+++	TR	-	-
Dilated	-	-	-	-	-	-	-	-	-	-	+	++	-	-	-
Yolk sac endoderm	TR	-	-	-	-	-	+	-	-	-	TR	-	-	-	-
Green border trophoblast	-	-	-	-	-	-	-	-	-	-	TR	TR	-	-	-
Green border epithelium	-	-	-	-	-	-	-	-	-	-	TR	TR	TR	TR	-
(late mid) 45 days															
Trophoblast	TR	TR	TR	-	TR	TR	++	-	-	-	+	TR	TR	TR	-
Spongy zone gland epithelium -															
Contracted	-	-	-	-	-	-	++	-	-	-	+++	+++	TR	-	-
Dilated	-	-	-	-	-	-	-	-	-	-	+	++	TR	-	-
Yolk sac endoderm	TR	-	-	-	-	-	++	-	-	-	+	TR	TR	+	-
Green border trophoblast	-	-	-	-	-	-	-	-	-	-	TR	TR	TR	TR	-
Green border epithelium	-	-	-	-	-	-	-	-	-	-	TR	TR	TR	TR	-

TABLE 17 (Continued)

	α3α	β3α	Δ5β	Δ3β	Δ3β	α3β	β3β	6β	C-ol	11β	16β (and)	16β (oest)	oest	test	20β
Dog (term) 60 days															
Trophoblast	TR	TR	-	TR	+	+	++	-	-	-	+	-	-	-	-
Spongy zone gland epithelium															
Dilated	-	-	-	-	-	-	-	-	TR	-	+	++	+	TR	-
Green Border trophoblast	-	-	-	-	-	-	-	-	-	-	-	TR	+	TR	-
Non placental trophoblast	TR	-	-	-	-	-	-	-	-	-	-	TR	++	+	-
Rat (early) 10½ days															
Giant cells 1st generation	+	++	++	+	++	+++	++++	+	-	-	++++	++++	-	TR	-
Ectoplacental cone	-	-	-	-	-	TR	-	-	-	-	TR	TR	-	-	-
Fibrinoid capsule	-	-	-	-	-	-	-	-	+	TR	-	-	-	-	-
(mid) 17½ days															
Giant cells 2nd generation	TR	TR	TR	-	TR	TR	+	-	-	-	++	+	TR	-	-
Labyrinth	TR	TR	-	-	-	-	-	-	-	-	-	-	+	++	-
Decidua basalis	+	TR	-	-	-	-	TR	-	TR	TR	TR	-	-	-	TR
Fibrinoid capsule	-	-	-	-	-	-	-	-	++	+	-	-	-	-	-
Yolk sac endoderm	-	-	-	-	-	-	-	-	-	-	-	-	TR	+	-

TABLE 17 (Continued)

	α3α	β3α	Δ3β	Δ3β	Δ3β	α3β	β3β	6β	C-ol	11β	16β (and)	16β (oest)	oest	test	20β
Rat (term) 20½ days															
Giant cells 2nd generation	TR	-	TR	-	TR	+	+	-	-	-	+++	++	TR	-	-
Labyrinth	-	-	-	-	-	TR	+	-	-	-	+	TR	+++	+++	-
Decidua Basalis	-	-	-	-	-	-	TR	-	TR	-	TR	-	-	-	-
Fibrinoid capsule	-	-	-	-	-	-	-	-	++	+	-	-	-	-	-
Yolk sac endoderm	-	-	-	-	-	-	-	-	-	-	-	-	TR	TR	-
Endoderm sinuses	-	-	-	-	-	-	-	-	-	-	+	TR	TR	TR	-
Endovascular plasmodium	-	-	-	-	-	TR	TR	-	-	-	+	+	++	++	-
Guinea Pig (mid) 20 days															
Trophoblast	TR	-	-	-	-	-	-	-	-	-	TR	TR	TR	-	-
Yolk sac endoderm	TR	TR	-	-	-	-	-	-	-	-	TR	+	++	+	-
Cells in decidua capsularis	TR	TR	-	-	-	-	TR	-	-	-	++	++	++	TR	-
Cells in uterine lumen	+	+	TR	-	TR	-	TR	-	TR	TR	++	++	++	TR	-
Decidua Basalis	-	-	-	-	-	-	-	-	TR	TR	TR	TR	-	-	-
(term) 63 days															
Yolk sac endoderm	-	-	-	-	-	-	-	-	-	-	-	TR	TR	-	-

TABLE 17 (Continued)

	α3α	β3α	δ3β	δ3β	δ3β	α3β	β3β	6β	C-ol	11β	16β (and)	16β (oest)	oest	test	20β
Rabbit (early) 13 days															
Trophoblast	-	-	-	-	-	-	+	-	-	-	TR	TR	-	-	-
Multinucleate decidual cells	-	-	-	-	-	-	-	-	-	-	-	-	++	+	-
Yolk sac endoderm	-	-	-	-	-	-	-	-	-	-	-	-	+++	+	-
(early mid) 17 days															
Multinucleate decidual cells	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Yolk sac endoderm	+	TR	-	-	TR	-	-	-	-	-	+	+	TR	-	-
Multinucleate bodies	+	TR	-	-	-	-	+	-	-	-	++	++	-	-	-
(late mid) 20 days															
Multinucleate decidual cells	-	-	-	-	-	-	-	-	-	-	-	-	TR	-	-
Yolk sac endoderm	TR	TR	-	-	-	-	-	-	-	-	++	++	TR	TR	-
Multinucleate Bodies	+	-	-	-	-	-	-	-	-	-	+++	++	-	-	-
Maternal epithelium	-	-	-	-	-	-	-	-	++	TR	-	-	-	-	-
(term)															
Yolk sac endoderm	TR	TR	-	-	-	-	-	-	-	-	+	TR	-	-	-

TABLE 17 (Continued)

	α3α	β3α	Δ3β	Δ3β	Δ3β	α3β	β3β	6β	C-o1	11β	16β (and)	16β (oest)	oest	test	20β
Human (term)															
Trophoblast	TR	+	+	TR	+	+	++++	+	TR	-	++++	++	+	-	-
Foetal blood vessels	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Amnion	-	TR	-	-	-	-	-	-	-	-	-	-	++	-	-
Amniotic mesenchyme	-	TR	-	-	-	-	-	-	-	-	-	-	TR	-	-
Chorionic mesenchyme	-	TR	-	-	-	-	-	-	-	-	-	-	TR	-	-
Trophoblast of chorion laeve	TR	+	+	TR	+	+	++	TR	TR	TR	+	TR	+	-	-
Decidua	-	TR	-	-	-	-	-	-	-	-	+	-	+	-	-

during pregnancy are presented.

With certain enzymes in the cat the reaction in the apical part of the trophoblastic cords was less than at their bases. This was seen particularly in the earlier specimen, with Δ^5 3β-, α3β- , β3β-, 16β (androgen)- and 16β (oestrogen)-HSD. At term, the tissue identified in the cat as "brown border symplasma" is that described by Bjorkmann (1957) and which may well be syncytiotrophoblast invading maternal tissues.

In the dog, only the epithelium of the dilated portion of the spongy zone glands is present at term - parturition specimen - as the plane of separation of the placenta is through this zone.

Two generations of giant cells are seen in the rat, one antimesometrial, identified here as the 1st generation, and one mesometrial and lateral to the foetal placenta, forming a zone 4 to 5 cells thick which decreases towards term. This is identified here as the 2nd generation. Distinct differences were observed in the levels of HSD activity in these two cells generations.

The active cells in the uterine lumen and decidua capsularis of the 20 day guinea-pig placenta did not

exhibit any specific morphological features in paraffin sections stained with routine histological stains. The identity of these cells requires further investigation. The marked differences in HSD activity patterns would make it improbable that both types could be trophoblastic in origin.

DISCUSSION

DISCUSSION

Enzyme specificity.

The question of the specificity of the histochemical methods used for the enzymes demonstrated here must be considered, particularly with reference to the specific alkaline phosphatases, and the dehydrogenases - both carbohydrate and steroid.

Alkaline phosphatases

Considerable discussion on the subject of the specificity of the alkaline phosphatases may be found in the recent literature, Freiman and Kaplan, (1960), Wachstein, Meisel and Niedwiedz (1960), Allen (1963), Barden and Lazarus (1963), Lazarus and Barden (1962). Certainly all of the substrates used are hydrolysed to a greater or lesser extent by non-specific alkaline phosphatase, but at the pH used (7.2) the activity of this enzyme is at a low level (Pearse, 1960). In this study the activity of non-specific alkaline phosphatase in various sites, for example, the rat decidua and glycogen wings, was always less than that observed with any of the other substrates, and the staining pattern was different with each, with the possible exception of ATP and ITP, both of

which are known, Barden and Lazarus (1963) to be hydrolysed in mitochondria by one enzyme. On these grounds it is felt that the methods used in this study have been specific, and allow some comparison to be made between the different enzymes at the different stages of pregnancy, and in the placentae of different species.

The identity of the enzyme demonstrated by the ATPase method requires further examination. This enzyme is known to exist in several forms, localised in different sites in the cell, and variously associated with different functions, for example, energy production, and transport across cell membranes, Hokin and Hokin (1963), including the transport of sugars, which may be linked to the ATP-activated "sodium pump", Crane (1962). However, recent analysis by Tormey (1966) has suggested that, in certain sites at least, "sodium pump" ATPase is not demonstrated by any histochemical method. Thus the possibility exists that the ATPase demonstrated here is the mitochondrial enzyme associated with energy production, rather than that localised in cell membranes and associated with transport. The staining of brush borders (not sites of mitochondrial localization) however, would point

to transport enzyme also being stained, and it is felt, therefore, that some comments on both functions may be made.

Dehydrogenases - carbohydrate

The specificity of the dehydrogenase techniques has also to be considered. Recently Kalina, Gahan, and Jones (1965) have suggested that all histochemically demonstrated dehydrogenase reactions merely show the localization of one or other pyridine nucleotide diaphorase. Were this the case, one would expect results demonstrating identical localizations of activity with all substrates, differing only in overall intensity depending on the degree to which the substrate used was dehydrogenated. However, this study shows totally different pictures with different groups of enzymes, for example, fig. 256, where separate enzymes, both NAD-linked, have stained single, and entirely different tissues. Also, considerable quantities of poly-vinyl-pyrrolidone were added to the incubation media to minimise diffusion of enzyme, a precaution which was not taken by Kalina, Gahan and Jones in their work.

The risk of enzyme diffusion applies particularly to the lactic dehydrogenase system, where the enzyme is

mucopolysaccharide in nature, and therefore more prone to diffusion into the medium, or into areas of tissue section which should give a negative reaction. Suggestions that the observed reaction is specific are found in Table 3 where negatively reacting tissue (e.g. the mesoderm at 8 days) lies adjacent to a positive area; also in the lack of discoloration of the incubation medium (due to diffusion of enzyme into it) for several hours after the sections had been removed from it, whereas the omission of PVP produced discoloration even before the sections themselves had stained.

Dehydrogenases - steroid

Similar considerations apply to this group of dehydrogenases, where the quantity of enzyme present in many tissues is considerably less than the quantity of carbohydrate dehydrogenases and the possibility of errors due to non-specific staining is correspondingly greater. However these methods have been extensively investigated by Baillie, Ferguson and Hart (1966) and have been shown to be specific, on the grounds both of differing distributions in different tissues, and of difference in co-factor (i.e. NAD or NADP) specificity.

Physiological significance of enzymes and other substances studied

(1) Glycogen

This substance exists in cells chiefly as a readily available source of energy, and is normally bound to protein, Stetten and Stetten (1960).

Associations have been noted between glycogen and embryonic morphogenesis, in the amphibian, Woerdemann (1933), in Amblystoma in the developing nervous system, Janosky and Wenger (1956), in the chick by Allen (1919), Jacobson (1938a,b) and McCallum and Wong (1956), and in fish embryos by Daniel (1947), and a diffuse staining, removal by diastase, and therefore presumably glycogen has been reported by Borghese (1957) in mammalian embryos. The existence of glycogen in the developing mouse embryo at the stage of gastrulation and primitive streak formation has been denied by Chiquoine (1957) however.

(2) Acid mucopolysaccharides

The occurrence of these substances in the ground substance of connective tissues, including cartilage and bone is well known. Recently they have also been shown to occur (Walker, 1961) in relation to the notochord, limb buds, cardiac jelly and other sites of morphogenetic

changes in developing embryos.

The concentration of acid mucopolysaccharides, particularly those which are sulphated, is influenced by a variety of factors, including cortisone, Bostrom (1958), and oestrogens, Bo and Smith (1965) all of which may affect the events taking place in the pregnant rat uterus, or in the embryo.

(3) RNA

The association of this substance with protein synthesis, and therefore with cell growth and differentiation is well known.

(4) Hydrolases

Of these enzymes, acid phosphatase and β-glucuronidase are known to be lysosomal, de Duve (1959), and are therefore associated with the absorption of foreign materials, including protein, into the cell, and also with the process of autolysis. β-glucuronidase has also been associated with tissue proliferation, Kerr and Campbell (1947), Karunairatnam, Kerr and Levvy (1949), Burton and Pearse (1952), Conchie, Hay and Levvy (1961), Stolk (1962) and with the conjugation of biologically active steroids, Lipschitz and Bending (1959), Fishman (1940), Fishman and Fishman (1944), Fishman and Anlyan (1947a,b), and Fishman (1947, 1950). Some doubt has been cast on the part played

by β-glucuronidase in conjugation of steroids, however, by Conchie and Findlay (1959) who suggest that its action is purely hydrolytic, affecting the turnover of mucopolysaccharides, and being concerned with degradation products of hyaluronic acid, and of chondroitin. Some association of acid phosphatase with secretion by cells became evident in this material, although the intracellular distribution of this portion of the enzyme is unknown.

Another hydrolytic enzyme, thought to be lysosomal in localization, and concerned with protein breakdown is cathepsin, which has been examined here as a C-esterase, demonstrated by the indoxyl acetate method, Hess and Pearse, 1958.

The localization (intra-cellular) and function of the remaining esterases is less certain, although here again the suggestion has been made, Novikoff (1961) that the organo-phosphate resistant A and C esterases are lysosomal and the sensitive B esterase cytoplasmic. Their action is probably to hydrolyse non-specifically ester linkages, either aliphatic (B esterase) or aromatic (A esterase). This would involve these enzymes in the hydrolysis of, among other things, neutral lipids, which have been studied here, and which, according to Novikoff

(1961) may be hydrolysed in the endoplasmic reticulum, where B esterase is found.

(5) Alkaline phosphatases

Non-specific alkaline phosphatase is concerned either with non-specific hydrolysis of phosphate esters, or with the detection of transphosphorylation between one compound and another, i.e. reactions of the type:-

$$ATP + UMP \rightleftharpoons ADP + UDP$$

That being the case its presence indicates the occurrence of many metabolic processes, e.g. the deposition of calcium phosphate in bone, the breakdown of glycogen - which requires phosphate ester formation, and the metabolism of RNA.

The significance of certain of the specific phosphatases is known, e.g. thiamine pyrophosphatase, which is localized to the Golgi region in cells, and whose intensity of reaction presumably is a reflection of the secretory activity of the cells, and also adenosine triphosphatase (discussed previously under "enzyme specificity") which is concerned with energy production in cells, and possibly with membrane transport.

The function of adenosine monophosphatase, and uridine

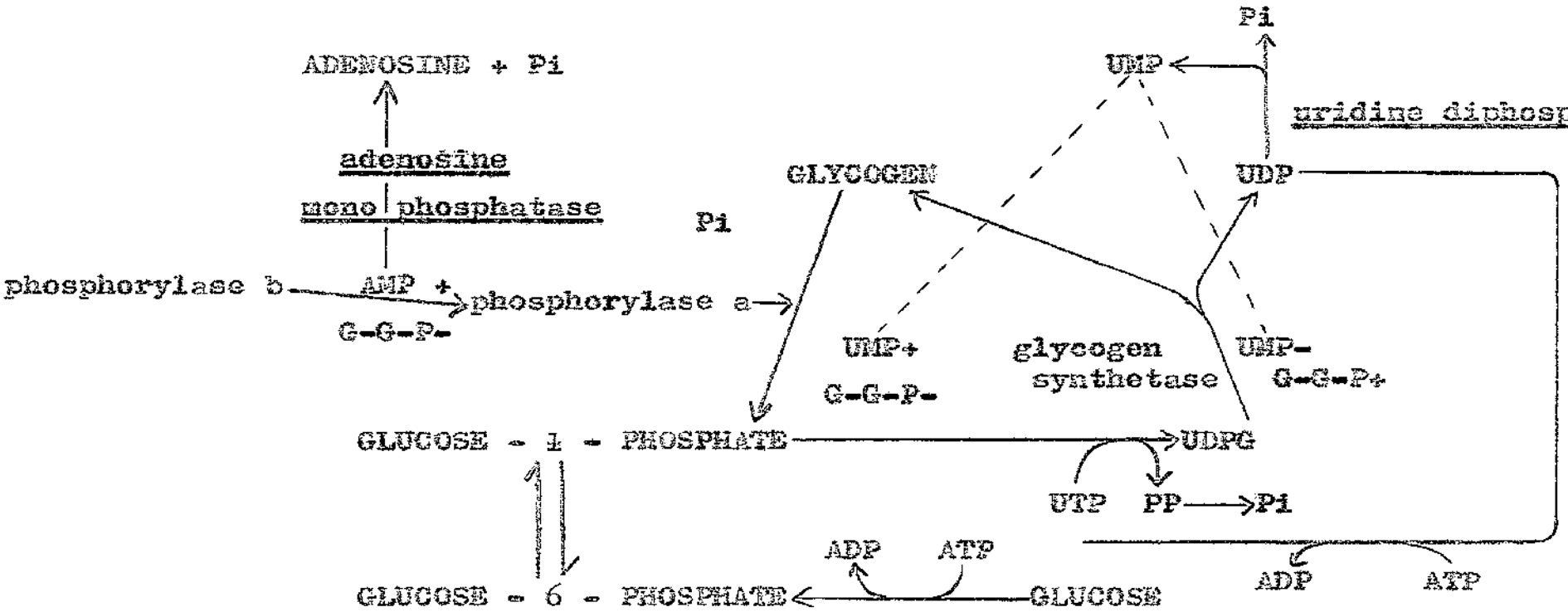
diphosphatase is less certain. On theoretical grounds, with regard to the mode of formation and breakdown of glycogen proposed by Stetten and Stetten (1960) - Table 18, functions for these enzymes in control of this process can be suggested, and some evidence in favour of these will be presented. Certainly it is known that UMP which would be produced by the action of uridine diphosphatase is inhibitory to glycogen synthetase, and activating to phosphorylase, Fridland and Nigam (1965) and that AMP, which would be destroyed by adenosine monophosphatase, is activatory to phosphorylase, in the conversion of the inactive "b" form of the enzyme to the active "a" form, Morgan and Parmeggiani (1964).

Inosine triphosphatase, which was also studied, is probably the same enzyme, when demonstrated histochemically, as mitochondrial adenosine triphosphatase, Barden and Lazarus (1963).

Of the enzymes glucose-6-phosphatase, fructose-6-phosphatase, and fructose-1:-6-diphosphatase specific staining was seen only in the rat with glucose-6-phosphatase, which is concerned with the production of glucose from glucose-6-phosphate.

TABLE 18

Biochemistry of glycogen synthesis and metabolism after Stetten and Stetten (19



UDPG - uridine diphosphoglucose

UTP - uridine triphosphate

UDP - uridine diphosphate

UMP - uridine monophosphate

AMP - adenosine monophosphate

ADP - adenosine diphosphate

ATP - adenosine triphosphate

Pi - inorganic phosphate

PP - pyrophosphate

\+ - activator

⟶ - inhibitor

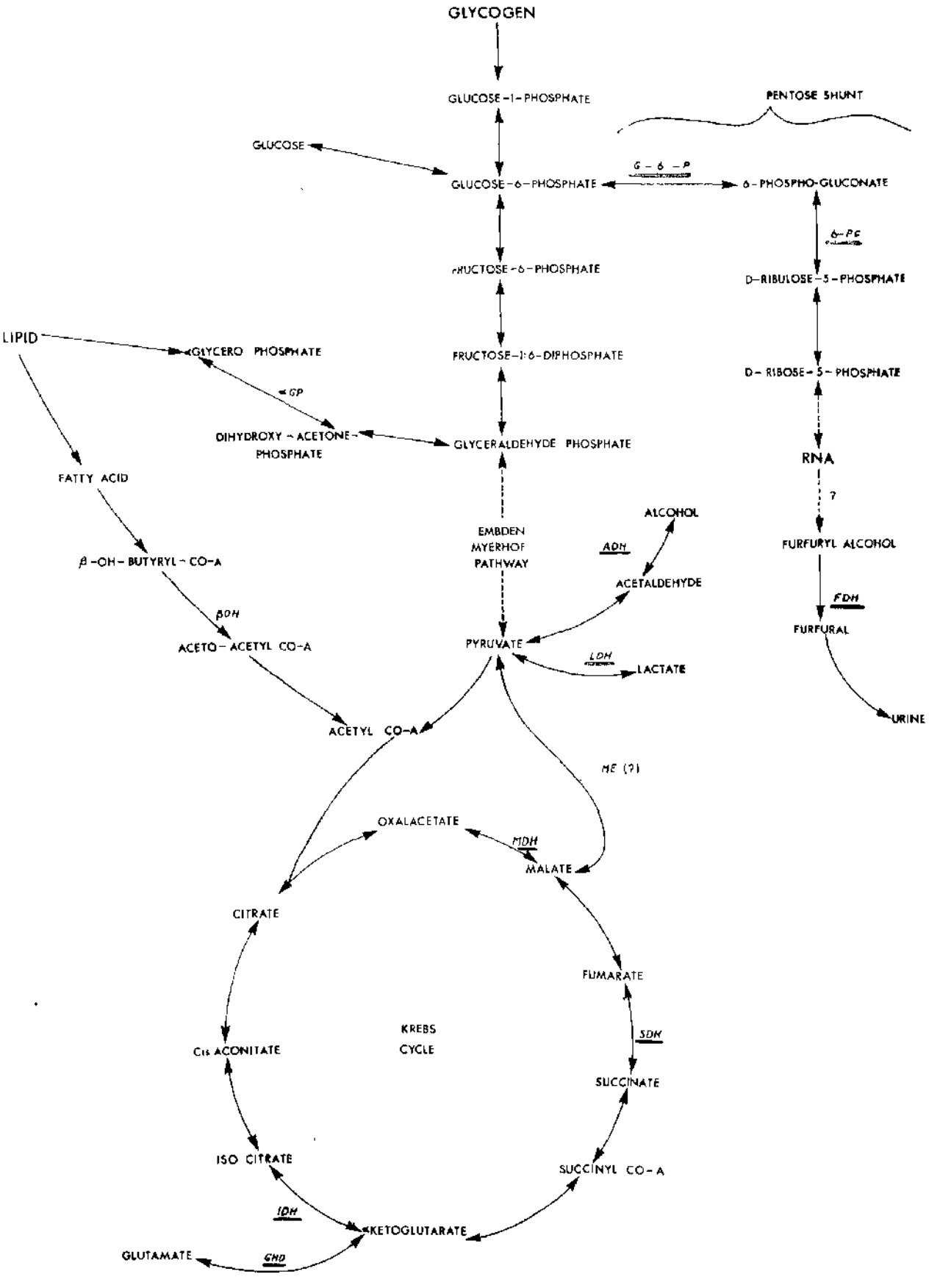
(6) Dehydrogenases - carbohydrate

The metabolic pathways relevant to this group of enzymes are shown in Text - fig. 8.

Glycogen can be broken down either to pyruvate, via the Embden-Myerhof pathway, or to ribulose-5-phosphate via the Pentose Shunt (enzymes underlined in blue). If degraded to pyruvate it then proceeds either to lactate (enzyme underlined in red) in anaerobic conditions, or to carbon dioxide and water via the Krebs cycle (enzymes underlined in green) in aerobic conditions, the function subserved in either case being the production of energy. If degraded to ribulose-5-phosphate, however, this may be passed either back into the glycolytic pathway, via xylulose-5-phosphate or to ribose-5-phosphate which may then be used for RNA synthesis.

Of the remaining dehydrogenases studied the function of αGP and βOH (underlined in yellow) is the introduction of α-glycerophosphate, and β-hydroxy-butyrate respectively (both derived from lipid breakdown) into the glycolytic pathway; that of GDH (underlined in brown) is the introduction of glutamate derived from protein breakdown into

Text-fig. 8. The metabolic pathways of lipid and glycogen breakdown, and RNA synthesis and breakdown, showing the points at which the carbohydrate dehydrogenases studied in this thesis act.

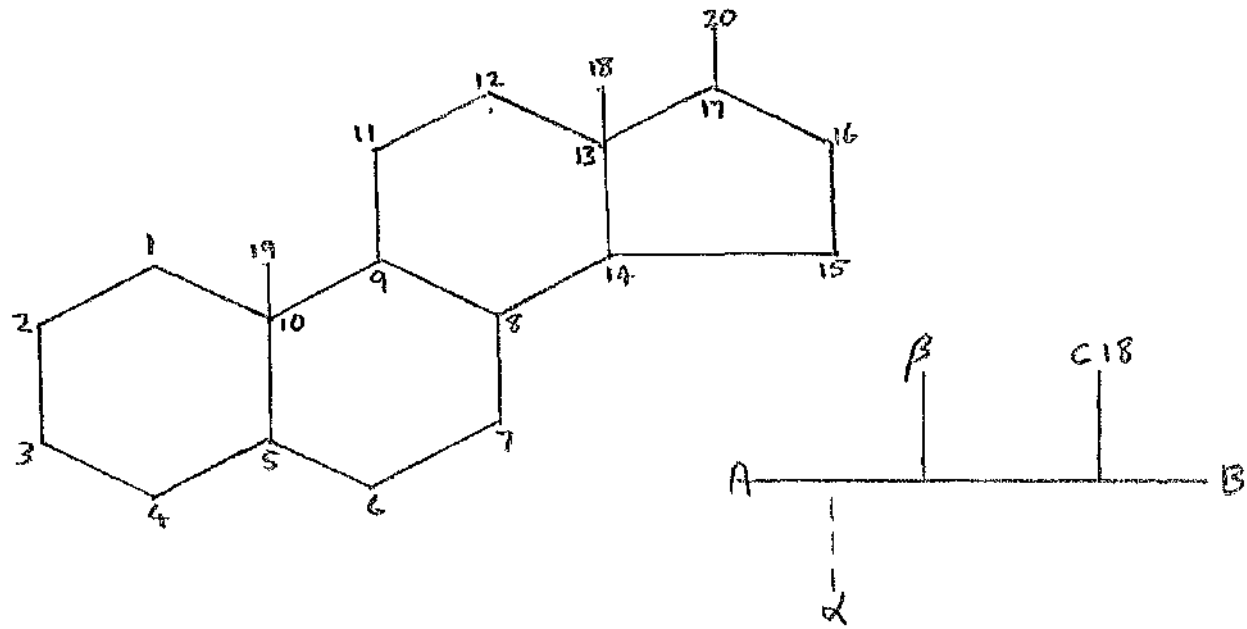

GLYCOGEN
GLUCOSE-1-PHOSPHATE
PENTOSE SHUNT
GLUCOSE
G-6-P
GLUCOSE-6-PHOSPHATE
6-PHOSPHO-GLUCONATE
6-PG
FRUCTOSE-6-PHOSPHATE
D-RIBULOSE-5-PHOSPHATE
LIPID
GLYCERO PHOSPHATE
FRUCTOSE-1:6-DIPHOSPHATE
D-RIBOSE-5-PHOSPHATE
GP
DIHYDROXY-ACETONE-PHOSPHATE
GLYCERALDEHYDE PHOSPHATE
RNA
FATTY ACID
?
ALCOHOL
EMBDEN MYERHOF PATHWAY
ADH
FURFURYL ALCOHOL
β-OH-BUTYRYL-CO-A
ACETALDEHYDE
FDH
βOH
FURFURAL
ACETO-ACETYL CO-A
PYRUVATE
LDH
LACTATE
URINE
ACETYL CO-A
ME (?)
OXALACETATE
MDH
MALATE
CITRATE
FUMARATE
KREBS CYCLE
SDH
Cis ACONITATE
SUCCINATE
ISO CITRATE
SUCCINYL CO-A
IDH
KETOGLUTARATE
GLUTAMATE
GHD

the Krebs cycle, or the divergence of α-keto-glutamate from the cycle into protein synthesis; that of ADH (underlined in purple) is the degradation of alcohols to the corresponding aldehydes; and that of FDH (underlined in black) is the degradation of furfuryl alcohol, which may be derived from RNA breakdown, to furfural which is excreted in the urine. Lastly the enzyme ME, which is the NADP-linked malate dehydrogenase demonstrated here, may be the malic enzyme concerned with the early stages of the reversal of glycolysis. Certainly in some of the material described here this enzyme showed an inverse relationship to NAD-linked malate dehydrogenase, and appeared in sites where glycogen synthesis was commencing.

(7) Dehydrogenases - steroid

The function of these enzymes is excellently described in detail in Baillie, Ferguson and Hart (1966) and will be summarised briefly here.

Prior to a discussion of function, however, the numbering of the steroid molecule, and the meaning of the terms "α" and "β" is shown below:



As can be seen the position of a hydrogen or hydroxyl group is described as "α" if it lies below the plane of the molecule (represented by line AB above), with respect to carbon 18 which is always considered to lie above, and the bond is represented as a broken line. Its position is "β", however, if it lies above the plane of the molecule, that is in the same plane as carbon 18, and there the bond is shown as a continuous line. Different enzymes act on different stereo-isomers of the same molecule, and their distribution as shown by staining is correspondingly different.

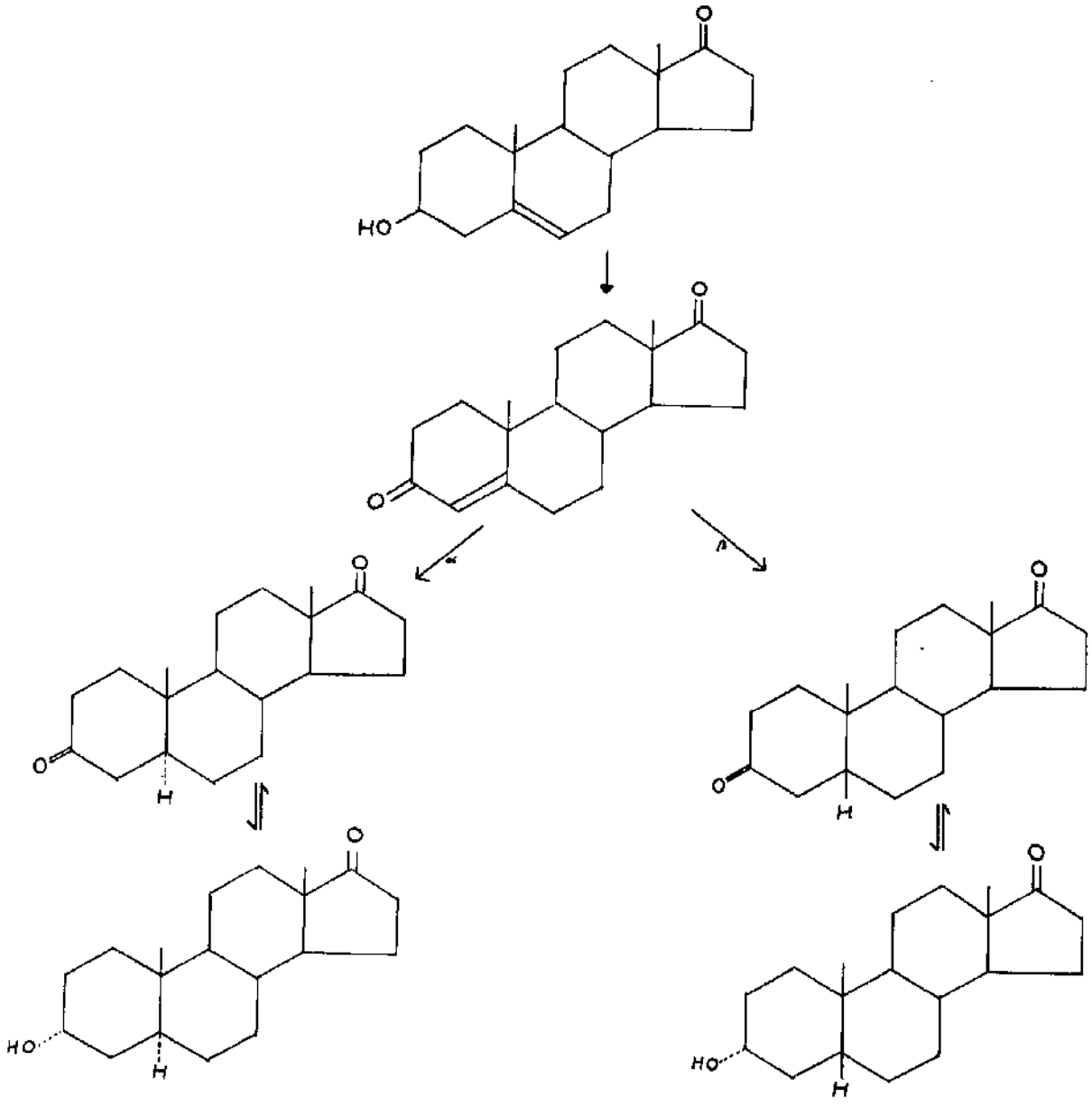
Text-fig. 9. The reactions of the $\Delta 3\alpha$ and $\beta 3\alpha$ HSD.

dehydroepiandrosterone

androstenedione

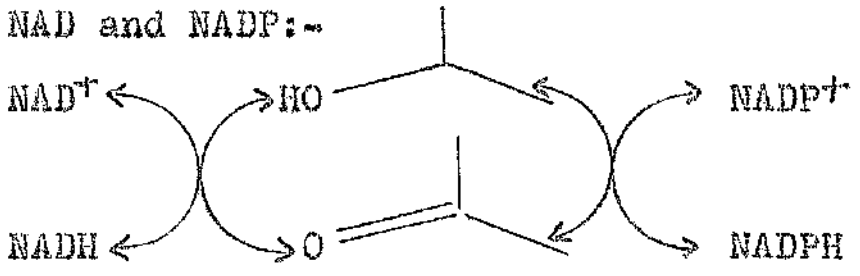
5α-androstan-3:17-dione 5β-androstan-3:17-dione

androsterone aetiocholanolone


HO
O
O
α
β
O
H
O
H
HO
H
O
H
HO
H

(A) $\alpha 3\alpha$ and $\beta 3\alpha$ hydroxy-steroid dehydrogenases. (Text-fig. 9)

The initial step in the metabolic pathway involving these enzymes is the conversion of dehydroepiandrosterone (above in Text-fig. 9) to androstenedione, this being a composite reaction involving an initial dehydrogenation of the 3β hydroxyl by a 3βHSD, followed by a shift of the Δ^5 double bond by a Δ^5 - Δ^4- isomerase. Androstenedione is then hydrogenated at the Δ^4 bond producing an α(left in Text-fig. 9) or β (right) hydrogen bond at the 5 position. Hydrogenation of the 3α oxygen by (reversible) $\alpha 3\alpha$, or $\beta 3\alpha$HSD leads to the final compounds androsterone (left) or aetiocholanolone (right). The resulting androsterone is known to act as an androgen. The function of aetiocholanolone is unknown. The possibility exists, however, that both $\alpha 3\alpha$ and $\beta 3\alpha$HSD may act as transhydrogenases between NAD and NADP:-

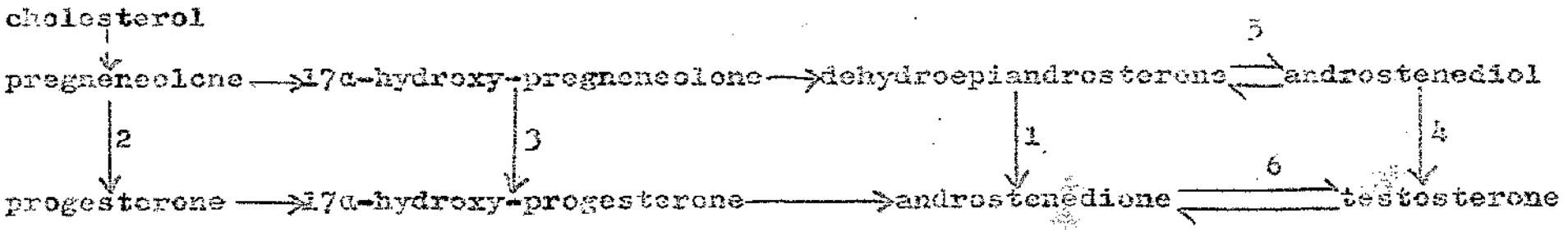


The production of NADH which would be increased by this mechanism, going from right to left, could be used for energy production, NADH entering the cytochrome system earlier than NADPH , while movement in the opposite direction would produce increased amounts of NADPH , needed for hydroxylation in steroid reactions, such as is known to occur in the metabolism of cortisol, Lipman, Katz and Jailer (1962).

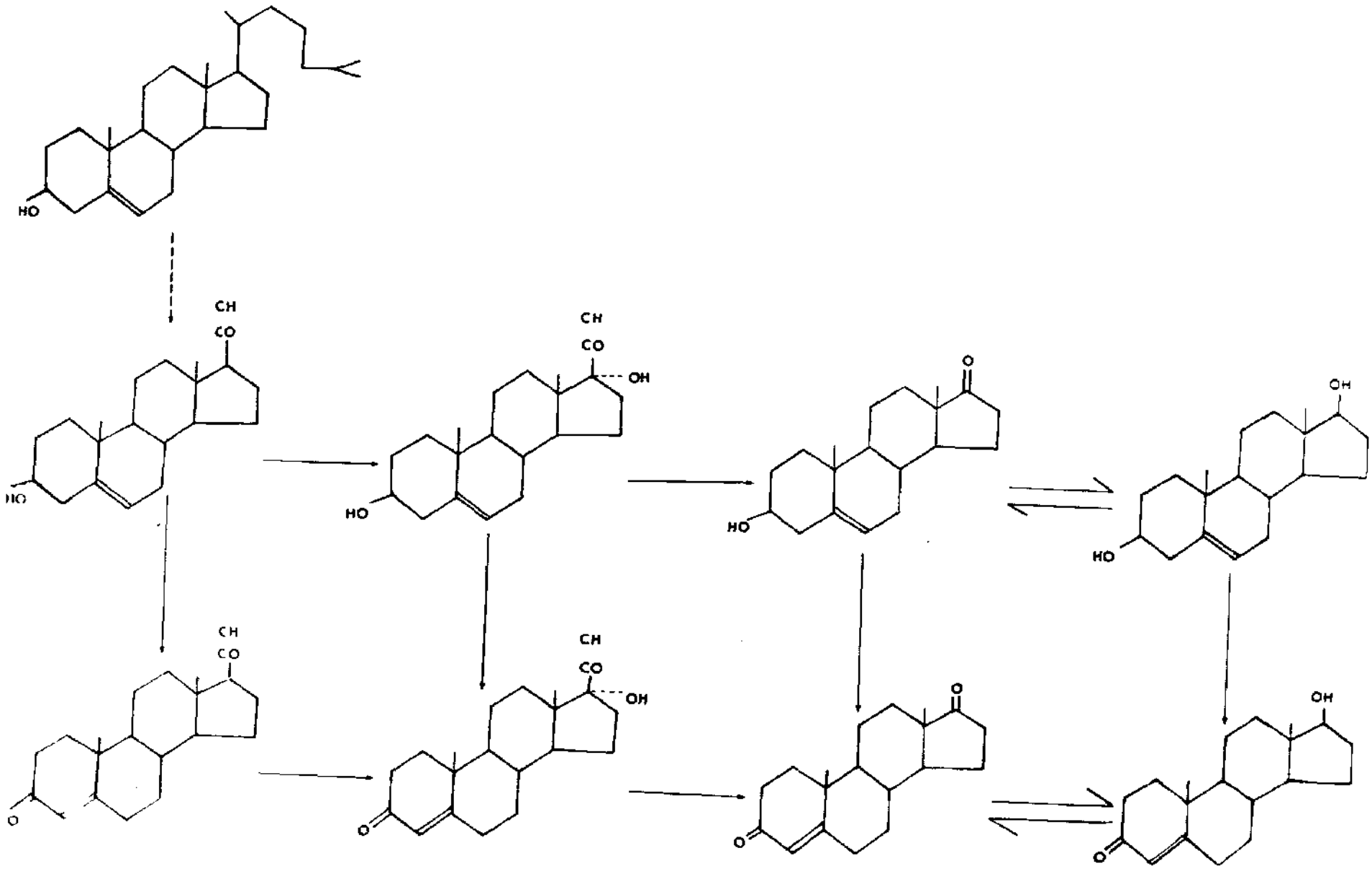
(B) Δ^5-3β hydroxy-steroid dehydrogenases

Enzymes acting on a number of substrates with a 3β hydroxyl group and a Δ^5 double bond exist. Their actions, and the inter-relationships of the compounds with which they are associated are shown in Text-fig. 10. From progesterone, and 17α-hydroxy-progesterone, corticosteroids can be synthesised; androstenedione can give rise either to androgens or oestrogens; and testosterone can give rise to oestrogens. Thus the presence of Δ^5-3β-HSD in a tissue indicates steroid biosynthesis. In practice it is found that reactions 1, 2 and 3 are usually present in the same site in tissues, the intensity of staining . decreasing in that order.

Text-fig. 10. The possible conversions utilising Δ^5-3β-HSD.



Reactions 1 to 4 are Δ^5-3β-HSD, reactions 5 and 6 are 17β-HSD. (see text-fig. 15).


HO
CH
CO
OH
O

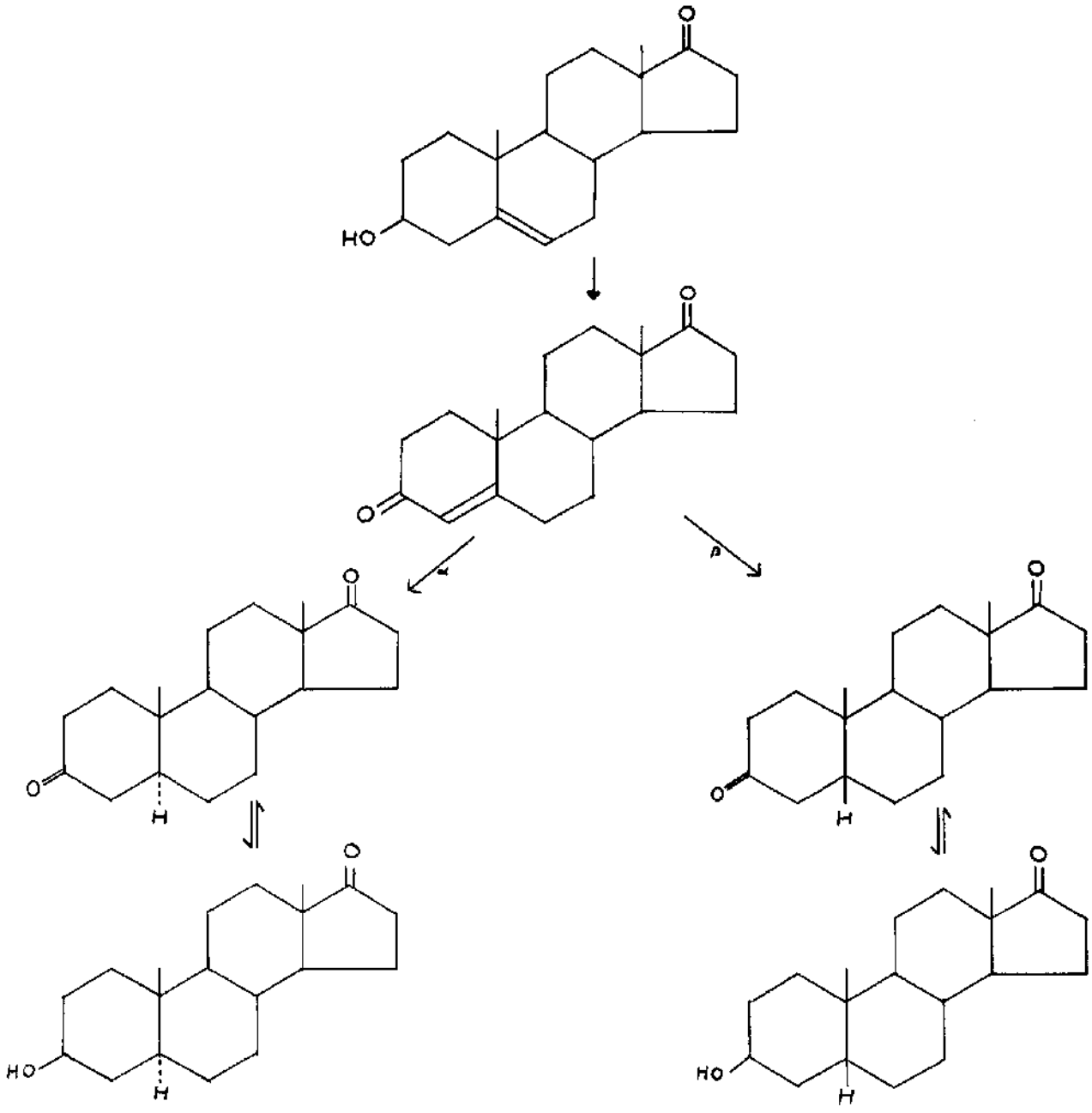
Text-fig. 11. The reactions preceding and involving the $\alpha 3\beta$- and $\beta 3\beta$-HSD.

dehydroepiandrosterone

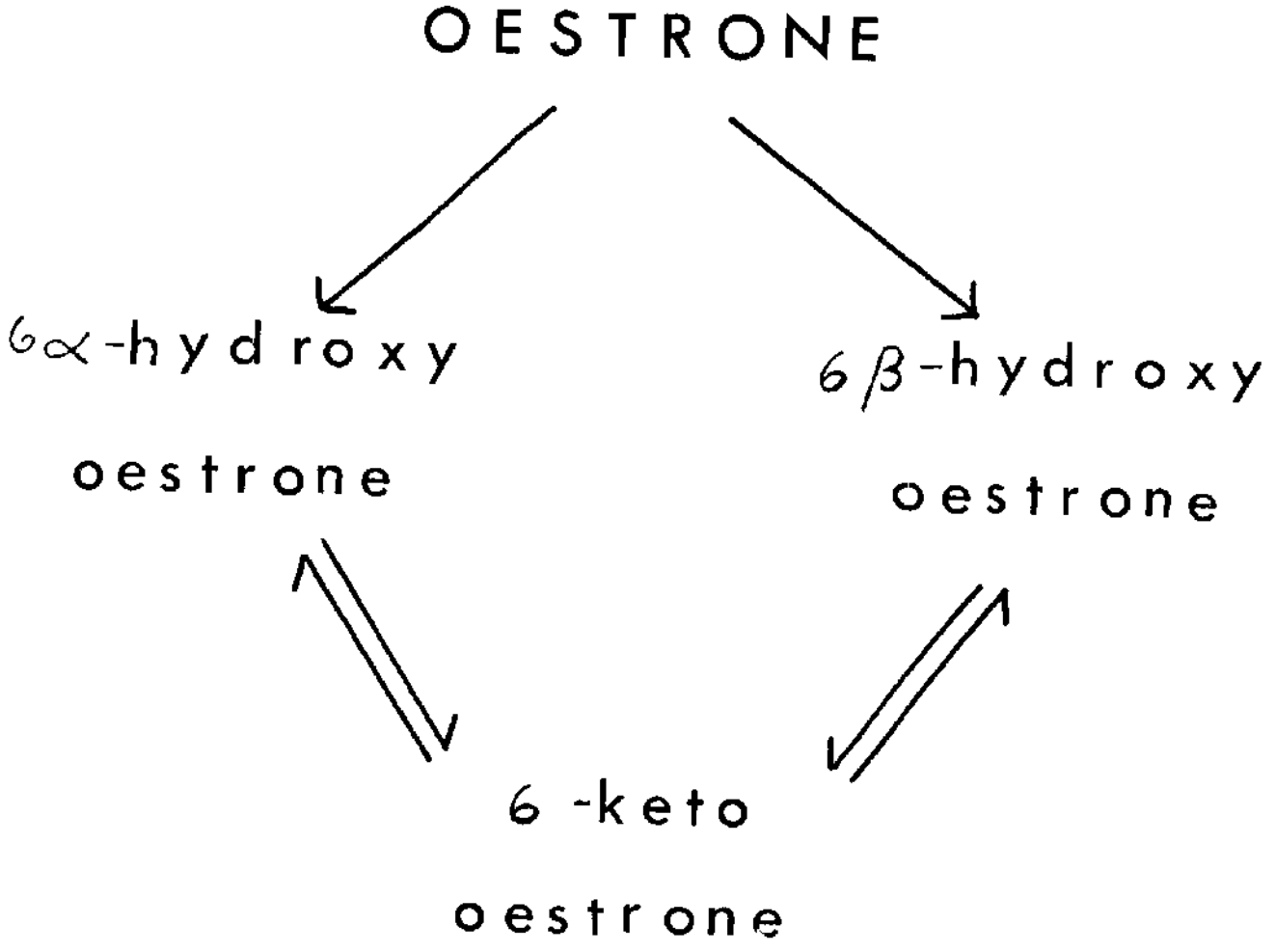
androstenedione

5α-androstan-3:17-dione 5β-androstan-3:17-dione

3β-hydroxy-5α-androstan-17-one 3β-hydroxy-5β-androstan-17-one


HO
O
O
O
α
β
O
H
O
O
H
HO
H
O
HO
H

Text-fig. 12. The reactions of the 6α- and 6β-HSD.


OESTRONE
6α-hydroxy
oestrone
6β-hydroxy
oestrone
6 -keto
oestrone

(C) $\alpha 3\beta$- and $\beta 3\beta$-hydroxy-steroid dehydrogenases

The significance of the presence of these enzymes in tissues is not clear. As can be seen from Text-fig. 11 their reactions are precisely similar to those of $\alpha 3\alpha$ and $\beta 3\alpha$ HSD except that the final compounds formed have the hydroxyl in the 3β position instead of 3α, and are 3β-hydroxy-5α-androsten-17-one, and 3β-hydroxy-5β-androstan-17-one. The evidence to date suggests that these compounds may be involved in an alternative pathway of steroid biosynthesis, Ferguson (1966).

(D) 6β-hydroxy-steroid dehydrogenase

This enzyme, with 6α-HSD may be involved in oestrogen metabolism, in the conversion of oestrone to 6-keto-oestrone (Text-fig. 12), these enzymes being responsible for the second part of the reaction, the first being mediated by a hydroxylase. It may also be involved in the production of 6β-hydroxy-cortisol, which is a polar steroid more easily excreted than the original compound.

(E) cortisol dehydrogenase and 11β-hydroxy-androstenedione dehydrogenase

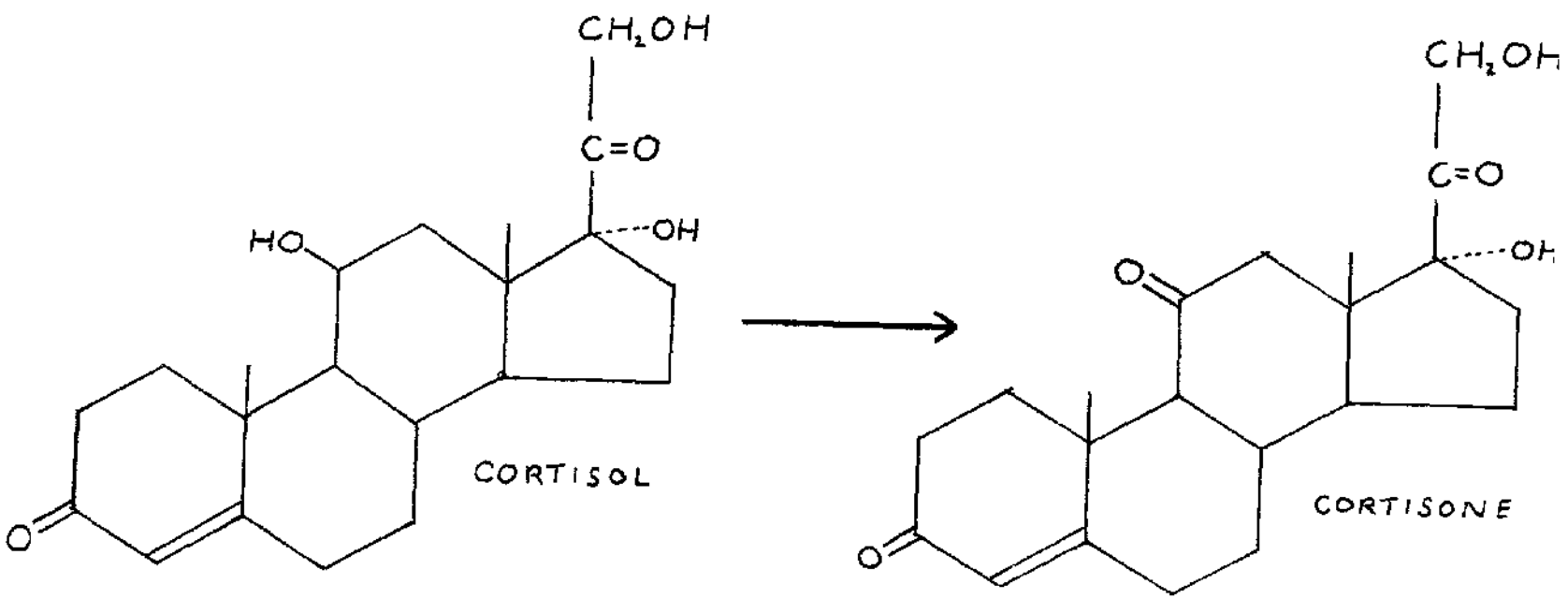
Commonly these two enzymes, both 11βHSD, are found together in tissues, although occasionally cortisol

dehydrogenase is found alone (Text-fig. 13). They are found in sites of androgen biosynthesis, and in sites of water and electrolyte transport across cellular barriers, for example, the renal collecting tubules, salivary gland ducts, and epidymal epithelium, Baillie, Ferguson and Hart (1966), McGadey, Baillie, and Ferguson (1966) and may be connected with the control of such transport, the presence of the enzyme indicating that the tissue concerned is a target organ for steroids with the 11β-hydroxy-molecular configuration.

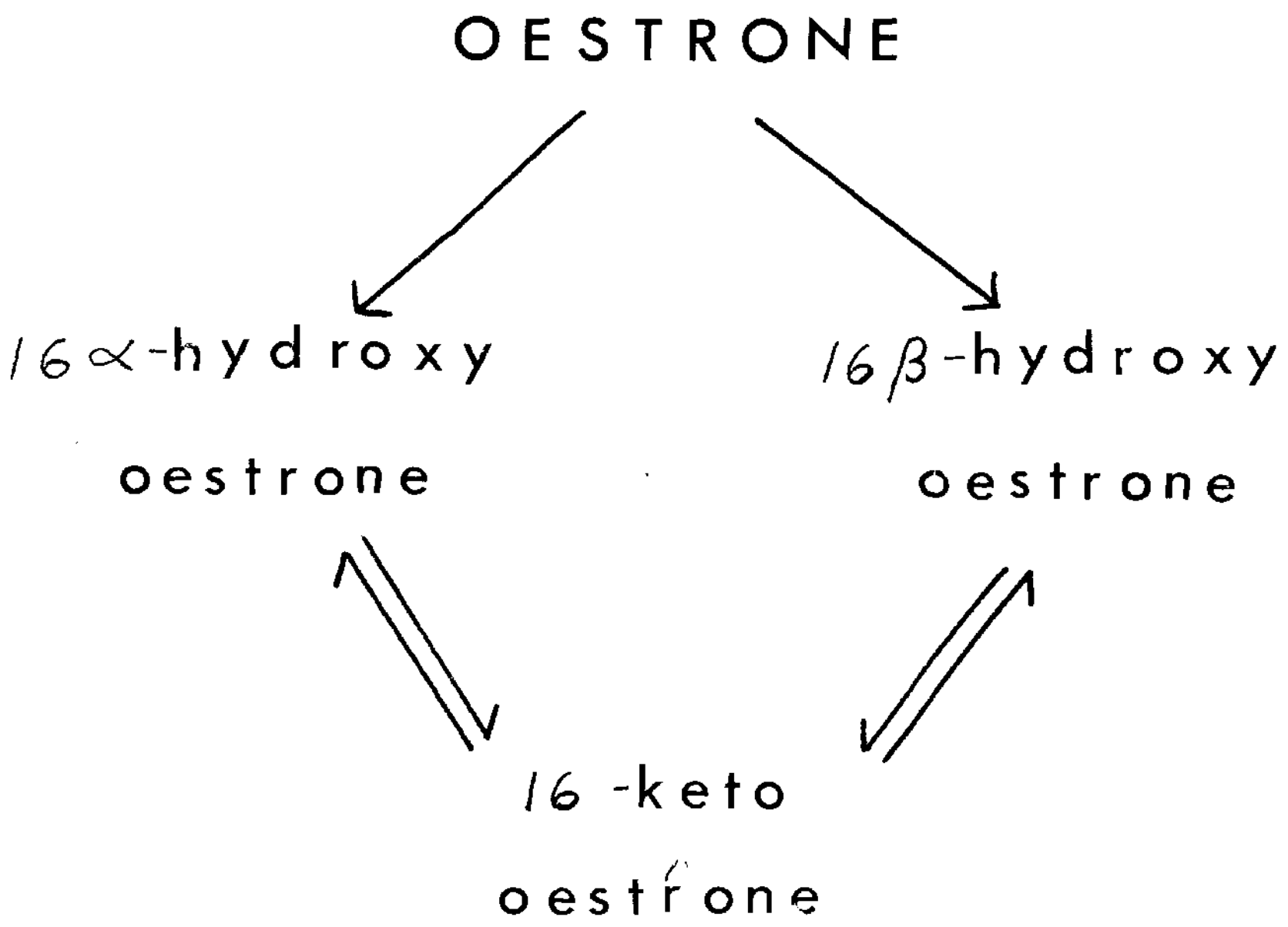
(F) 16β-(androgen)-hydroxy-steroid dehydrogenase, and 16β-(oestrogen)-hydroxy-steroid dehydrogenase. (Text-fig. 14)

These two enzymes, concerned with dehydrogenation at the 16β position of 16β-hydroxy-androst-4-ene-3-one, and 16β-hydroxy oestrone respectively are probably concerned with oestrogen and androgen metabolism, but our knowledge of their function, despite the fact that they stain quite heavily in some sites (vide infra) is at the moment scanty. In a similar manner to the 6β-HSD they are responsible for the second part of the reaction shown in Text-fig. 14.

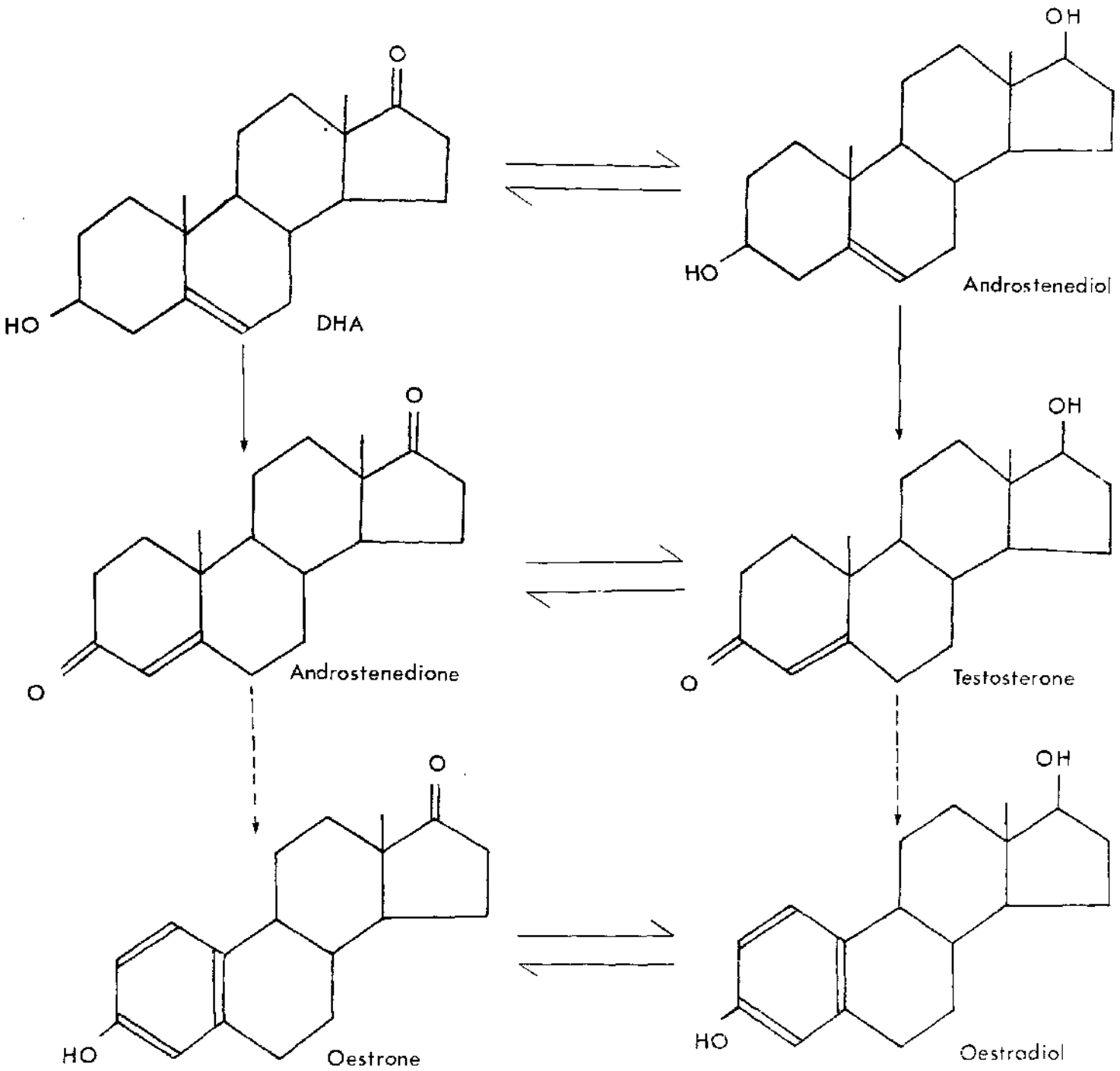
Text-fig. 13. Cortisol dehydrogenase in the conversion of cortisol to cortisone.


CH₂OH
C=O
HO
OH
O
CORTISOL
CH₂OH
C=O
OH
O
O
CORTISONE

Text-fig. 14. The reactions of the 16β-(oestrogen)-HSD. That of the 16β-(androgen)-HSD is similar.


OESTRONE
16α-hydroxy
oestrone
16β-hydroxy
oestrone
16-keto
oestrone

Text-fig. 15. The reactions of the 17β-HSD, including conversions 5 and 6 from Text-fig. 10, and the conversion (7) from oestrone to oestradiol. The reactions androstenedione to oestrone, and testosterone to oestradiol are shown as dotted lines as they are composite reactions.


HO
O
DHA
HO
OH
Androstenediol
O
O
Androstenedione
O
OH
Testosterone
HO
O
Oestrone
HO
OH
Oestradiol

(G) 17β-hydroxy-steroid dehydrogenases (Text-fig. 15)

These enzymes are further subdivided into the 17β-N-HSD concerned with androgen metabolism, and 17β-A-HSD concerned with oestrogen metabolism, the former being concerned with the first two conversions of Text-fig. 15 (conversions 5 and 6) and the latter with conversion 7 where the A ring is aromatic. These enzymes have also been studied, particularly the oestrone → oestradiol conversion, with respect to transhydrogenation (as shown for α3αHSD and β3αHSD) Langer and Engel (1958), Talalay and Williams-Ashman (1958), Talalay, Hurlock, and Williams-Ashman (1958). Their third possible function is as an indicator of target organs for the hormones oestradiol, and testosterone.

(H) 20β-hydroxy-steroid dehydrogenase

This enzyme is concerned with the production of 20β-hydroxy-progesterone from progesterone, and the production thereby of a progestational agent.

Implantation in rodents, prior to formation of the chorio-allantoic placenta

The depth of implantation in rodents varies widely, from superficial in rabbits to completely interstitial

in the guinea-pig. For this section of the thesis, two widely differing types were chosen, in both of which localization of the blastocysts just prior to, and during implantation is fairly easy - the rabbit, superficial implantation, where the blastocyst remains in the uterine lumen, and the rat, variously termed eccentric, oralmost interstitial implantation, in which the blastocyst rapidly comes to lie in a deep pocket of uterine mucosa, which quickly seals off, the maternal epithelium degenerating and bringing the trophoblast into contact with the stroma. These two types were also chosen for the wide difference which exists between their respective deciduas; and the rat because it presents two areas of very different glycogen metabolism.

The hormone requirements of implantation vary between these two species also. In both increase in circulating progesterone is required, Mayer (1963) - rat; Hapez and Pincus (1956). In the rat, an absolute essential is a surge of oestrogen, Mayer (1963) commencing on the 4th day after mating and extending to the 5th day. In the rabbit, on the other hand, addition of oestrogen in minute amount

will prevent implantation, Greenwald (1957). Some evidence linking these endocrine changes to the histochemical findings emerged during this investigation and will be presented below.

Previous histochemical studies on implantation have been concerned only with the rat, and have described the distribution of glycogen, (Krehbiel, 1937; Bridgman, 1948a, b; Christie, 1966a) lipid (Krehbiel, 1937; Bridgman, 1948a, b; Alden, 1947) or RNA (Krehbiel, 1937); or have been concerned with later stages (Wislocki, Deane and Dempsey, 1946; Padykula, 1958; Bulmer and Dickson (1960, 1961); Dickson and Bulmer (1960, 1961); Bulmer (1963); Padykula and Richardson,(1963); Bulmer (1965).

Non-specific alkaline phosphatase has also been studied, and its distribution in this investigation correlated well with that described by Pritchard (1947) and Wislocki, Deane and Dempsey, (1946), and with the recent description in the mouse by Finn and Hinchcliffe (1964), all of which describe an intense reaction for the enzyme in the antimesometrial decidua. As in Pritchard's results no staining of the embryonic tissue was observed with this method, but slight staining of the inner cell

mass and its derivatives (as observed in the mouse by Finn and Hinchcliffe) was observed using an azo dye method. No nuclear staining was seen, however, all activity being located in the cytoplasm. In any case such nuclear staining with the Gomori method, particularly on paraffin sections, is of doubtful significance, the general opinion at the moment being that it is artefactual.

Epithelial removal

The mode of disappearance of the epithelium in relation to the implanting blastocyst is one of the most vexed questions of reproductive physiology, and it was hoped that some evidence might be forthcoming from this material as to the part played therein by the blastocyst. In both species processes of trophoblastic cells were observed insinuating themselves between the antimesometrial epithelium and its basement membrane, and, in the rat at least, inclusions suggestive of pinocytosis were observed in the giant cells. However, in neither case was the activity of hydrolase enzymes exceedingly high, as might have been expected were the cells actively destroying the epithelium. In the rat these findings accord with those of Blandau (1949) who could not demonstrate proteolytic activity of

rat trophoblast, and of Cole (1965) who showed that the abembryonic giant cells of the rat will "roll back" a monolayer of cells in tissue culture, without obviously destroying them. Further evidence in favour of epithelial removal being purely passive, at least in the rat, comes from the recent experiments by Finn and Hinchcliffe (1965) in the mouse. Their results confirmed those of Krehbiel (1937) and many others on rats in showing that antimesometrial epithelial degeneration occurs in artificially (but not traumatically) induced deciduomata. Thus it would seem likely that in these two species, in the antimesometrial region at least, epithelial removal is not effected by active trophoblastic ingestion.

Mesometrially disappearance of the epithelium in the rat is again associated with the appearance of inclusions in the ectoplacental cone, whose cells show only moderate hydrolase activity and it would seem likely that here again the process of removal is not mediated by active trophoblastic ingestion. In the rabbit, however, differentiation of syncytiotrophoblast, and its contacting of the maternal epithelium is accompanied by increase in hydrolase

activity, and the possibility of some form of active removal is present, particularly as the maternal epithelium in this site becomes converted into a symplasma, and invasion thereof by trophoblastic cells is histologically evident. With the electroh microscope, Larsen (1961) has observed vacuoles in the syncytial trophoblast immediately following its fusion with the maternal epithelium, end, while these are not typically lysosomal the possibility that they contain degenerating products of the maternal epithelium in the process of absorption by the trophoblast cannot be excluded.

Although the question of the mode of epithelial removal cannot be answered fully histochemically, the metabolic changes leading to its destruction can be followed.

In both species glycogen content increases in the epithelium prior to degeneration, this increase being accompanied by increase (rat, and antimesometrially in rabbit) or no change (rabbit - mesometrially) in dehydrogenase activity with lactate, isocitrate, malate, and succinate. In the rat all of these enzyme activities fall off immediately prior to degeneration, but increase in the rabbit when symplasma formation occurs. The fall

off of activity in the rat is presumably an expression of metabolic death, while the increase in the symplasma of the rabbit may be a result of increased ease of access of nutritive materials from one cell to another when the intervening membrane is removed, or alternatively an expression of increase in the number of mitochondria, which are said (Larsen, 1961) to be numerous and closely packed in the symplasma. Unlike the rabbit, the rat shows an increase in lipid content in the epithelium, which is accompanied by an increase in acid phosphatase and esterase activity (possibly concerned in lipid degradation). Immediately prior to degeneration, increase in αGP and βOH activities occurs, and these enzymes may be acting on lipid metabolites produced by the strong esterase activity. Certainly no increase in αGP and βOH activities occurs in the rabbit epithelium, where lipid content is minimal.

Increase in hydrolase activity does occur, however, in rabbit epithelium, where very marked activity of acid phosphatase, in particular, is seen. As this accompanies symplasma formation, and is not seen in the deeper parts of the glands (which remain cellular), it may indicate

breakdown of intercellular barriers by lysosomal enzymes i.e. a form of autolysis. Why this should occur is uncertain, unless the localized extreme alkalinity postulated by Boving (1963) is sufficiently damaging to the cells to release the enzymes contained in the lysosomes.

In both species degeneration of the epithelium is accompanied by increase in furfuryl dehydrogenase (possibly concerned with RNA degradation), alcohol dehydrogenase, and glutamic dehydrogenase, suggesting that these enzymes are responsible, in part at least, for further metabolism of products of cellular degeneration.

Uterine secretion

In both species histochemical evidence of uterine secretion was present, and the material seen in the lumen contained acid mucopolysaccharide.

In the rat the volume of secretion appeared to be rather less in amount than in the rabbit. It was composed, histochemically speaking, mainly of debris and glycogen derived from epithelial breakdown, and increased considerably in amount when the latter commenced. Its function is presumably for embryonic nutrition, and certainly inclusions of similar staining reaction were seen in the abembryonic giant cells, and ectoplacental cone.

In the rabbit, the amount of uterine secretion appeared histochemically to increase particularly over the period immediately following shedding of the lemmas by the blastocyst. This is in contradiction to the findings of Lutwak-mann, Boursnell, and Bennett (1960) who state that the uterine fluid was thin and ample 1 to 3 days after mating, but scanty and viscous later in the progestational phase. However, the histochemical methods used here detect the content of the secretion, rather than the quantity, and show the increase in acid mucopolysaccharides (which would increase the viscosity) and in PAS-positive mucoprotein, as described by Boving (1963). It is of interest that the period of increased secretion described above corresponds to the period in which thiamine pyrophosphatase became visible histochemically localised in the Golgi apparatus of the mesometrial and paraplacental epithelium, suggesting that, prior to implantation, these are the sites of production of the secretion.

Further evidence in favour of this suggestion is seen in the fact that mucoprotein granules increase in this site up to the period of increasing secretion, and

then disappear. Parallel changes in G-6-P and 6-PG activities, alone of all the dehydrogenases, over the same period, also suggest increase in secretory activity as these enzymes are involved in RNA synthesis, presumably for protein production. The decrease in these enzymes and the RNA content is accompanied by an increase in FDH which may be related to RNA breakdown. That the uterine glands continue to secrete, presumably still under the influence of progesterone, is seen in the accumulation of masses of mucopolysaccharides in their lumina, after their openings are blocked by trophoblastic invasion. The functions of the uterine secretions will be considered later (see "Histiotrophe nutrition").

Decidua

As the studies described in this thesis cannot contribute to the problem of the induction of the decidua, it will not be considered here.

In the rat the decidual cells are derived from stromal cells, which enlarge and form the primary decidua. Simultaneous accumulation of RNA and G-6-P occurs (although not 6-PG) and their appearance there together

suggests that ribose for RNA production in this site can arise from glycogen via the pentose shunt (Text-fig. 8). The absence of 6-PG from this site remains inexplicable.

The RNA increase is of particular interest, as primary decidual development corresponds temporally with the oestrogen "surge" (Mayer, 1963) and oestrogen injection increases RNA synthesis in the uterus of immature rats (Gorski and Nelson, 1965). This finding suggests that the oestrogen surge may primarily alter the levels of ATPase activity which was observed to increase in the uterus, RNA synthesis being known to decrease uterine ATP levels under oestrogen stimulation (Aaronson, Natori, and Tarver, 1965).

An association between the pentose shunt and RNA production is again suggested by the decrease in RNA concentration with conversion of primary to secondary decidua, and the concomitant marked decrease in G-6-P and 6-PG activities.

The low RNA, G-6-P, and 6-PG concentrations in the glycogen wings, must be interpreted with caution, as the enzymes depend for their demonstration on NADP diaphorase activity whose activity is at a low level in this site.

In the antimesometrial secondary decidua, and stroma, glycogen accumulation and disappearance is accompanied by alterations in the activities of LDH, SDH, MDH, and ME which show (from 6½ to 8½ days) intense staining decreasing laterally. At 8½ days the activity begins to decrease. Thus the enzyme is distributed in the area of glycogen breakdown and, when all glycogen has been metabolised, the enzyme activity tails off. The intense glycolytic activity in this site, with much less activity of G-6-P and 6-PG, suggests glycogen breakdown via the Embden-Myerhof pathway, rather than via the pentose shunt. Beyond pyruvate marked activity of both anaerobic (LDH) and aerobic (MDH, SDH) pathways is here demonstrated, but it seems unlikely that cells will metabolise simultaneously aerobically and anaerobically. Evidence favouring the LDH pathway in this actively proliferating tissue is seen in Dickson (1966), who has shown that cancer cells tend to metabolise anaerobically; in the observations of Villee and Hagerman (1953), Gordon and Villee (1955), and Villee (1962) who have shown that decreasing oestrogen concentration, which occurs in the maternal blood supplying these cells, is accompanied by less complete oxidation of

glucose; and in the effect noted by Bever (1959) who, working on the uterus of ovariectomized rats, showed that progesterone increase (present in pregnant rats) is accompanied by increasing LDH activity. That the cells are gaining some of their energy requirements from aerobic metabolism, however is suggested by the intense SDH activity, and slightly less MDH. In any event, whatever the route, considerable energy production is obviously proceeding in these cells, and the breakdown of glycogen may be not only to glucose-6-phosphate and thence to glucose as suggested by the observed occurrence of glucose-6-phosphatase in the decidua, (vide infra) but also to more complete oxidation. The quantities of RNA present in the decidua suggest that the energy produced may be used for protein synthesis.

In the glycogen wings glycogen accumulation is not accompanied by any alteration in these enzyme activities, and the only change suggestive of decreased oxidation of glucose is a fall off of SDH concentration at 10½ days. It can only be assumed either that decrease in glucose oxidation for glycogen synthesis in this site is too

gradual to be detected histochemically, or that the glucose for glycogen synthesis is coming directly from the maternal blood stream.

Some evidence for control of the processes of glycogen metabolism in these sites can be gained from a consideration of the changes taking place in the intensity and localisation of staining for the specific alkaline phosphatases - adenosine monophosphatase, glucose-6-phosphatase, uridine diphosphatase, in relation to the pathway of glycogen synthesis and breakdown proposed by Stetten and Stetten (1960). These authors suggested that glycogen synthesis in most animals takes place through the action of UDPG-glycogen transferase (Table 18) and branching enzyme, while its breakdown is mediated by phosphorylase to glucose-1-phosphate. These enzymes have been studied in induced deciduomata by Bo, Smith and Colborn (1964), who considered that glycogen synthesis in this site took place from glucose-1-phosphate by phosphorylase. However, their results were very variable, and no definite conclusions can be drawn from them.

The importance, at first of the decidua and then of the glycogen wing region, in the production of glucose,

presumably for the nutrition of the embryo, can be seen from the changes in glucose-6-phosphatase. This enzyme builds up initially in the antimesometrial decidua, and here some correlation exists between the changes of concentration of glycogen, and of the enzyme observed. The glycogen increases to a maximum antimesometrially at 7½ days, falling off thereafter to none at 9½ days, and disappears, first from the centre of the region, while the enzyme builds up steadily over the period from about 6½ days onwards, starting in the centre. Thus the final step in the disappearance of glycogen from this site may well be due, in part at least, to this enzyme. Build up of glucose-6-phosphate is inhibitory to phosphorylase (Fridland and Nigam, 1965) possibly by a direct action on the enzyme system, possibly due to its inhibitory action on the activating effect of adenosine-5'-monophosphate (Morgan and Parmeggiani, 1964) in the conversion of phosphorylase b to phosphorylase a. For this reason also the build up of glucose-6-phosphatase by removing this substance would again aid in the breakdown of glycogen. Once the glucose is produced it could then be transferred

to the blood vessels of the region whose direction of flow is towards the embryo whose nutrition would thus be assured.

The changes seen in the other enzymes studied in the antimesometrial decidua, are more difficult to correlate with the alterations in glycogen content. As can be seen from Table 18, UMP, which would be produced by the action of UDPase, is inhibitory to glycogen synthetase, and activating to phosphorylase (Fridland and Nigam, 1965) and the destruction of UDP by the enzyme would make less available for re-phosphorylation and re-circulation in the UDPG-glycogen synthesis system. AMPase, on the other hand, by destruction of AMP would remove its activating action on phosphorylase (Morgan and Parmeggiani, 1964). Thus the two enzymes would seem to be competitive in action, with respect to the metabolism of glycogen. However, correlation of these results may be possible if one suggests that the control of synthesis and breakdown of glycogen in this site is mainly mediated by alterations in the level of UDPase, which builds up to a maximum in the centre of the decidua at 8½ days, starting at 6½ days,

when the accumulation of glycogen is quite well advanced. Accompanying the build-up of enzyme, the glycogen level begins to fall off, starting in the centre of the decidua, until, by 8½ days, it has almost all disappeared and the enzyme level is at a maximum. Thereafter the UDPase level falls off, presumably as there is no further glycogen to be acted upon. AMPase in this site would serve the function of destroying AMP after it had activated the phosphorylase required for the breakdown of the glycogen, and also by limiting the amount of phosphorylase available for breakdown, could act as a brake on the speed with which the glycogen is removed.

By 9½ days the importance, as regards supply of foodstuffs to the embryo, has shifted to the mesometrial region, and a reflection of this is seen in the build up of glucose-6-phosphatase in the glycogen wings, where it is presumably associated with the breakdown of glycogen and its release into the blood stream. While the blood vessels in this region are generally considered to be venous channels draining the placental site, rather than supplying blood to it (Young, 1956), it seems likely that the flow in them will be very variable in direction due to uterine contractions, and that the glucose released

into them will reach the embryo.

If the suggestion made with respect to the anti-mesometrial decidua, that the control of the glycogen level is through the action of UDPase, is to be followed in this site, one would expect the concentration of glycogen to fall rather abruptly to 8½ days and thereafter rise, thus exhibiting an inverse ratio to the concentration of enzyme. This does not occur, however, the quantity of glycogen increasing rapidly in this region from 7½ to 9½ days. A possible explanation of this apparently anomalous situation, however, can be seen in the abrupt rise in AMPase concentration, starting at 7½ days and continuing to increase over the entire period. This enzyme, by applying a firm brake on the quantity of phosphorylase available could counteract the increase in UDPase from 7½ to 8½ days, and allow glycogen accumulation to occur. After 8½ days, when the concentration of UDPase falls off, the conditions for glycogen synthesis would become favourable, and the rapid increase in glycogen which occurs at this time would be expected.

The significance of the intense reactions seen for TPPase in the decidua and glycogen wing sinusoids is less

certain. In the decidua the histochemical picture is different from that seen with any of the other substrates examined, although the distribution and alterations in this site are similar, and the concentration is generally less. Although this is by no means conclusive it would suggest that the localisation is to the Golgi apparatus, where it can only be assumed that it is concerned with the secretion of substances (possibly protein in view of the accumulation of RNA in these cells) into the blood stream to supply the embryo. Certainly if the localisation was to the intercellular spaces, as has been suggested for the rat adrenal (Penney and Barrnett, 1965), one would expect a similar picture to be present when β-glycerophosphate was used as substrate, which is not so.

Yet another site in which this enzyme may be associated with the Golgi apparatus is in the endothelium lining the sinusoids of the glycogen wings, where staining becomes very prominent between 7½ and 9½ days, and where secretion into the blood stream to supply nutrition to the embryo is presumably occurring. The increase in prominence in this site may be associated with thickening of the sinusoid lining due to swelling of the endothelium which commences at 7½ days, and is not to be confused

with the development of endovascular plasmodium, which occurs later and chiefly affects the maternal blood vessels in the mesometrial region supplying the octo-placental cone. The changes in ATPase distribution are also of significance in relation to the function of the decidua, and the hormone balance required for its induction.

In the antimesometrial decidua, which is an area of rapidly growing and enlarging cells, metabolically very active, considerable quantities of ATPase accumulate up to 8½ days. Presumably the enzyme in this site is associated with transport of substances to the embryo, whose nutrition at this stage is purely histiotrophic, and with synthesis of materials necessary for its continued growth and survival. As regards its function in transport, it is of interest that an inverse relation-ship exists between the concentration of ATPase (more centrally, less peripherally) and that of glycogen (more peripherally, less centrally) suggesting that the enzyme is involved, in this site, in transport of glucose derived from the breakdown of glycogen (vide infra) to the embryo.

With decrease in importance of the antimesometrial decidua, as the ectoplacental cone becomes bathed in maternal blood, and the source of embryonic nutrition tends to shift towards the mesometrial region, the concentration of ATPase falls off slightly, although the region immediately surrounding the embryo - the zone of trophoblastic giant cells in which the maternal blood is thought (Bridgman, 1948) to circulate - remains intensely positive in reaction. However, the reaction of the lining of the maternal blood vessels supplying the ectoplacental cone becomes very intense, as does that of the sinusoids in the glycogen wing regions. The staining in this site would seem to be associated with the swollen endothelium visible on histological preparations, endovascular plasmodium as described by Bridgman not yet having developed, and is probably associated again with transport across cells either of glycogen precursors into the glycogen cells, or of glucose derived from them into the maternal blood for the nutrition of the embryo.

The changes seen in the concentration of ATPase between $3\frac{1}{2}$ and $4\frac{1}{2}$ days are of interest. This is the period of the oestrogen surge (Mayer, 1963) which is followed, at

4 days and 16 hours in our strain of rats, by the appearance of the positive "blue" reaction (Psychoyos, 1961) which he considered to be indicative of an increase in capillary permeability. Certainly the accumulation of water in rat uterus is known (Boettiger, 1946) to be associated with oestrogen, and transport of water across cell membranes is associated with ATPase. Thus the primary action of the oestrogen surge would again appear to be to cause an increase in ATPase activity in the capillaries and stroma of the uterus, thereby producing an increase in their permeability, which leads to the 'blue reaction'. The localisation of the increase in enzyme activity to the antimesometrial region may be a factor in bringing about the preferential implantation of the embryo in that part of the uterus.

The significance of the presence of hydrolytic enzymes in the rat decidua and glycogen wings is not clear. Antimesometrially the increase in activity towards the end of the implantation period may be associated with the incipient death and degeneration of this area, but the presence of large quantities of acid

phosphatase, but not esterase, in the glycogen wings is inexplicable, unless one suggests that glycogen release from this site (the occurrence of which was previously suggested due to the presence of glucose-6-phosphatase) is mediated by hydrolysis of the glycogen-protein bond thought to exist in cells, Stetten and Stetten (1960), by lysosomal enzyme. It is of interest to note that the occurrence of acid phosphatase in the glycogen wings was not observed by Bulmer (1965), who also stated that the majority of the esterase activity which he observed in these tissues was due to organophosphate resistant (i.e. A or C) enzyme, whereas in this material considerable quantities of B esterase (organophosphate sensitive) occurred. Some explanation of this difference may be found in his use of fixed sections, which fixation is known (Pearse, 1960) to destroy considerable quantities of enzyme. Again unlike Bulmer's results, no evidence of a zone of stronger hydrolase activity immediately surrounding the embryo was observed.

It would seem likely, therefore, that the function of the decidua is three-fold:-

(1) to provide glucose for the embryo.

(2) to provide protein for the embryo, due to its RNA content and high metabolic activity,

(3) to provide various other foodstuffs later in the implantation period by autolysis, rather than by a process of active ingestion by the trophoblast.

To these must be added, in view of the experiments of Kirby (1965) the function of acting as a barrier to excessive invasion by the trophoblast.

As judged histochemically, the decidua of the rabbit is metabolically, and anatomically, rather similar to the glycogen wings of the rat.

In both, thickened endothelium lines the blood vessels, and reacts strongly for adenosine triphosphatase, inosine triphosphatase and thiamine pyrophosphatase. However, in the rabbit, activity is seen with adenosine monophosphatase and uridine diphosphatase also, and, unlike the rat, activity decreases later in the implantation period. A similar spectrum of phosphatases is seen in the decidual cells, and decreases similarly, the decrease in AMPase preceding that of the remainder by 6 hours.

The dehydrogenases gradually increase in the decidual cells until 8 days and then decrease slightly, whereas

in the rat glycogen wings, no decrease occurs.

In both sites glycogen accumulates, and small quantities of RNA, and hydrolytic enzymes (including esterase in the rabbit) are seen.

Correlation of these findings with the control of glycogen metabolism by UDPase concentration suggested above cannot be obtained, unless one suggests that the rabbit decidua is not only synthesizing glycogen, but also - up to 8 days at least degrading it again for further use.

However, the overall picture in the rat glycogen wings, and the rabbit decidua, would suggest that both are storing glycogen for use later in the processes of implantation and placentation, or as a reserve of easily available energy.

Mesometrial stroma

Despite the fact that this area will form the decidua basalis of the placenta in both animals, the histochemical appearances are totally different. The accumulation of RNA, acid mucopolysaccharide, G-6-P, and 6-PG, seen in the rat is not paralleled in this area in the rabbit, whereas the latter shows increase in all alkaline phosphatases, which occurs only in the centre of the area in the rat.

The reason for these differences remains unsolved, unless it be that this area in the rabbit will largely

degenerate as the separation zone (although this would suggest that considerable increase in hydrolases, which is not seen, should occur), while in the rat it will persist until term, when it is split by the reforming uterine lumen at the plane of separation. Maintenance of the tissue in the rat would require the continuation of dehydrogenase activity which is seen, although the concomitant increase in RNA, G-6-P, and 6-PG, while again indicating RNA synthesis via the pentose shunt, would not be expected unless increase in the volume of tissue in this area occurs. No measurements to indicate whether such increase is present have yet been made.

Giant cells

Two generations of giant cells arise in the rat, one antimesometrial - the primary trophoblastic giant cells, and one mesometrial, which will form a base for the chorio-allantoic placenta.

From a functional point of view, the trophoblastic knobs which arise antimesometrially in the rabbit are more akin to the rat primary giant cells than the obplacental giant cells which represent the remains of the antimesometrial trophoblast in the rabbit, and are

found under the maternal epithelium where they greatly enlarge later in pregnancy. No homologue of the rat mesometrial giant cells is found in the rabbit.

Histochemically too, the trophoblastic knobs are similar to the primary giant cells. Both show moderate activity with hydrolytic enzymes, and activity of all the dehydrogenases examined, although in the rat the latter increase, while remaining steady in the rabbit. Both are moderately PAS-positive, contain more positive inclusions, and moderate amounts of RNA, and are negative for acid mucopolysaccharides. In the rat, however, glycogen is present, whereas the rabbit "knobs" are negative for this substance. Finally the abembryonic giant cells in the rat show chiefly TPPase activity, while the trophoblast knobs in the rabbit are active with ITPase and UDPase.

The differences between these two tissues can probably be resolved on a functional basis. In the rat the abembryonic giant cells are probably concerned with removal of the maternal epithelium initially, and then with absorption, and possibly breakdown, of food materials from the uterine lumen, from the maternal blood circulating

round the chorionic vesicle, and from the degenerating decidua. Thus a source of energy, in the form of glycogen, and utilization of that energy, in the form of increase in dehydrogenase activity is always required during the implantation period. In the rabbit, however, the trophoblast knobs are probably primarily concerned with insinuation between the maternal epithelial cells (which may even be largely passive - Boving, 1963), the absorption of food materials being from the uterine secretion at this stage. Thus little energy is required, no glycogen is present, and the activity of the dehydrogenases does not increase. Further evidence in favour of this suggestion is seen in the increase in cytoplasmic inclusions which occurs in the rat giant cells (indicative of absorption), no corresponding increase being observed in the rabbit trophoblast knobs, and in the increase in dehydrogenase activity which occurs in the trophoblast between the knobs, indicative of increased energy production, possibly for the absorption of nutrients.

The question of the part played by these cells or processes in epithelial removal has already been considered (see "Epithelial Removal" above).

Histochemistry of morphogenesis

Associations between glycogen or acid mucopolysaccharides and morphogenesis have been noted by previous authors (vide "Physiological function" above), and further research by Bullough (1952) and Sorokin, Padykula and Herman (1959) has suggested a possible association between glycogen and mitosis and differentiating epithelia. Also Runner (1957) has suggested that morphogenesis is associated with carbohydrate metabolism - in particular the Krebs cycle enzymes, and Solomon (1958) described surges of enzyme activity (particularly LDH and MDH) in relation to differentiation in the developing chick. It is of interest, therefore, to study the distribution of glycogen and carbohydrate dehydrogenases, and of acid mucopolysaccharide, in relation to the developing structures in the rat and rabbit.

Acid mucopolysaccharide was observed in relation to embryonic structures in the rat only, in the cavity of the yolk-sac when the visceral endoderm was differentiating, and in the cavity of the inner cell mass when the ectoderm was differentiating. Thus in each case developing tissues lay in contact with this substance, suggesting an association

between it and differentiation. The nature of the association is not, however, clear.

In the rat embryo glycogen is seen in wave-like cycles of deposition and loss. One extends from the commencement of the period examined to 9½ days in the trophoblast and certain structures derived therefrom - abembryonic and lateral giant cells, although not the ectoplacental cone where central accumulation occurs. A second extends from 6 to 9½ days in the inner cell mass, parietal endoderm, and visceral, later to become yolk-sac, endoderm and a third from 7½ to 9½ days in the ectoderm, mesoderm, embryonic endoderm, and amnion.

The enzymes LDH, IDH, MDH and SDH show in embryonic structures, as might be expected, decreasing staining with lessening importance of the trophoblast and inner cell mass. However, the tissues derived from these two structures diverge markedly in staining pattern. Enzyme activity accumulates in the ectoplacental cone and giant cells, while a wave-like increase and decrease occurs in the ectoderm, mesoderm and endoderm (except the parietal layer). In the ectoderm, mesoderm, and endoderm, the

main energy requirements at this stage are for mitosis. Thus in this study, the peak of the wave of enzyme activity in each of these tissues follows 12 to 24 hours after the decrease in glycogen concentration, suggesting that glycogen is metabolised in this site via glycolysis for energy production for mitosis, enzyme activity falling off, when the glycogen has been expended, to "resting" levels.

A definite association between morphogenesis, glycogen, and carbohydrate dehydrogenase activity in these sites is thus observed.

Similar correlation is seen in the developing foetal placenta in the rabbit. Glycogen accumulation in the trophectoderm is associated with increase in dehydrogenase content. Syncytial differentiation shows loss of glycogen, but a considerable increase in LDH, MDH, IDH and SDH activities, and this is accompanied by lessening of these enzyme activities in the cytotrophoblast. Thus the association of a peak of glycogen deposition and loss, followed by a rise in dehydrogenase activity, to be followed by a decrease later (vide infra) is seen in the syncytiotrophoblast, which is again an area of differentiation.

The correlation in the cytotrophoblast is not quite so clear, presumably the continuance of glycogen content here is associated with continuing development of syncytiotrophoblast, which appeared in this material to be developing from cell division in the cellular layer.

The association between RNA, and G-6-P and 6-PG noted for the decidua was also seen in the embryo, and is of interest in view of the known association between RNA and cellular differentiation.

In the rat embryo correlation between RNA content and G-6-P and 6-PG activities is seen in most differentiating areas, for example, in the trophoblast and its giant cells. However, in the ectoplacental cone considerable quantities of G-6-P and 6-PG activities accumulate (particularly centrally) towards 10½ days without a corresponding increase in RNA and with a rapid accumulation of glycogen. The explanation of these observations may perhaps be found in the rapid enlargement of the ectoplacental cone which is taking place; this presumably requires increased protein synthesis and therefore increased RNA turnover. In this respect the concomitant increase in FDH in this site is of interest in view of its possible function (suggested

earlier) in RNA degradation.

In the rabbit, RNA accumulation is seen initially in the trophoblast knobs, where it is associated with their differentiation, and later in the developing cyto-, and syncytiotrophoblast. In the latter correlation between glycogen disappearance, G-6-P and 6-PG activities, and RNA synthesis is again seen (as in the mesometrial stroma of the rat) suggesting that glycogen breakdown in this site may be via the pentose shunt.

The overall picture therefore is one of correlated enzyme activities, leading to RNA synthesis where required, or to production of energy for mitosis; peaks of glycogen deposition and loss, followed by peaks of dehydrogenase activities, are seen in association with morphogenesis and differentiation, and confirm the separate findings of previous authors.

Comparative post-implantation Placentation

In this section certain tissues which are common to all, or nearly all of the species examined will be considered first. Thereafter certain individual tissues from different animals, where observations on their function can be made, will be discussed.

Trophoblast-placental

In general the histochemical findings reported here confirm those of other workers where relevant. Certain discrepancies exist, however, for example in the occurrence and distribution of glycogen, the presence of which was denied in cat trophoblast, Wislocki and Dempsey,(1946b) and rat trophoblast, Wislocki, Deane and Dempsey (1946), whereas it has been observed here in both situations. A possible explanation for this is the greater sensitivity of the PAS-dimedone method compared to the older Best's carmine or silver methods. Better histochemical methods may also explain the discrepancy as regards the distribution of β-glucuronidase in the trophoblast of the labyrinth and spongy zone of the rat, between the results of Bulmer (1963) who described high activity in both sites decreasing in the labyrinth towards term, and the present results which indicate low activity in the labyrinth increasing towards term, and no activity at all in the spongy zone. However, increased sensitivity of the method used does not explain Bulmer's (1965) description of lack of esterase activity in the spongy zone trophoblast

of the rat, where here is observed moderately strong activity, as the same method was used in eachcase. The only possible discrepancy here was that Bulmer used fixed sections, which is known to destroy considerable enzyme activity (Pearse, 1960). The present results are confirmed by those of Padykula (1958), although she observed acid phosphatase activity in the labyrinth and giant cells only over the last quarter of pregnancy, while here it is observed throughout, with a terminal increase. Finally, in the cat labyrinth, Wislocki and Dempsey (1946b) stated categorically that no acid phosphatase was seen in the foetal placenta, and further, that towards term the alkaline phosphate content decreased considerably in the interstitial matrix while increasing in the syncytiotrophoblast. The present results do not confirm their observations with either enzyme, and it is possible that this is due to the increased accuracy of localization obtained with more modern methods. Certainly they observed considerable nuclear staining with their acid phosphatase method, which staining is now considered to be artefactual and due to enzyme diffusion.

The most striking finding, as regards the trophoblast in this study, was the very great variability in histochemistry, not only between placental types, but also within the one type. This variability affected all classes of enzymes, and as an example one might take acid phosphatase in the term cat and dog, both endotheliochorial placentae, but with high activity in the former, and relatively low activity in the latter.

Another feature which varied considerably from species to species was the degree to which activity of the Krebs cycle enzymes fell off towards term, indicative of placental ageing. Despite the fact that decrease in permeability (possibly due to decrease in active, energy-requiring transport) is known to occur from nine-tenths of the period of gestation onwards in all placental types except the epithelio-chorial, Flexner and Gellhorn (1942), only the sheep, rabbit, and human showed decrease in LDH, MDH, IDH and SDH activities towards term. The sheep

also showed decrease towards term in ATPase activity, which may be concerned with sodium transport, in the inter-cotyledonary maternal epithelium which the results of staining for 11βHSD (possibly concerned with electrolyte transport) suggest may be concerned with that process in this species. No similar decrease was observed in the antimesometrial epithelium of the rabbit, however, although here again 11βHSD activity was observed. The sheep, rabbit and human also showed decrease in acid phosphatase activities (although not of non-specific esterase or βglucuronidase) towards term, again suggestive of decreased breakdown of materials presumably for utilisation or transport to the foetus, with placental ageing. In certain other species, particularly the cat and dog, alteration in enzyme patterns towards term occurred, decrease in activity of the steroid dehydrogenases in particular being seen. Whether these changes are indicative of placental ageing, or merely of a change in the direction of metabolic activity is questionable, however.

Glycogen was noticeably absent from the placental trophoblast of all species except the sheep, where it

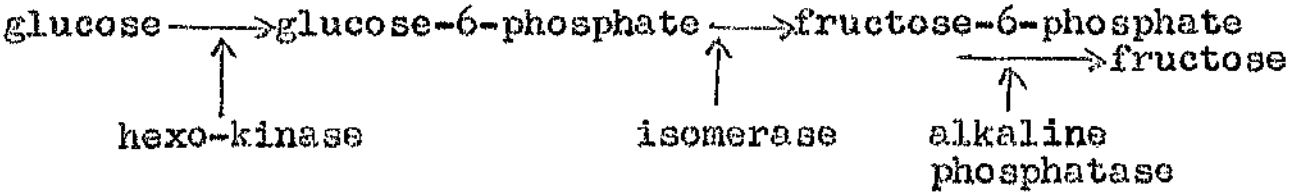
appeared briefly early in gestation in the diplokaryocytes, contrary to the findings of Wimsatt (1951); the cat, where its content decreased in the syncytial layer towards term; the rat, where the content increased markedly towards term; and the rabbit which showed traces at the earlier stage of gestation. The significance of trophoblastic glycogen is not certain, and certainly its distribution does not correspond to the "glycogen-rich" and "glycogen-poor" division proposed by Huggett (1961). Glycogen in the placenta has been correlated with both high, Szendi (1934) and low, Dempsey and Wislocki (1945) rates of metabolism. Certainly in this study all cells containing glycogen also contained high activity of dehydrogenases, thus confirming, as did Huggett (1961) Szendi's interpretation. A high level of metabolism would be expected in placental trophoblast where considerable activity with respect to transport, degradation, and synthesis of materials, is taking place. These findings do not confirm the suggestion of Fahmy and Huggett (1954) that glycogen and alkaline phosphatase do not occur together in the same site, as the cat trophoblast exhibited both activities, as did the early sheep diplokaryocytes. It is doubtful whether the suggestion made

previously, that glycogen is associated with morphogenesis applies here, as if this were the case, one would expect it to persist in the sheep diplokaryocytes throughout pregnancy when they are thought to be forming the syncytium on the walls of the placental crypts, Wimsatt (1951), and to be present in greater quantities at the growing bases of the cat trophoblastic cords than at the luminal ends, instead of the reverse. Also one would have to assume that the placenta of the cat and rat grew more actively than that of the other carnivores, or rodents, respectively, for which there is no evidence. Thus the function of trophoblastic glycogen would seem to be uncertain. However it is of interest that the concentration of trophoblastic glycogen in the placenta is inversely proportional to that in the haematoma trophoblast in the carnivores, or the decidua in the rodents, and it may be that it is acting as a store of readily available energy in these species, to be drawn on in case of emergency.

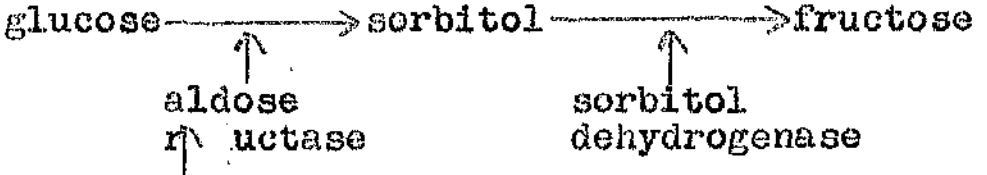
Placental glycogen, as opposed to trophoblastic, showed in this material the classification of Huggett

(1961) into the glycogen-poor sheep and horse placenta, in both of which it was only found in the blood vessels and allantoic endoderm, and the glycogen-rich. In this class were the carnivores, particularly, cat and then ferret in both of which the staining in allantoic endoderm and blood vessels was supplemented by trophoblastic (placental - cat, haematoma - ferret) and yolk-sac endodermal staining; less so, dog, which resembled ferret in its distribution; rodents, in which considerable decidual staining was seen; and human, where the foetal stroma contained glycogen.

Considerable interest has been roused by the observations of Hugget (review, 1961) and others that the glycogen-poor placentae are associated with fructose as the foetal blood sugar, while the glycogen-rich placentae are associated with glucose. Several investigators have attempted to define biochemically the enzyme pathway involved. Two possible routes have been suggested, either the Embden-Myerhof-Cori cycle, as below:-



or the sorbitol pathway:-



Hers (1957a, b) suggested that the sheep placenta converted glucose to sorbitol, which was passed in the blood stream to the foetal liver and was there converted to fructose, and Andrews, Britton, and Nixon (1959) demonstrated that sheep placentae perfused through the umbilical artery yielded sorbitol. However such a hypothesis would not explain the ability of sheep placenta to form fructose when perfused with glucose, Huggett (1961).

Histochemically alkaline phosphatase is found in small quantity in the trophoblast of both horse (chorionic plate) and sheep (syncytiotrophoblast) placentae. Sorbitol dehydrogenase is found in the same situation in the horse placenta, and particularly in the chorionic non-cotyledonary trophoblast of that of the sheep, although some activity is seen in the cytotrophoblast also. Thus the possibility of either metabolic pathway exists in these species although the presence of the enzymes does not necessarily indicate their use, Neil, Walker and Warren (1961). Biochemical evidence of the presence or absence of hexokinase and isomerase in sheep placenta is inadequate and clearly further research is required before

the problem of the route of metabolism is solved. Ironically the concentration of alkaline phosphatase (and, in the carnivores sorbitol dehydrogenase) is histochemically greater in the placentae of the species in the foetal blood of which only small quantities of fructose are found; these results for alkaline phosphatase have been confirmed biochemically, Ainsworth, Parr and Warren (1950).

RNA occurs in lesser quantity in the horse placenta then in that of the other species studied. Its significance is not certain, but it has been suggested that placental structures containing RNA are probably concerned with protein hormone production, Weber (1964). In this respect it is thought that the placenta, and in particular the Langham's cells, in the human produces chorionic gonadotrophin. The rat is known, Bourdel and Jacquot (1956) to produce luteotrophic hormone, although whether this is protein in nature is not definitely established. Thus the moderately high levels of RNA seen in the trophoblast of these species would be anticipated. In the horse, on the other hand, pregnant mare serum gonadotrophin is produced not from the trophoblast,

but from specialised endometrial "cups" in the first half of gestation, and therefore the lower levels of trophoblastic RNA would not be unexpected.

It would seem likely therefore, that other species which exhibit marked RNA concentration in the trophoblast may also be secreting protein hormones. Unfortunately evidence on this subject is lacking.

In the trophoblast of the placenta (with the possible exception of the horse and dog) no distinct differentiation of hydrolase activity into lysosomal - acid phosphatase, C-esterase, and β-glucuronidase, and non-lysosomal - B-esterase as described by Novikoff (1961) could be made, nor was there any correspondence either between the number of layers separating the two blood streams and the intensity of hydrolase activity, or between the thickness of the barrier and the enzyme activity. In the horse the distribution of activity of acid phosphatase, A and C esterases, and β-glucuronidases corresponded, although even here the β-glucuronidase activity was more extensively distributed, while B esterase was more widely found, and appeared alone in the allantoic mesenchyme. In the later

dog specimens, too, the lysosomal enzymes appeared together, while the activity of B esterase did not change. The cat trophoblast, in contrast, showed marked acid phosphatase, but no C esterase or β-glucuronidase, and moderate B esterase, and similar discrepancies occurred in other placental types. Thus it would seem that, in trophoblast at least, the intra-organellar distribution of hydrolytic enzymes is not so clear as suggested by Novikoff (1961).

It is of interest to speculate upon the significance of hydrolases in the placenta.

In the horse the activity is high, particularly in the areas of chorionic plate between the bases of the primary villi, and such an enzyme distribution would accord with the suggestion of Amoroso (1952) that the intervillous areas absorb material from the uterine milk, whichcontains a relatively high percentage of protein in this species (18 Gm % - Amoroso, 1952) while the villi, where enzyme activity of acid phosphatase and C esterase is less, are concerned with haemotrophic nutrition. The reason for the high β-glucuronidase activity here is uncertain, unless it be associated with conjugation of the

considerable amounts of steroid which appear histochemically (vide infra) to be produced.

In the sheep considerable quantities of uterine milk are again produced, but are probably absorbed by intercotyledonary trophoblast, which would account for the higher hydrolase activity seen in that site than in the cotyledon. Activity in the cotyledonary trophoblast is consistently higher in the syncytiotrophoblast than in the cytotrophoblast suggesting that a process of degradation of absorbed materials occurs through these two layers. In this species peak activity was again seen at 15 cm and then decreased (except for a terminal increase in B esterase) suggesting decreasing placental function as gestation proceeded.

Amongst the carnivores the degree of hydrolase staining was not proportional to the thickness of the placental barrier as judged histologically, the cat, which appeared to have the thinnest barrier being most active, with the ferret, whose barrier, due to the thickened maternal epithelium, was widest, next. In the dog acid phosphatase was least active, but β-glucuronidase and C esterase appeared. The level of B esterase showed no significant differences. The significance of these

findings is uncertain. Presumably the hydrolases (except B esterase) are concerned in protein degradation, and it is known, Buglia (1913) that injection of protein hydrolysate into the mother in the dog increases the non-protein nitrogen of the foetal blood. Comparative studies on other species have not, unfortunately been carried out. The B esterase content of the placenta here, as in all species, is presumably associated with lipid degradation to glycerol and fatty acids, Needham (1931).

All of the hydrolase enzymes are found in trace to moderate quantities in the haemochorial placentae, but, with the exception of the rabbit, and human, never reach the levels of activity seen in the cat, ferret, or horse. Their function in these species may be similar to that described above, but here again comparative study of transplacental passage is lacking. In certain species, also, notably the rabbit, the passage of protein across the placenta is complicated by the presence of the accessory yolk sac placenta, which is known to absorb unhydrolysed antibodies from the maternal circulation, Brambell (1954).

The functional significance of non-specific alkaline phosphatase is uncertain, and beyond confirming the observation of Dempsey and Wislocki that a layer of alkaline phosphatase constantly (with the exception of the horse placenta) intervenes between foetal and maternal blood streams its distribution will not be discussed.

Deductions regarding function can be made from the distribution of certain of the specific alkaline phosphatases, however. As might be expected ATPase activity was seen in the trophoblast, where it stained particularly in the brush border in the horse and human, in the cells in the sheep, and in the syncytium in the rabbit, and probably rat and guinea-pig, although I feel that the localisation in the last two might be disputed. In the carnivores less activity was seen, but marked staining was still found in other sites intervening between foetal and maternal blood (vide infra - "carnivores"). These localisations of this enzyme correlate well with its probable function, either in energy production, or directly in transport across the placental barrier. As regards the latter, the levels of ATPase activity in

certain of the species examined, particularly the horse, rat, cat, and extra-cotyledonary chorion of the sheep, were approximately proportional to the rate of transport of sodium across the placenta as measured by Flexner and Gellhorn (1942) suggesting a possible connection. The correlation suggested earlier between AMPase, UDPase activities and glycogen is again seen in these specimens, but only to a certain extent, and in the glycogenic placentae. Thus the glycogen found in cat trophoblast is accompanied by marked enzyme activity, with less in the interstitial matrix which is the usual site for other alkaline phosphatases in the carnivore placenta. Similarly the increase in glycogen in the rat labyrinth is paralleled by increase in AMPase and UDPase activities, and the decrease in the rabbit trophoblast is accompanied by decrease in enzyme activity also. However, both enzymes are seen in dog, ferret, guinea-pig, and human placental trophoblast, where no glycogen is observed. Thus the connection suggested can only be considered tentative, and further investigation, of a biochemical nature, into the metabolic role of these enzymes is

clearly required. TPPase activity is seen in the placentae of the horse, sheep, cat, dog, human, and traces in the guinea-pig and rabbit. It is of interest that the staining intensity corresponds fairly closely to that for steroid production (vide infra) and it may be that some connection exists, possibly of a secretory nature, in view of the known localisation of TPPase to the Golgi apparatus. The distribution of ITPase in these specimens tended to confirm the idea that this substrate is hydrolyzed by the same enzyme as ATP, Barden and Lazarus (1963).

The activity of αGP and βOH was never very high in the trophoblast, more activity being seen in the areas which exhibited lipid, i.e. the trophoblast of the chorionic plate in the horse, and of the cat labyrinth, where presumably the enzymes are involved in lipid degradation. The significance of the presence of both enzymes in the sheep cytotrophoblast and extra-cotyledonary trophoblast is uncertain, although in the latter site, they may be associated with the absorption of uterine milk, known to contain 1.2 gm % of fatty matter in the ewe (Amoroso, 1952). The traces of activity of both enzymes seen in the placentae of dog, rat, guinea-pig, and human, unless they be associated

with degradation of lipid material absorbed from the maternal blood stream, remain inexplicable.

The presence of LDH, MDH, IDH, and SDH activities in the placenta indicates the high metabolic activities of this organ, and show the production of energy for a variety of metabolic processes. All areas of trophoblast showed high activity, which increased during gestation particularly in the rat, in the human(with a terminal slight decrease), and in the sheep syncytiotrophoblast, although that in the cytotrophoblast remained constant after 15 cm. In the cat the activity was greater basally in the labyrinth, which correlates with the findings as regards steroid production in this animal (vide infra). The significance of the terminal drop in enzyme activity in the rabbit and human with this group of enzymes has already been discussed with respect to placental ageing. In the sheep the decrease is in the other carbohydrate dehydrogenases. The increase in activity noted in the rat, human and sheep may indicate greater placental transport of nutriment to the foetus, whose increase in weight in

these species is very marked in the later part of pregnancy. However, similar increases in foetal weight occur (so far as data are available) in the species in which no increase is present, and the significance of this finding is not clear. Certainly, with the possible exception of the rat, it is not associated with increase in steroid production as will be observed from the results to be presented below.

G-6-P and 6-PG activities are seen in all areas of trophoblast where they may indicate either RNA synthesis, or the generation of reduced NADPH which is required for steroid hydroxylation, Deane, et al (1962). In this material, trophoblast of the rat, guinea-pig, and rabbit, which did not exhibit steroid synthesis, but contained considerable quantities of G-6-P and 6-PG, also exhibited quite marked RNA concentration. On the other hand, the horse, dog, cat, human, and sheep cytotrophoblast, all of which were concerned in steroid metabolism, showed low or decreasing quantities of RNA, but contained quite marked G-6-P and 6-PG activities. The sheep syncytiotrophoblast is in an intermediate position, in that these both RNA and steroid metabolic enzymes decrease throughout pregnancy.

Here, however, only traces of G-6-P or 6-PG are seen. In species not exhibiting increase in RNA synthesis the ribulose-5-phosphate produced by 6-PG must presumably be passed back into the glycolytic cycle.

The activities of ADH, FDH, GDH and Sorb.DH were never very high in trophoblast. FDH tended to be present in sites of decreasing RNA concentration, further confirming its function in the degradation of that substance. ADH appeared in the same sites, and in approximately the same concentrations as αGP, suggesting that some non-specific glycero-phosphate dehydrogenation might be taking place through this enzyme. The only trophoblast in which GDH appeared in any quantity was that of the horse and sheep, suggesting that it might be concerned with the degradation of the protein content of the uterine milk. The function of Sorb.DH in relation to fructose formation has already been examined.

The activity of hydroxy-steroid dehydrogenases (HSD) was very varied from species to species, both in occurrence of activity, and in degree.

In the horse, only the trophoblast exhibited any HSD activity, and a wide range of hydroxy-steroids was

well utilized, indicating active steroid biosynthesis by this tissue. 20βHSD activity was more intense than that previously observed in any site in any species (review - Baillie, Ferguson and Hart, 1966). Short (1957) isolated 20β-hydroxy-progesterone from equine placenta, and it is possible that this is a major progestogen in this species. As has been mentioned previously (see "Physiological Function") the presence of Δ^5-3β- and 17β-HSD activity in this trophoblast is indicative of steroid hormone biosynthesis in contrast to metabolism, but the function of the high level of 3β- and 16β-HSD observed is unclear, and the possibility of other major metabolic pathways involving these compounds exists. The intense reaction indicative of biosynthesis in the equine trophoblast may be related to the finding that ovariectomy during pregnancy in the mare does not lower the urinary excretion of oestrogens, suggesting that the placenta is an active site of production of these hormones, Allan and Dodds (1935) and in further support of this Amoroso (1955) states that ovariectomy does not terminate pregnancy in this species after the early months.

The striking feature of sheep placenta is the marked absence of histochemically demonstrable Δ^5-3β-HSD from all tissue components, although a high level of α3β- and β3β-HSD was found. This compares to the Leydig tissue of ram testis where similar findings were recorded, Baillie (1965). As the cytotrophoblast and syncytiotrophoblast are examined throughout gestation changes occur in HSD activity, and differences appear between the two tissues. The 3α-, and β3β-HSD activities increase in the cytotrophoblast but <u>decrease</u> in syncytiotrophoblast, these changes not being occasioned by alteration in general level of cellular metabolism which remain unchanged in cytotrophoblast but <u>increases</u> in syncytiotrophoblast. Thus all trophoblast in the sheep is probably capble of steroid metabolism, and probably synthesis, although the biochemical route of the latter, in view of the absence of Δ^5-3βHSD is uncertain.

For this section a goat placenta (foetal part only) was also obtained, although under rather adverse conditions, being passed four hours after the birth of the foetus. However, although it was rather degenerate, the findings in trophoblast tended to confirm those for the sheep.

In the cat the trophoblast exhibits a wide range of HSD activity and is probably concerned in steroid biosynthesis. The striking differences in levels of activity between apices and bases of trophoblastic cords correlates with the similar distribution of enzymes of the Krebs cycle and probably reflects regional differences in trophoblastic activity, that nearer the base of the placenta being more recently formed. As mentioned previously decrease in HSD activity with increase in gestation occurred, but whether this reflects placental ageing, or merely an alteration in metabolic pathways is uncertain. Certainly no decrease in Krebs cycle enzymes was observed, suggesting that the latter possibility was the case. The presence of 11βHSD in the placental trophoblast contrasted with the situation in the ferret (vide infra) suggesting that electrolyte transport may take place in this site. Another possible explanation exists, however, that 11βHSD is associated with general steroid biosynthesis, for example of cortisol, the 11β-androgens, and oestradiol. Such an association has been noted in other tissues, Baillie, Ferguson and Hart (1966).

The dog trophoblast, like the cat, utilized a wide range of hydroxy-steroids and would appear to be involved in steroid biosynthesis. The decrease seen fromm id-pregnancy to term raises the same problems regarding placental ageing as in the cat, and similar conclusions pertain.

The ferret trophoblast does not exhibit HSD activity.

The labyrinthine trophoblast in general contrasts markedly with that of other placental types, in its lack of HSD activity, and does not appear to constitute a major source of oestrogens, Amoroso (1955). Previous workers, Deane et al (1962), Botte, et al (1966) failed to demonstrate Δ^5-3βHSD activity in the rat labyrinth and the present findings support these. The significance of the increase in α3β- and β3β-HSD towards term in the labyrinth is uncertain, although the possibility that the rat labyrinth is producing some oestrogen towards term cannot be denied. Similar increase of these enzymes occurs in the sheep placenta, and the observations of Cassida and Warwick (1945) that pregnancy can continue after ovariectomy in the ewe, suggest again that these enzymes may be involved in an alternative route of steroid

biosynthesis. Further evidence suggesting that this may be so is found in the parabiosis experiments of Muneaitsu and Segal (1959) who suggested oestrogen secretion by the rat placenta, although the site was not investigated. In our material, the activity of 17β-HSD increased markedly towards term. A similar increase was seen by Botte et al (1966) and ascribed by these authors to the second generation of giant cells (which lie mesometrial to the spongy zone) which they placed, so far as can be judged from their photomicrographs, in the middle of the labyrinth. This increase observed by them would appear to be the same as described here in the labyrinthine trophoblast. The function of the 17β-HSD here is uncertain, although its role as a transhydrogenase would correlate with the transfer of hydrogen from NADPH produced by G-6-P and 6-PG activity in the placenta (which was shown previously to increase) to NAD for increased energy production. Another possibility is the production of testosterone to maintain the increased anabolism characteristic of pregnancy in the rat, which is said to be placenta-dependent, Bourdel and Jacquot (1956). However, that this is unlikely is shown by the observations of Canivenc and Mayer (1953) who showed that rat placenta, on bioassay, contains no androgens.

Another labyrinthine haemochorial placenta - that of the guinea-pig - again showed no histochemically demonstrable steroid biosynthesis, weak HSD activity with few hydroxy steroids only being present and that only at 20 days. This confirms the observations of Deanesley (1960) who stated that the endocrine function of the guinea-pig placenta at this stage is comparatively low, and suggested that oestrogen secretion that was taking place was at a level which produced less than 1 mg daily.

The rabbit placenta was again disappointing, and the labyrinthine trophoblast showed only low activity with 3- and 3-, and 16-HSD, in early gestation, decreasing thereafter. In view of the similar decrease seen later with the Krebs cycle enzymes the possibility of placental ageing must be seriously entertained in this species. The multinucleate bodies, which are derived from the trophoblast, exhibit a similar picture of enzyme activity, with the exception that the activity of 16 HSD increases markedly towards term. The existence of the 3-, 3-, and 16-HSD activities in these cells may represent an alternative biosynthetic pathway as has been suggested

for the sheep, particularly in view of the suggestion by Amoroso (1955) that the rabbit placenta may be an oestrogen source.

In the human placenta, a wide range of oestrogens and progesterones have been isolated, Diczfalusy and Lindkvist (1956), Schmidt-Clemendorf (1961), and this tissue as a whole has been shown to be capable of converting acetate and cholesterol to a variety of compounds, including pregneneolone, progesterone, oestrone, oestradiol, cortisol and cortisone, Levitz, Cordon and Dancis (1956), Endroczi, Telegdy and Martin (1958), Osinski (1961), Sybulski and Venning (1961), Troen (1961), Suzuki, Takahashi, Hirano and Shindo (1962), Baulieu, Wallace, and Lieberman (1963). Histochemically a wide variety of hydroxy-steroids were well utilized by the human trophoblast of both placenta and chorion laeve, and the enzyme pattern observed correlated with the above biochemical findings. This accords with previous histochemical findings in trophoblast, Baillie, Calman, Ferguson and Hart (1966), Hart (1966a, b, c) and supplements them with the demonstration of $\alpha 3\beta$- and $\beta 3\beta$-HSD, activities. These findings correlate well with the accepted fact that the human trophoblast is involved

in active steroid biosynthesis. In agreement with Koide and Mitsudo (1965) and Hart (1966c) with NAD 17 -oestradiol dehydrogenase was found in both trophoblast and foetal blood vessels, but 17 -testosterone dehydrogenase in the vessels only, although the significance of this finding is not clear.

Decidua

The degree to which decidualization of the maternal stroma takes place varies markedly from species to species, and is most marked in those showing a more intimate relationship between the foetal and maternal blood streams. Thus the decidual reaction is sparse in the epithelio-chorial placenta of the pig, or syndesmo-chorial placenta of the sheep, goat, and cow; moderately developed in the carnivores, particularly the cat, less in the dog, and less still in the ferret; and pronounced in the placentae of man, and the rodents (Amoroso, 1952). However, from a histochemical basis it is of interest to include the maternal stroma in the sheep in this section.

In general the decidua exhibits marked hydrolase activity, particularly in the sheep, rat, guinea-pig and rabbit multinucleate decidual cells, and considerably less

in the dog, cat, and rabbit uninucleate decidual cells, those of the human occupying an intermediate position. The level of non-specific and specific alkaline phosphatases varies, too, both in degree of activity, and in the presence or absence of certain enzymes, and the cat, dog, and human exhibit the least activity, more being seen in the rodents, and marked activity in the sheep. All areas of decidua show moderate to pronounced activity of Krebs cycle enzymes, indicative of a fairly high rate of metabolism, and RNA synthetic enzymes are also seen particularly in the cat, rodents and human, and less in the remainder. Steroid dehydrogenase activity is only observed in the human decidua, and the rabbit multinucleate decidual cells, and these only with 16β-HSD and 17β-HSD not indicative of biosynthesis.

In the sheep the activity of all enzymes is highest around 15 cm and decreases thereafter, suggesting maximal metabolic function at that time. The association of high hydrolase and alkaline phosphatase activity would suggest breakdown of substances for foetal nutrition

and their transport to the foetal tissues. Similar enzyme associations, and possible functions are seen in the guinea-pig decidua basalis (the degenerating decidua capsularis exhibiting chiefly hydrolase activity), and in the cells lining the blood vessels of the rabbit decidua where glycogen synthesis and transport into the less active surrounding uninucleate decidual cells is presumably occurring. Conversion of these uninucleate cells to multinucleate ones is also associated with increase in enzyme activity including ATPase, but not hydrolases, possibly indicative of glycogen metabolism, breakdown, and transport out of the cells to the invading trophoblast. Further evidence in favour of this comes from the observation of 17 -HSD activity in these cells, which could indicate transhydrogenation from the reduced NADPH (produced therein by the active G-6-P, and 6-PG) to NAD for further energy production, although it could also be indicative of target organ utilization. Much less activity is seen in the dog and human decidua, and possibly correlates with the poor decidual development of the former, where less active transport and metabolism is required. The presence of 16β- and 17β-HSD in the

human decidua, not observed by Hart (1966b), may be related to transhydrogenase activity to increase the energy production in this not too active area. Alternatively the 16β-HSD activity may represent target organ utilization of oestrogens. The cat decidual cells exhibit high energy production, including activity of G-6-P and 6-PG, despite the histochemically demonstrable lack of RNA accumulation. The significance of this finding is uncertain. Finally the rat decidua basalis, which exhibits less energy production, but high hydrolase, and AMPase and UDPase activity would seem to be concerned with glycogen metabolism, probably breakdown for supply to the blood stream supplying the placental labyrinth. Here again the association previously noticed between acid phosphatase, AMPase, UDPase and glycogen degradation is observed.

Giant cells

A characteristic feature of the rodent placenta is the presence of trophoblastic giant cells, both antimesometrially earlier in gestation in the rat and rabbit but possibly not in the guinea-pig (vide infra), and mesometrially, where the giant cells of the rat and guinea-pig are

paralleled by the appearance in the rabbit of multinucleate bodies derived from the trophoblast of the placental fringe.

From a histochemical point of view the antimesometrial giant cells in the rat and rabbit appear to be homologeous, those of the rabbit being more active with all enzymes except β glucuronidase. The function of these cells is uncertain. In the rat they are presumably associated with destruction of the decidua capsularis, but in the rabbit the latter is never formed and it may be that these cells represent merely isolated masses of trophoblast lying beneath the antimesometrial epithelium. Certainly their staining reactions are similar, and they appeared, in this material, to degenerate and disappear around the same time as decrease of the metabolic activity of the placenta, suggesting that both are under the same endocrine control. In the decidua capsularis of the guinea-pig, and in the adjacent uterine lumen, cells, indistinguishable histologically, are present, whose histochemical reactions are similar to those of the decidua capsularis, and also of the antimesometrial giant

cells of other species, more particularly the rabbit, with the exception that they exhibit certain HSD activities - a wide range in those in the uterine lumen including $\Delta^5 3\beta$-HSD, indicative of steroid biosynthesis; slightly less in those in the decidua capsularis, but particularly 16β- and 17β-HSD. The nature of these cellsrequires further examination. As regards the luminal cells two possibilities spring immediately to mind. Firstly, in the guinea-pig the parietal trophoblast disappears early from a histological point of view, and the suggestion could be made that these cells are homologous with the trophoblastic giant cells in the rat placenta. In this respect the pattern of HSD activity is very similar. The second possibility is that these are macrophages en route for the decidua capsularis which is at this stage degenerating. It is known that oestrogens stimulate phagocytosis, Nicol, Bilbey, Charles, Cardingley and Vernon-Roberts (1964) and the utilization of 16β- and 17β- hydroxy-steroids in this site may reflect this possibility, as a target organ. Similar function may be ascribed to the cells in the decidua capsularis,

the apparent decrease in steroid utilization being due to lower levels of activity.

Steroid biosynthesis is one possible function of the mesometrial, (and, early, the antimesometrial) giant cells in the rat, although another possibility, the erosion of the decidua basalis, is suggested by their mucoprotein inclusions, and fairly high content of hydrolase activities, Dickson and Bulmer (1960). Previous authors have ascribed steroid biosynthetic activity to these cells, Deane et al (1962), Botte et al (1966), and present findings confirm their results. The first (antimesometrial) generation were intensely active with a wide range of steroids at 10½ days, but had lost all signs of activity by 17½ days. The second (mesometrial) generation showed activity in the 17½ day placenta which decreased towards term, with the exception of 16β-HSD which increased. Thus the function of the giant cells in this species is clearly one of steroid production.

The mesometrial trophoblastic multinucleate bodies in the rabbit were described by Sansom (1927) as "inactive degenerate structures". That this is not so has already

been observed (vide "Trophoblast" steroid enzymes). The possibility that they secrete cytolytic enzymes to aid in the digestion of the yolk sac contents also exists, in view of their high level of Krebs cycle enzymes, of G-6-P and 6-PG, and content of RNA, and of their alkaline phosphatase activity possibly concerned with transport out of their cells.

Trophoblastic giant cells are found in the sheep also, as diplokaryocytes. Increase in mucoprotein, acid mucopolysaccharide content, non-specific alkaline phosphatase activity found only in the cotyledon which decreases to 15 cm, and specific phosphatase content similarly distributed which increases to 25 cm and then remains steady, were the only differences observed in the staining reactions of these cells from those of the trophoblast in which they lay. The functions of these cells are uncertain. Wimsatt (1951) suggests that they form the syncytium lining the maternal crypts, and that they may possibly secrete some material into the lumen. It seems to me that this suggestion may be accurate earlier in gestation, and would accord with the decrease in mucoprotein, dialyzed iron staining and non-specific alkaline

phosphatase activity which occurs during this period. Later, however, when they are undiminished histologically but much less active with non-specific alkaline phosphatase their function would seem to be different. Possibly they are concerned with transport across the placental barrier also as suggested by their content of hydrolases, and specific alkaline phosphatases.

Yolk sac endoderm

Several different types of yolk sac were studied in this material, ranging from the simple one of the chick, and viviparous fish to the inverted yolk sac of the rodents.

The simple yolk sac, as found in the fish, chick and human (although the last named is said to be non-functional) shows moderate hydrolase activity, low to negative non-specific alkaline phosphatase, (but specific phosphatase in the chick and human, including AMPase, whose presence in human yolk sac was denied by McKay et al (1959) moderate Krebs cycle enzymes, and lower activity of the other carbohydrate dehydrogenases. G6P and βOH activities show interesting changes in the endoderm of the chick yolk-sac, where they appear after 5 days and increase. This corresponds to the change over from carbohydrate to lipid

as the primary energy source in the developing embryo, as described by Mahler, Wittenberger and Brand (1958). The presence of 17β-oestradiol-HSD in human yolk-sac is presumably associated with transhydrogenation and energy production, Hart (1966c).

The main difference observed between the simple absorptive yolk sac of the fish and cat, and the secretory yolk sac of the carnivores, Amoroso (1952), is the greater activity of the Krebs cycle enzymes, the degree of staining of which correlates, within the carnivores, with the glycogen content. Correlation with the degree of secretion, as observed histochemically was also present. The significance of the brush border staining with specific phosphatases is uncertain, unless the endoderm is absorbing certain materials and secreting others, as appears to happen in other sites, e.g. the epididymis. RNA is present in these cells as might be expected in a site of protein synthesis. All three yolk sac endoderms exhibited 16β- and 17β-HSD activity. The utilization of testosterone (17β-HSD) may be indicative of androgen metabolism and related to protein anabolism, which is known to be affected by androgens. Increase in this

enzyme, and of 16β-HSD in the cat yolk sac towards term paralleled the increase in histochemically demonstrable secretion. In the ferret α3β- and β3β-HSD activities were also present, suggesting that steroid secretion in this species may take place in this site - certainly no activity was present in the trophoblast.

As judged by the intensity of hydrolase activity, and the number of PAS-positive inclusions, the function of the rodent inverted yolk sac is mainly absorptive. Activity seems to increase after rupture of the parietal wall of the yolk sac, and peak hydrolase activity is seen at 17 days in the rat with a terminal drop in acid phosphatase content not observed by Padykula (1958), mid-gestation in the rabbit, and late in gestation in the guinea-pig. The presence of a brush border exhibiting specific phosphatase activity also suggests absorption, and this is further confirmed by the high Krebs cycle enzyme content. High activity of G-6-P and 6-PG are also present, but not associated with increasing RNA content, and the possibility of further energy production via transhydrogenation from NADPH to NAD is suggested by the presence in this site of 3α- and 17β-HSD activities. The presence of 16β-HSD in

this membrane is of interest, in view of the possible stimulatory effect of oestrogens on phagocytosis, Nicol et al (1964). In the guinea-pig GDH activity increases markedly towards term, as does that of β-glucuronidase whose activity is at a maximum in the yolk sac endoderm of this rodent placenta, suggesting that marked protein absorption and metabolism is occurring in this site.

The parietal endoderm is much less active in all rodent placentae, and exhibits activity of β-glucuronidase and FDH correlating with tissue, and RNA breakdown.

Insufficient material was available to compare the endoderm of the chorio-vitelline placenta with that of the free yolk sac in the carnivores, but results on the two early dog specimens suggested that the only difference was the presence of αGP and βOH prior to separation of the yolk sac endoderm from the placenta, which would correlate with the presence in the endoderm of lipid, Amoroso (1952). The presence of non-specific alkaline phosphatase in this site later in pregnancy, reported by Amoroso, is confirmed in this material.

Non-placental chorion and maternal epithelium, and uterine secretion

Certain limited deductions, concerning the chemistry of the uterine secretion may be made from its histochemical

reactions. Conklin (1963) suggested that alcian blue staining and colloidal iron positivity represent the presence of free carboxyl groups, and also of sulphate while azur A staining at pH 1.5 is indicative of sulphate or phosphate, and its absence is proof of their absence. Azur A at pH 4.5 is less specific. The decrease in azur A (pH 1.5) staining after methylation indicates sulphate groups also. On this basis the uterine secretion of the horse, cat, and dog contains sulphated acid mucopolysaccharides, while those contained in the secretion of the sheep, and ferret are non-sulphated but contain free carboxyl groups. All secretions studied, being PAS-positive, but diastase resistant would also appear to contain some muco- or glyco-protein. Glycogen is present in the secretion of the sheep, dog, and ferret, and that of the rodents. These results differ markedly from those of Buchanan (1966) who stated that neither glycogen nor acid mucopolysaccharide were present in the uterine secretion of the ferret.

Evidence for the passage of materials from the uterine secretion into the trophoblast is seen in the horse, and

carnivores in the form of PAS-positive intra-cellular apical inclusions, a moderate to high content of hydrolases, specific phosphatases (except the ferret) and, as suggested by marked Krebs cycle enzyme staining, high activity in the cells. In the horse these features were found particularly in the chorion between the bases of the primary villi, this area in particular being associated with absorption of the uterine milk, Amoroso (1952). Functions of this area in relation to steroid secretion have already been described. In the sheep the activity of the uterine glands is very high with non-specific alkaline phosphatases and, in the Golgi region, with TPPase, but hydrolase activity is less. These features correlate with their function in producing uterine milk, and the changes in the Krebs cycle enzymes suggest that their activity becomes maximal at about 15 cm and thereafter remains steady. Such a situation exists in the horse, Amoroso (1952) where the "milk" is copious earlier in gestation, and could be expected in the sheep also as this early period is that of the development of definitive cotyledonary attachment when histiotrophic nutrition would be most required. The presence of 11βHSD in the maternal

epithelium would suggest the possibility that electrolyte transfer from mother to foetus could occur, or be controlled, in this extra-cotyledonary site also, and certainly the barrier to this is decreased here by the presence of intra-epithelial capillaries, intensely active with specific alkaline phosphatases including ATPase, in the maternal epithelium and trophoblast. The absorption of uterine milk by the trophoblast is accompanied by the presence of hydrolases, and a brush border staining intensely with specific alkaline phosphatases. Here again activity seems to be greatest at 15 cm and thereafter decreases slightly. The presence of αGP and βOH (concerned in lipid degradation) correlates with the presence in the uterine milk of lipid.

In the rodents the maternal epithelium lining the glands (in these antimesometrially) which presumably produces the uterine secretion, exhibits a common picture of hydrolase activity, and phosphatases - specific, in the apical part of the cell, and non-specific, in the cat and guinea-pig later in gestation. Activity of the Krebs cycle enzymes is also present indicating energy production by the cells. In the rabbit 11βHSD activity

appears here at mid-pregnancy and increases to term, its appearance immediately preceding the increase in electrolyte transport across the placenta described by Flexner and Gellhorn (1942) and suggesting some causal connection; although no connection with water absorption would appear to be present, Paul, Enns, Reynolds, and Chinard (1956).

A final feature of the non-placental region, in this case, of the sheep maternal epithelium is worthy of mention. Most authors are agreed that this maternal epithelium is destroyed early in pregnancy, and not regenerated until the fourth month (foetal length - 38 cm). In this material intact epithelium was observed from 35 days of gestation on.

Allantois

The allantoic endoderm uniformly exhibited the presence of glycogen with the exception of that of the cat. Hydrolase activity was also present and specific alkaline phosphatases, except in the dog and ferret. Carbohydrate dehydrogenases were seen, greatest in the horse, less in the sheep, cat, and dog, and negative in the ferret. Presumably the function of this tissue is to absorb fluid

from the allantoic cavity, which would accord with the histochemical findings. Certainly in the cat the volume of allantoic fluid decreases towards term, with a terminal rise. In the horse it is very variable. The presence of high GDH activity in the horse allantoic endoderm is possibly connected with this absorption, although data on the composition of the allantoic fluid is lacking. In the sheep 17β-oestradiol hydroxy-steroid dehydrogenase is present in this membrane, a pattern of activity common to other endodermal derivatives such as intestinal epithelium, yolk sac, and pancreas, Ferguson (1966). The presence of the 17βHSD in this site may be related to energy production by transhydrogenation, or may be an expression of target organ steroid utilization.

Haematoma region

Certain changes are observed when the maternal epithelium and trophoblast are compared to those of the non-placental region, in the dog "green border", cat "brown border" and ferret "haematoma".

In the maternal epithelium of the cat and dog non-specific esterase, and GDH are increased, and 16β- and 17β-HSD are present. In the trophoblast, PAS-positive

inclusions are seen, and esterase activity is again increased (C-esterase appearing), as is that of G-6-P and 6-PG, and of ADH, FDH, GDH, and SorbDH. These enzymes activities are similar to those observed in the yolk sac of rodents, which is thought to play a significant part in iron absorption in these species, Bothwell, Pribella, Mebust, and Finch (1958), Campbell and Nylander (1951), Lambson (1966), Nylander (1953), Wohler (1955). Iron transport across the gut is regulated by ferritin (an iron-protein complex), Granick (1946), and ferritin is known to be transported across the rat yolk-sac, Nylander (1953), Wohler (1955), and has recently been demonstrated in the visceral endoderm of the latter, Lambson (1966), where it has been observed in apical vacuoles. G-6-P, 6-PG, and GDH can be concerned with protein metabolism and might well be involved in conjugation of iron with a protein in the maternal epithelium, the material in the lumen as judged histochemically being in a conjugated form, and subsequent de-conjugation in the trophoblast. The activity of esterase seen in both sites may also be concerned in protein metabolism, certainly

the C-esterase observed in the trophoblast and the inclusions seen in the trophoblast could be conjugated material prior to degradation. The significance of the 16β- and 17β-HSD in the maternal epithelium is uncertain, unless the 17β-HSD is acting as a trans-hydrogenase to ensure the ferrous state of the iron required for its absorption. The 16β-HSD may represent target-organ oestrogen utilization, although no information regarding the effect of oestrogens on iron metabolism is available.

In the cat, the masses of "symplasma" observed are histochemically very similar to syncytiotrophoblast. Bjorkmann (1957) suggested that they are derived from maternal connective tissue. It seems to me, however, that it is far more probable that this tissue is foetal syncytiotrophoblast invading the maternal tissues, possibly to contain the haematoma contents in place, by attaching the trophoblast bounding them on one side, to the maternal epithelium on the other.

Sheep

Two aspects of this placenta require further discussion, the first being the special staining reactions

of the foetal mesoderm.

This tissue is intensely metachromatic at 15 cm, and decreases thereafter, a reaction which indicates the presence of acid mucopolysaccharide, probably with free carboxyl groups, and possibly sulphated. The possibility exists here, as in the developing rat embryo, that this material is associated with morphogenesis, as proliferation of the villi is associated with decrease in its quantity. 16β-HSD is present here also, and the following possible association between it and the acid mucopolysaccharides exists. This enzyme is indicative of oestrogen metabolism, and Zuckermann (1955) has shown that the material which accumulates in the subcutaneous connective tissues of sexual skin, under oestrogenic stimulation, contains large quantities of hyaluronic acid, and chondroitin sulphate.

The second subject is that of the origin and nature of the tissue lining the maternal crypts, which is variously described as foetal, Assheton (1906), Andresen (1927), or maternal, Wislocki (1941). Lawn, Chiquoine and Amoroso (1963) in origin, and of its relation to the binucleate giant cells. Histochemically the syncytiotrophoblast resembles the maternal epithelium more than

the cytotrophoblast, the reactions of the hydrolases and most of the dehydrogenases being similar, and varying quite markedly from those of the cytotrophoblast. However, similar variations between syncytiotrophoblast and cytotrophoblast are frequently observed in a variety of species, and the steroid reactions were similar, although the degree of staining varied in opposite directions. Also Lawn's paper described absence of a basement membrane external to the layer he called maternal epithelium, and elongated micro-villi extending into maternal tissues. Thus it would appear in the light of histochemical evidence that the "syncytiotrophoblast" of the sheep placenta may possibly be true syncytiotrophoblast, but modified in morphology, and that the placenta may be truly syndesmo-chorial in nature. The function of the diplokaryocytes is uncertain, but, as their numbers do not seem to vary during gestation, while their staining reactions, at least for non-specific alkaline phosphatase, decrease towards term, the possibility exists that they are secreting alkaline phosphatase into the space between the cytotrophoblast and the adjacent tissue to aid in absorption of nutritive materials.

Wimsatt (1951) ascribed a secretory function to these cells, and the presence therein of TPPase activity would confirm this. The decrease of staining of certain cytoplasmic constituents, notably G-6-P, 6-PG, RNA and GDH (all concerned with protein metabolism directly or indirectly) would suggest that the degree of enzyme synthesis and secretion decreases towards term. This would accord with the increasing attenuation of the syncytiotrophoblast, and presumably, therefore, of the barrier between foetal and maternal blood streams.

Carnivores

In these the nature of the interstitial matrix, the thickened maternal endoderm of the ferret, and the function of the spongy zone are of interest.

The interstitial matrix, from its acidophilia and histochemical staining reactions, particularly the highly positive diastase-resistant PAS reaction, the negative staining for acid mucopolysaccharides, and the intense positivity for alkaline phosphatase, ATPase, ITPase, and UDPase, would appear to be little more than a basement membrane for the maternal epithelium, similar

to that described in the Chiroptera by Wimsatt (1958), rather than a condensation of maternal connective tissue.

The thickened endoderm of the ferret placenta appears to be concerned at least partly with steroid production, although the activity of the HSD demonstrated was not very high. Histochemically it also exhibits several features adaptive towards absorption of materials and their transport to the external syncytiotrophoblast, in the form of hydrolase activity, which is higher here than in the endothelium of the other carnivores, and of brush border activity, seen with ATPase and ITPase, and quite intense AMPase and UDPase activities. Carbohydrate dehydrogenase activity was also quite marked.

The spongy zone varies from one carnivore to the other, the amount of histiotrophe lying in the glands beneath the basal cytotrophoblast being greater in the cat, less in the ferret, and less still in the dog. The presence of RNA, G-6-P, 6-PG, FDH, and GDH would suggest protein synthesis in the basal cytotrophoblast possibly associated with cellular proliferation, and active Krebs cycle activity is also present for energy production. The presence of hydrolase activity would also suggest

active absorption of material from the histotrophe, as would the presence of specific alkaline phosphatases which stain a brush border.

The maternal epithelium in the cat and ferret also shows hydrolase activity, particularly near the encroaching trophoblast, suggesting that active cell autolysis may contribute to the histiotrophe. In the dog, acid phosphatase activity is higher in the contracted parts of the glands than the dilated parts nearer the trophoblast and the same is observed with the alkaline phosphatases and with 3β- and 16β-HSD the presence of which may be related to the copious acid mucopolysaccharide secretion by these glands. Certainly association between 16β-HSD and acid mucopolysaccharide has already been noted in the foetal mesoderm of the sheep. The carbohydrate dehydrogenase activity in this species is localised in reverse concentrations to the remaining enzymes and the significance of this finding, apart from showing that the differences in HSD concentrations are not merely due to energy alterations, is uncertain. Presumably the energy produced is used for secretory processes. In the cat the hydrolase activity in the basal parts of the glands is less. Energy for

secretory processes in these cells can be derived as judged histochemically from the glycogen content, both directly from glycolysis and oxidation through the Krebs cycle, and indirectly via the pentose shunt and trans-hydrogenation, as 17β-HSD is present here. Finally in the ferret, where the histiotrophe is considerably less the function of the spongy zone may be similar to that in the cat, similar histochemical findings being present. Also observed, however, in the maternal epithelium is 11β-HSD, suggesting that electrolyte transport may be regulated here, rather than in the labyrinth, where maternal and foetal blood streams are separated by a thickened endothelium, interstitial matrix, syncytiotrophoblast, and foetal endothelium, possibly a rather thick barrier to electrolyte transport.

Rodents

Several as yet unmentioned aspects of the rodent placenta are of interest. In the rat and guinea-pig an area of trophoblast supplied purely by maternal blood, with no foetal blood stream, and known as the spongy zone is present. This area seems to act as a reserve of trophoblast for extension of the placental labyrinth.

Morphologically it differs in the two species, that of the guinea-pig being syncytial, but of the rat cellular. Histochemically the labyrinthine trophoblast of each animal, when compared to that of the spongy zone, showed greater activity with all enzymes studied (except with acid phosphatase, and ATPase, ITPase, UDPase and TPPase in the guinea-pig), which would accord with the greater functional activity of the labyrinth in foeto-maternal exchange. The increased activity of acid phosphatase, and certain alkaline phosphatases in the guinea-pig spongy zone, which was most marked immediately adjacent to the labyrinth may be associated with the extension of the latter which appears to proceed at a very high rate in this species. Such a suggestion would also explain the increase in degradative dehydrogenases (ADH, FDH), found in this site also.

Also found in the spongy zone of the rat are vacuolated cells, which store glycogen - the glycogen-cells - and the question of their origin, which was disputed for some considerable time, Bridgman (1948b) is thought to have been decided, in favour of a maternal origin, Padykula and Richardson (1963). No contribution to this question

can be made here, but, in view of the variation of glycogen deposition seen here, increasing to a maximum at 17 days and thereafter remaining steady, it is of interest to note a wave of carbohydrate dehydrogenase activity, reaching its maximum at 17½ days and then decreasing, with LDH, IDH, SDH, and MDH where the later fall off in activity might be expressive of decreased glycogen utilization, and with G-6-P and 6-PG which could be concerned via RNA with synthesis of the protein to which glycogen is said to be bound in cells, Stetten and Stetten (1960).

The origin of the endovascular plasmodium has also been questioned in the rat, Mossman (1937), Bridgman (1948). Histochemically, its cells are almost identical with the labyrinthine trophoblast, with the exception of acid phosphatase. Thus it would appear likely that it is of foetal trophoblastic origin.

Certain enzymes are associated with the fibrinoid capsule, adjacent to which the tissue is split to reform the uterine lumen, in the rat. Particularly evident are the hydrolases, including β-glucuronidase, and 11βHSD. The carbohydrate dehydrogenases are poorly active in this site. These changes could be explained on the basis of

water transport into the tissues, under the control of 11β-HSD, and splitting of acid mucopolysaccharides (β-glucuronidase, Conchie and Findlay, 1959) followed by tissue splitting and re-epithelialization to reform the uterine lumen. GDH, which is associated with protein synthesis is seen in the regenerating epithelium.

In both the rabbit, and guinea-pig an <u>area of degenerating tissue</u> is present in the placenta, in the former the separation zone, in the latter the junctional zone. As might be expected all hydrolases were present here, including β-glucuronidase, and also seen fairly intensely were ADH, FDH, GDH, SorbDH, αGP and βOH. It would appear therefore that these enzymes can be considered to be at least partly concerned with tissue breakdown, the products being absorbed for either foetal or maternal nutrition.

Associated with the junctional zone in the guinea-pig is the <u>subplacental trophoblast</u>, which contains considerable quantities of RNA, and of LDH, IDH, MDH and SDH, less of G-6-P and 6-PG, and is almost negative for phosphatases and hydrolases. These findings confirm those of Wislocki, Deane and Dempsey (1946) and Davies, Dempsey and Amoroso

(1961) and would suggest that the subplacenta is concerned in absorption by diffusion rather than by active ingestion, and possibly in gonadotrophin (or at least protein) secretion, as suggested by Davies et al (1961).

Finally it is of interest to examine the histochemistry of embryonic nutrition in its three phases. The first of these is histiotrophic, prior to development of the yolk-sac placenta - the second phase, and the third is that of the definitive chorio-allantoic placenta.

Histiotrophic nutrition

This is seen prior to implantation, when the embryo gains its nutrition from the uterine secretions, and also at the base of the carnivore placenta in the spongy zone. It occurs, too, in the non-placental chorion of the carnivores and sheep, and in the areas of chorionic plate between the bases of the primary villi in the horse. In the trophoblast in all of these situations hydrolytic enzymes are observed, the quantity varying from species to species. Differentiation between the trophoblast of the pre-nidation blastocyst, and that of post-implantation stages was observed, the former containing much less activity. With the exception of the rat, specific phosphatase is seen in the trophoblast of histiotrophic nutrition, and is particularly active earlier in the rabbit blastocyst, and

decreases later. Krebs cycle dehydrogenases are also seen in moderate to high activity in all areas.

All of these findings suggest active absorption by the trophoblast. The changes in the rabbit are particularly interesting, in view of the findings of Lutwak-Mann, Boursnell and Bennett (1960) on the uptake of radio-active ions in the early blastocyst of this species. They concluded that an active process of selection was taking place and showed that phosphate, potassium, and sulphate in particular, were absorbed in considerable quantities over the period 6 to 8 days after mating. The earlier part of this period corresponds with the decrease in phosphatase activities noted above, and it is tempting to suggest that some connection with ion absorption as described by Lutwak-Mann may exist. The lack of phosphatase activities in the rat blastocyst may be related to the fact that it does not expand very markedly during the early part of gestation.

Yolk sac nutrition

The enzymes of absorption by the yolk-sac have already been considered (vide "Yolk-sac") and the association of hydrolase activity with protein absorption in yolk-

sacs concerned with that function, particularly that of the rabbit, Brambell, Hemmings, Henderson and Oakley (1952), Brambell (1954), Hemmings and Oakley (1957) has been noted, although much of the work cited above is concerned with the non-hydrolytic absorption of proteins. Possibly this may explain the higher activity seen in the yolk-sac of the rat, which does not absorb unhydrolysed proteins.

The differences in the staining reaction of the dog yolk-sac and that of the rabbit, with respect to hydrolases, the former being almost negative, and the latter moderately positive, may perhaps explain the findings of Whipple, Hill, Terry, Lucas and Yuile (1955) that C^{14}-lysine- or I^{131}-labelled protein does not readily pass the dog placenta, but does cross that of the rabbit. However, the possibility of trans-placental (as opposed to yolk-sac) passage exists Dancis and Shafran (1958), and these results should not be extrapholated without caution.

The association of lipid absorption and αGP and βOH in the chick yolk-sac after 5 days of gestation has also been noted, as has that of RNA and FDH in that of the chick and Limia Maculata. These findings along with the presence

of acid phosphatase would accord with the suggestion of Thomas (1938) that yolk is a lipo-protein complex which is broken down by intra-cellular proteases and lipases to protein and glycorides, which are further degraded to amino-acids and fatty acids. RNA is also known to occur in yolk, Romanoff (1960) and the FDH activity in the yolk-sac endoderm would correlate with its degradation.

Alterations of function in the yolk-sac have recently been suggested, Beck and Lloyd (1966) as a possible cause of abnormalities in the offspring of tryphan-blue injected rats. Several dyes are known, Everett (1935) and Gerard (1925) to be absorbed by the yolk sac and stored in supra-nuclear vacuoles. These also contain hydrolases, and Beck and Lloyd have shown biochemically, that trypan blue inhibits these enzymes, and have suggested that it may thus interfere with embryonic nutrition. Such an inhibition would also explain the alterations in thyroid release (which requires hydrolase activity) seen in these animals, Christie, 1964, 1966.

The chorio-allantoic placenta

Many studies of a biochemical nature have been made on placental transport in a limited number of species, and

these have been reviewed by Huggett (1954), Hagerman and Villee (1960), Hertig (1962), Villee (1962), Adamsons (1965), Bloch (1965). Some of the factors affecting placental permeability have also been examined, Dancis, Brenner, and Money (1962). A basic lack of information of a comparative nature is present, however, and limits the deductions that can be made from the histochemistry of the placenta. Correlation of enzymic activity with certain generalized metabolic activities only is possible.

It was hoped that certain enzymes might be found, the degree of staining for which correlated with the number of layers intervening between foetal and maternal blood streams, an obviously expected example being the hydrolases. Although a layer of these was constantly found intervening except in the villi of the horse placenta, the degree of activity correlated in only a general way with the thickness of the placental barrier, and certain notable exceptions, for example the horse, and cat were present. Similarly, a layer of specific phosphatases, albeit discontinuous in the sheep, was found between foetal and maternal blood streams, but here again no correlation with thickness was observed.

It is generally agreed that neither proteins, nor lipids pass across the placenta without being degraded, Popjak and Beeckman (1950), and in this respect the presence of acid-phosphatase and C-esterase on the one hand, and B-esterase on the other, between the two blood streams, is of interest, in view of their suggested functions in such degradation. Relevant to lipid transport after degradation the frequent observation of αGP and βOH in the trophoblast of various species is of interest, in view of their action in further metabolism. The functions of various parts of the placenta containing 11β-HSD in relation to water and sodium transport have been detailed previously, and a possible association between alkaline phosphatases and other ions is seen in the early blastocyst, where uptake is known to be active and selective, Lutwak-Mann, et al (1960), although similar correlation in mature placentae is less certain. Association between certain of these enzymes (particularly AMPase and UDPase) and carbohydrate transport is also possible, in view of their association with glycogen breakdown, and in view of the fact that simple diffusion does not account for such transport at least in the rabbit,

Davies (1955). Little can be said here on the subject of the transport of drugs across the placenta, the mechanism of which is largely unknown.

In conclusion it is of interest to compare the chorio-allantoic placenta of mammals, concerned in the transport of many substances, and the synthesis or degradation of several, with the simple chorio-allantoic placenta of the chick, concerned purely with gaseous exchange, although the allantoic endoderm may be involved in reabsorption of water from the allantoic sac. Here the ectoderm applied to the inner aspect of the shell membrane, and presumably most concerned with oxygen and carbon dioxide exchange showed intense NADP diaphorase activity, unaccompanied by activity of any NADP-linked enzymes, and much less activity with NAD diaphorase. This is the reverse of the situation in the analogous trophoblast of all species examined except the horse, where NADP diaphorase activity is again higher. The significance of this finding is uncertain. The allantoic endoderm showed activity with both diaphorases (NAD greater than NADP) and of FDH, LDH, MDH, IDH, G-6-P, and αGP. This wide range of enzyme activities would accord

with its function in fluid resorption, and also possibly in energy production for its own extension, and is similar to the range seen in the allantoic endoderm of other species.

CONCLUSIONS

CONCLUSIONS

Implantation.

(1) Wide differences are seen, histochemically, in the tissues related to and including the implanting blastocyst of the rat, and rabbit.

(2) Antimesometrial epithelial removal in both instances would appear not to be effected by trophoblastic ingestion, but rather by mechanical removal by invading trophoblast and subsequent autolysis.

(3) Mesometrial epithelial removal in the rat would appear to be similar, but in the rabbit the possibility of active trophoblastic "ingestion exists.

(4) The metabolic changes leading to cell death and epithelial destruction can be followed histochemically in both species, and differ markedly.

(5) Histochemical changes in the rabbit uterine glandular epithelium leading to increased secretion just prior to, and after implantation, can be seen, and it is suggested that this increase in secretion is similar to that observed around a foreign body and may be stimulated by the same mechanism.

(6) The metabolic changes leading to RNA production, and glycogen utilization in the rat decidua can be followed

histochemically, and the previously undescribed localization of glycogen in the antimesometrial stroma is noted.

(7) It is suggested that the primary action of the oestrogen surge known to precede implantation in the rat is to increase uterine stromal ATPase activity in the antimesometrial region, bringing about implantation on that aspect of the uterus. Subsequent changes include RNA synthesis via the pentose shunt.

(8) Glycogen deposition in the glycogen wings of the rat and the decidua of the rabbit is intended as a store of readily available energy for the foetus, some of which is normally used during late implantation in the former species.

(9) Glycogen synthesis and breakdown in the rat may be in some way controlled by uterine AMPase and UDPase levels.

(10) The rat decidua may subserve the functions of (a) providing glucose for the embryo, (b) synthesizing protein for embryonic use, and (c) acting as both a barrier to excess trophoblastic invasion, and as a pabulum for embryonic nutrition by autolysis.

(11) Functionally and histochemically the abembryonic giant cells in the rat, and the trophoblast knobs in the rabbit are similar, and are primarily concerned in embryonic

nutrition, the former more so than the latter where the intervening trophoblast is also very active.

(12) An association between waves of glycogen deposition and loss, LDH, IDH, MDH and SDH activity increase and decrease, the presence of acid mucopolysaccharides, and morphogenesis is seen in the embryo of the rat and developing placenta of the rabbit. Some connection between G-6-P, 6-PG and RNA and cellular differentiation is also observed.

Comparative Placentation

(1) Wide differences and similarities exist in the histochemical picture observed in different placental types.

(2) Some evidence for placental ageing was found in the rabbit, sheep and human.

(3) Glycogen in the trophoblast appears to be acting as a store of readily available energy for the foetus.

(4) The division of placentae into glycogen-rich, with foetal blood glucose, and glycogen-poor, with foetal blood fructose is confirmed, and the possibility of fructose production either via sorbitol, or via phosphorylated intermediates is present histochemically.

(5) RNA appears in the trophoblast, and may be associated with the production of protein (? gonadotrophic) hormones.

(6) Hydrolase activity in the trophoblast is presumably associated with protein and lipid degradation prior to passage to the foetus, and these enzymes are almost constantly present between maternal and foetal circulations.

(7) The function of non-specific alkaline phosphatase in the placenta is unknown, unless it is related to fructose formation. However its concentration in the placentae of non-fructogenic species is higher than that in fructogenic species.

(8) Specific phosphatases occur in the trophoblast, where their presence correlates with a function in energy production and placental transport. TPPase activity, normally considered to lie in the Golgi apparatus, correlates with the level of steroid production and may be associated with their secretion.

(9) αGP and βOH activities correlate with lipid concentration in the trophoblast and may be concerned with its degradation.

(10) LDH, IDH, MDH and SDH activities in the trophoblast demonstrate the high energy utilization of that tissue.

(11) G-6-P and 6-PG appear to be used in the trophoblast to generate reduced NADPH either for transhydrogenation and energy production, or for steroid hydroxylation.

(12) FDH is associated in the trophoblast with decreasing RNA concentration, confirming its function in the degradation thereof.

(13) ADH appeared in sites parallel with αGP suggesting some non-specific degradation of glycero-phosphate.

(14) GDH appeared in the trophoblast of the horse and sheep only, correlating with the breakdown of the protein content of uterine milk in these species.

(15) The trophoblast of the horse, cat, dog, and human probably produce steroids, but not that of the ferret, guineq-pig or rabbit. The sheep and goat, if they produce steroids, do so by a different metabolic route from the normal one, and a similar finding is seen in the late rat trophoblast. The horse trophoblast also produces a progestogen. In the rabbit, a possible route of oestrogen biosynthesis similar to that observed in the sheep is seen in the multinucleate trophoblastic bodies.

(16) 17β-HSD is seen in the placentae of the horse, carnivores, and rat, but its significance is uncertain.
(17) The enzyme activities of the decidua would suggest that it plays a part in the breakdown of materials and the subsequent transport of their metabolites to the foetus.
(18) The antimesometrial trophoblastic giant cells of the rat, and obplacental giant cells of the rabbit are histochemically homologous. The mesometrial giant cells of the rat appear to be concerned in steroid biosynthesis.
(19) Cells were observed in the degenerating decidua capsularis and uterine lumen of the guinea-pig which could be, as judged histochemically, the remains of the parietal trophoblast of that species.
(20) The trophoblastic multinucleate bodies in the rabbit appear to secrete hydrolases to aid in the digestion of the yolk sac contents.
(21) The sheep diplokaryocytes may possibly be concerned in the secretion of enzymes to aid in the transport of material across the placental barrier.
(22) Absorption in the yolk-sac is associated with hydrolase, alkaline phosphatase, and carbohydrate dehydrogenase activities. In the chick, the known change over from

carbohydrate to lipid absorption at 4 days is confirmed by histochemical demonstration of alteration in metabolic pathways.

(23) The ferret yolk sac may be a site of steroid biosynthesis.

(24) The rodent inverted yolk sac is much more active than that of other species in absorption, particularly as regards protein absorption later in gestation.
The parietal endoderm shows enzyme content consistent with its degeneration.

(25) A significant amount of absorption appears to take place in the non-placental chorion, particularly that of the sheep, and of the horse between the bases of the primary villi, in both of which species uterine milk is copious, and the possibility exists that electrolyte transfer to the sheep foetus is mediated in this site.

(26) No destruction of uterine epithelium was observed in the sheep over the wide period of pregnancy examined.

(27) The histochemical reactions of the allantois in all species examined suggest a function in fluid re-absorption.

(28) In the haematoma region of the carnivores, a possible mode of iron transport by conjugation to protein and

excretion in the maternal epithelium, and active absorption and de-conjugation in the trophoblast is suggested by the histochemical findings. Possibly ferritin may be involved. Oestrogen utilization is possibly observed in this site.

(29) The cat maternal "symplasma" in the brown border appears histochemically to be syncytiotrophoblast, which may be attaching foetal and maternal tissues to enclose the brown border contents.

(30) In the sheep acid mucopolysaccharide is again associated with morphogenesis in the development of the foetal villi, and its presence in the mesoderm may be controlled by oestrogens.

(31) The tissue lining the maternal crypts in the sheep would appear histochemically to be syncytiotrophoblast, and not modified maternal epithelium.

(32) The carnivore interstitial matrix would appear to be a thickened endothelial basement membrane.

(33) The ferret thickened maternal endothelium appears to be concerned with steroid function and also exhibits enzymes concerned with transport.

(34) The carnivore spongy zone is concerned with histiotrophe production, in the dog also with uterine secretion, and

possibly steroidogenesis, and in the ferret also possibly with electrolyte transfer across the placenta.

(35) The rodent spongy zone acts as a reserve of trophoblast for labyrinthine extension, and enzymatic changes are seen at its inner edges suggesting that such a process is taking phace. A possible role in protein (? gonadotrophic) hormone production is also suggested by its histochemistry.

(36) The endovascular plasmodium in the rat is identical histochemically with labyrinthine trophoblast.

(37) Histochemical evidence of water deposition and acid mucopolysaccharide degradation in the region of mesometrial reformation of the uterine lumen in the rat, and of protein synthesis in the regenerating epithelium, is seen.

(38) ADH, FDH, GDH, and Sorb DH are often associated with tissue breakdown.

(39) The guinea-pig subplacenta is possibly concerned in protein (? gonadotrophic hormone) elaboration.

(40) Histiotrophic nutrition is mediated by hydrolase, specific alkaline phosphatase, and carbohydrate dehydrogenase enzymes.

(41) Correlation between the degree of specific phosphatase staining, and the absorption of phosphate, potassium and

sulphate in the implanting rabbit blastocyst may be present.

(42) The enzymes of yolk sac nutrition are basically similar to those of histiotrophic nutrition, and the considerable hydrolase activity would accord with protein absorption and degradation.

(43) No correlation between activity of any one enzyme, and thickness of the placental barrier in the chorio-allantoic placenta could be found.

(44) Acid and specific alkaline phosphatases formed almost complete barriers between foetal and maternal blood streams in the chorio-allantoic placenta.

(45) Acid phosphatase and C-esterase, and B-esterase, appear to be associated with the degradation of protein, and lipid, respectively during their passage across the placenta.

(46) Further degradation of lipid via αGP and βOH and entry into the glycolytic cycle can occur.

(47) Specific alkaline phosphatases may be associated with the passage of ions, and of carbohydrates, across the placenta.

(48) The chorio-allantoic barrier of the chick exhibits certain similarities histochemically to that of mammals.

SUMMARY

The histochemistry of implantation in the rat and rabbit is examined, with respect to hydrolases, non-specific and specific alkaline phosphatases, and carbohydrate dehydrogenases. The question of uterine epithelial removal, the significance of the uterine secretion, the functions of the decidua and glycogen wings, and of the mesometrial stroma, the homology of the giant cells and trophoblastic knobs, and the histochemistry of morphogenesis are discussed.

Similar studies are made on the placentae of the term horse, sheep throughout pregnancy, cat at mid-gestation and term, dog throughout pregnancy, ferret at mid-gestation, rat, rabbit, and human throughout pregnancy, and guinea-pig at mid-gestation and term are made, and the yolk sacs of Limia Maculata, the chick at various stages, and early human embryo, and the chorio-allantoic placenta of the chick are studied. A similar range of enzymes, and also dehydrogenases acting on a large number of hydroxy-steroids are examined. The results are described, discussed and compared and certain conclusions regarding the functions of placental structures in each species are drawn.

ACKNOWLEDGEMENTS

It gives me great pleasure to acknowledge the co-operation and encouragement I have had from Professor G.M. Wyburn of the Anatomy Department, University of Glasgow, during the preparation of this thesis. Help with the steroid section from Mr. M.M. Ferguson, a research student in that department is also acknowledged. My veterinary friend, Mr. P. Rossdale of Newmarket, provided the horse specimens, and Mr. R. McCance of the Glasgow Veterinary Inspectors those of the sheep. The specimens of cat and dog were obtained through the kind co-operation of another veterinary friend, Miss I. Towers, of Hamilton, and the co-operation of all of these is gratefully acknowledged. Mr. G. Marshall and Mr. J. Lees were of great assistance technically, and my wife's help in the sorting and collaboration of the reference list was invaluable. Secretarial help provided by Professor R.E. Coupland of Queen's College, Dundee, and his secretary, Mrs. R.E.MacDonald, is gratefully acknowledged. The excellently reproduced diagrams and reaction tables are largely the work of the departmental artist, Miss J. Young, and my thanks are due to her for her help. Finally the printing of the photographs illustrating the thesis was skilfully carried out by Mrs. W. Hiddlestone, Miss A. Macmillan, and Miss L. Cresswell, and my thanks go to them also.

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COMPARATIVE HISTOCHEMICAL STUDIES ON IMPLANTATION AND PLACENTATION.

VOL. 2.

FIGURES.

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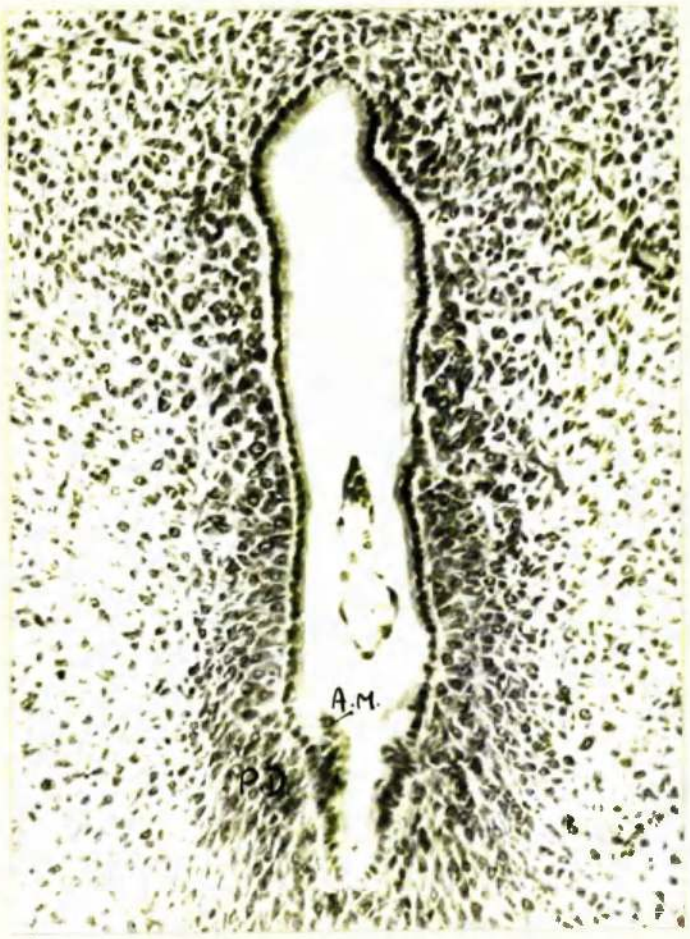
Fig. 1. $5\frac{1}{2}$ day pregnant rat, showing the primary decidual reaction (P.D.) around the antimesometrial end of the uterine lumen. The antimesometrial shelf of pseudo-stratified columnar epithelium (A.M.) is well seen. H & E. X 82.

Fig. 2. The same section as Fig. 1. at a higher magnification showing the embryo, with its outer shell of trophoblast (T.) and inner cell mass (I.C.M.) H & E. X800

Fig. 3. $5\frac{1}{2}$ day pregnant rat, showing the earliest attachment (arrowed) of the embryo to the uterine epithelium in the region of the uterine shelf (A.M.). The abembryonic giant cells (G.C.) and primary decidual reaction (P.D.) are visible. H & E. X 500.

Fig. 4. $6\frac{1}{2}$ day pregnant rat , showing the removal of the antimesometrial epithelium by processes of the abembryonic giant cells (arrowed) and the development of the ectoplacental cone (E.C.) and inner cell mass (I.C.M.) on whose surface the visceral endoderm (V.E.) is differentiating. H & E. X 300.



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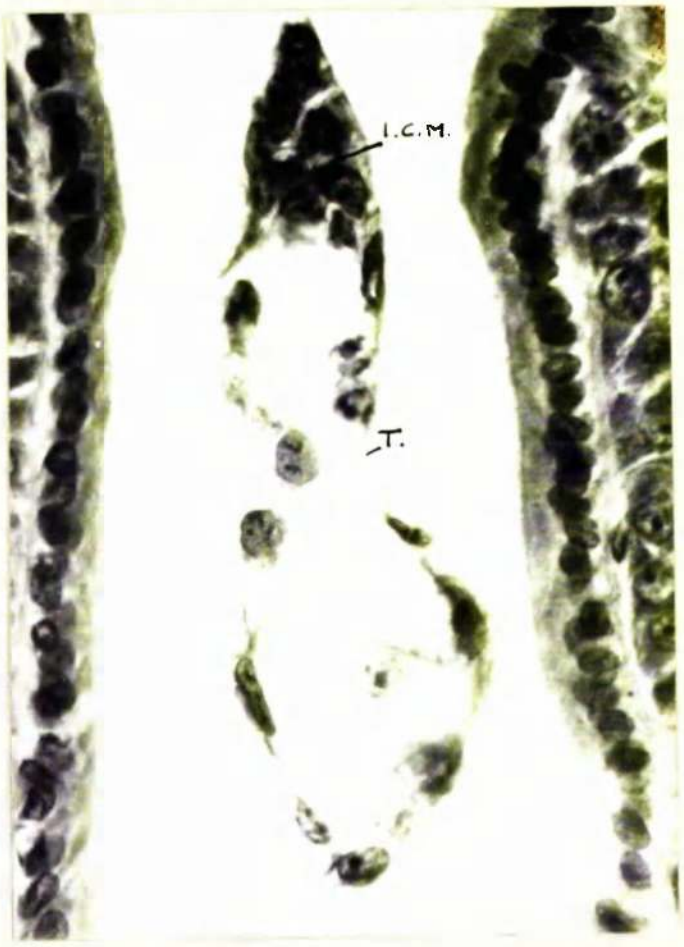

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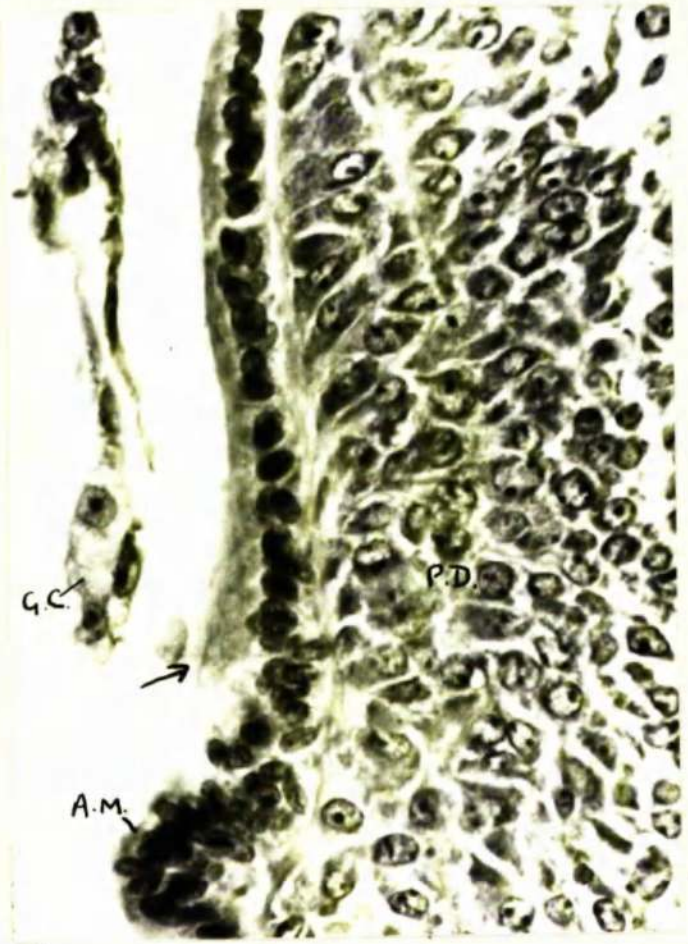

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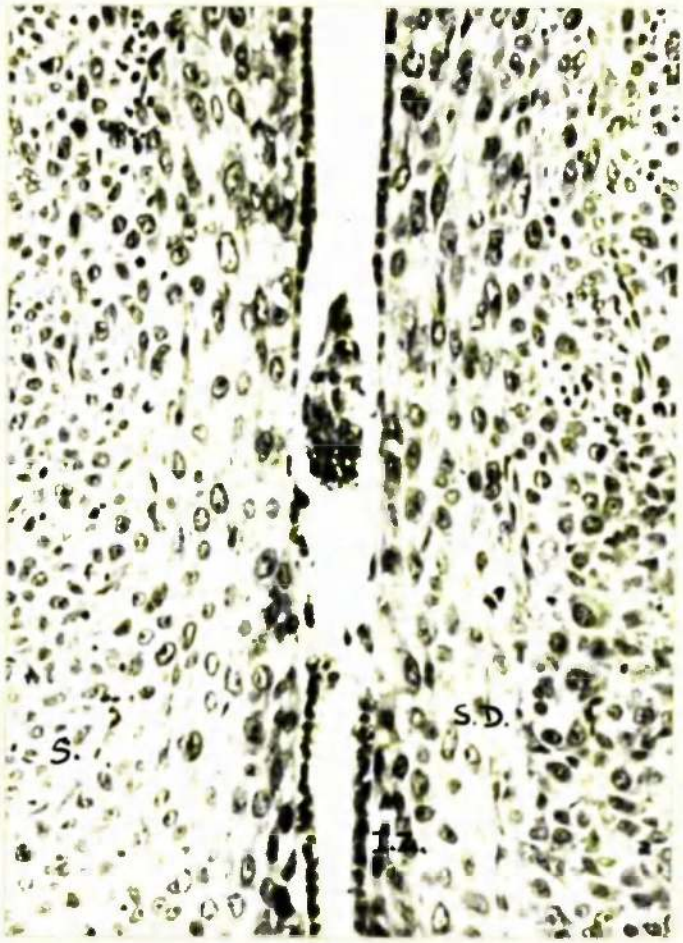
Fig. 5. 6½ day pregnant rat, showing the commencement of degeneration of the uterine epithelial basement membrane (arrowed) at the antimesometrial end of the implanting embryo. Gordon and Sweet. X 300.

Fig. 6. 6½ day pregnant rat, showing the implantation zone (I.Z.) and the conversion of primary decidua into secondary decidua (S.D.). The increased cellularity of the stroma (S.) is also visible. H & E. X 200.

Fig. 7. Typical secondary decidual cells, mono- and bi-nucleate, with some in mitosis. H & E. X 1000.

Fig. 8. 9½ day pregnant rat, showing the great enlargement of the secondary decidua (S.D.), the lateral compression of the antimesometrial stroma into the fibrinoid capsule (F.), and the region of the glycogen wings (G.W.) lateral to the ectoplacental cone. H & E. X 27.5.

5



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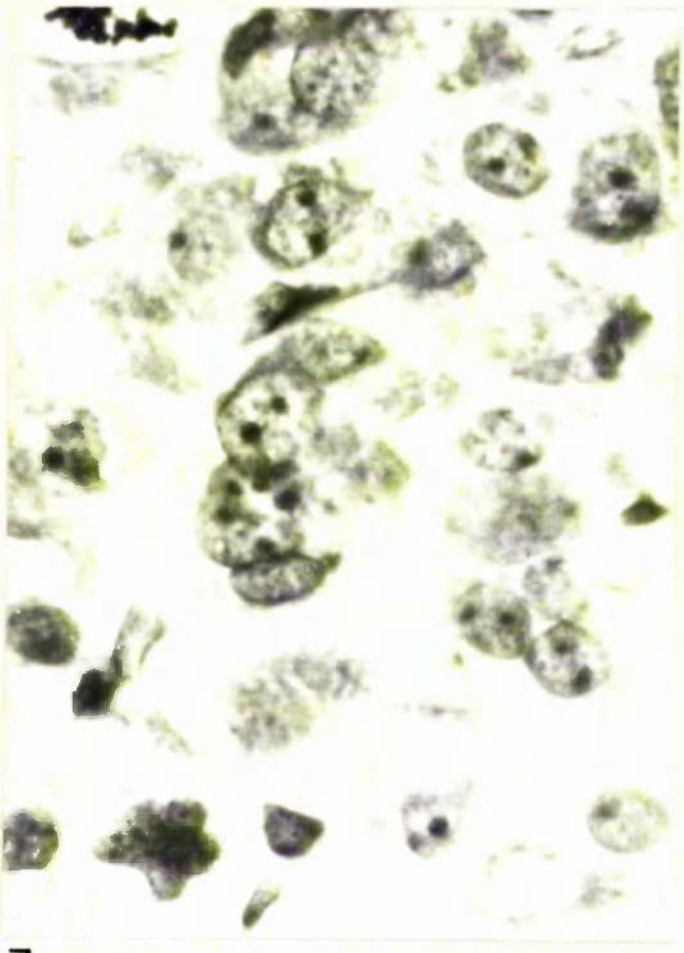

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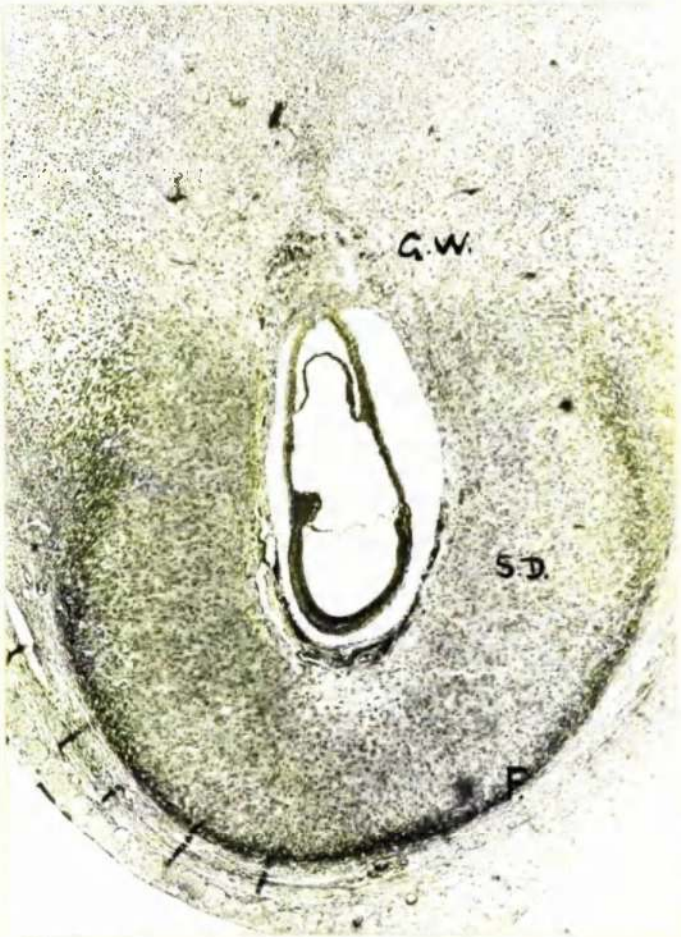

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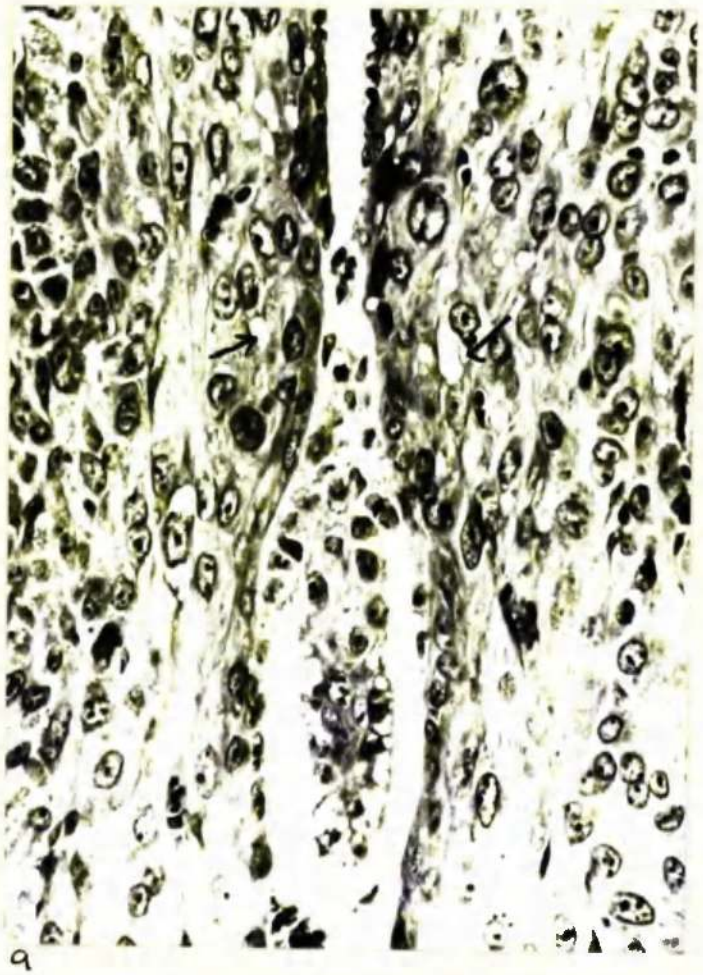
Fig. 9. 7 day pregnant rat, showing the vacuolation of the cells mesometrial and lateral to the embryo (arrowed). Masson. X 150.

Fig. 10. 8½ day pregnant rat, showing the accumulation of glycogen in the wings (G.W.) mesometrial and lateral to the embryo. PAS-dimedone. X 45.

Fig. 11. 10½ day pregnant rat showing lateral giant cells (L.) and ectoplacental cone giant cells (E.C.G.C.). The fusion of the allantoic outgrowth (A.) to the base of the ectoplacental cone (E.C.) is visible. Masson. X 180.

Fig. 12. 6½ day pregnant rat, showing the visceral (V.E.) and developing parietal (P.E.) layers of endoderm. Masson. X 300.



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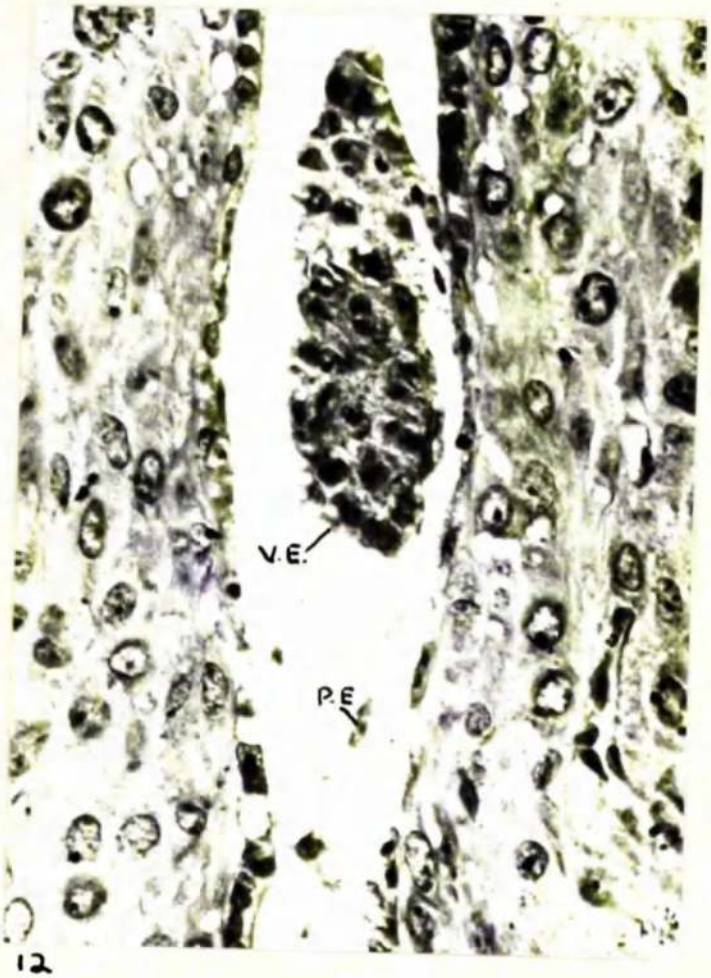

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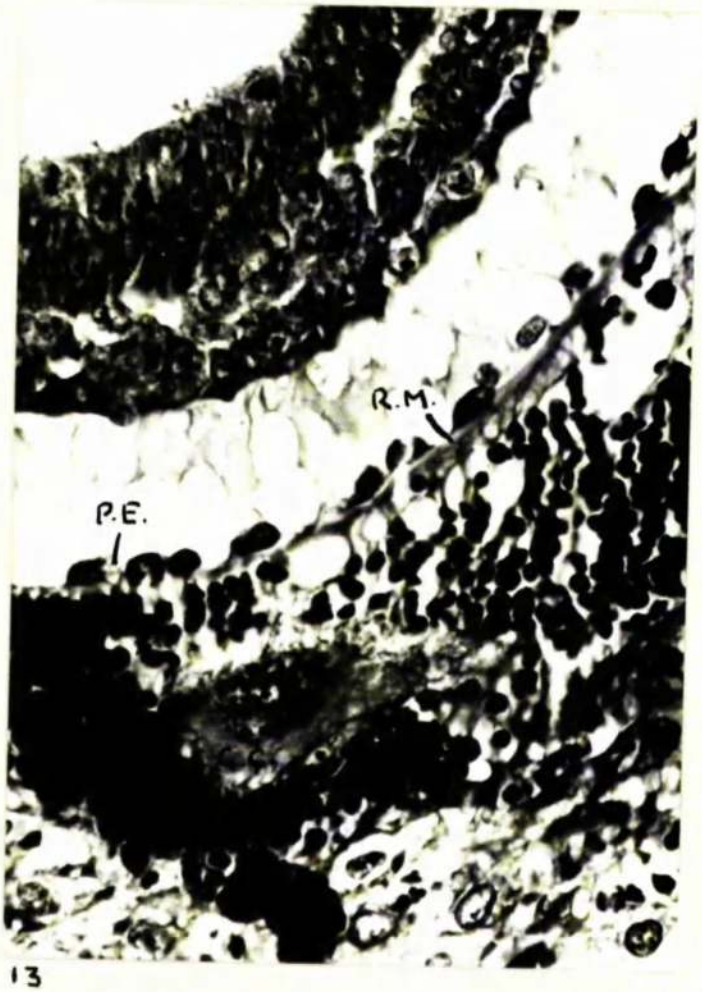
Fig. 13. 9½ day pregnant rat. Reichert's membrane (R.M.), the parietal endoderm (P.E.), and abembryonic giant cells (G.C.) are visible. Masson. X 800.

Fig. 14. 9½ day pregnant rat, showing the ectoplacental cavity (E.C.C.), the lamina (L.), the extra-embryonic coelom (C.), the amnion (A.), the amniotic cavity (A.C.), and the developing allantois (All.). The yolk-sac cavity surrounds the embryo at this stage due to the inversion of the germ layers. H & E. X 60.



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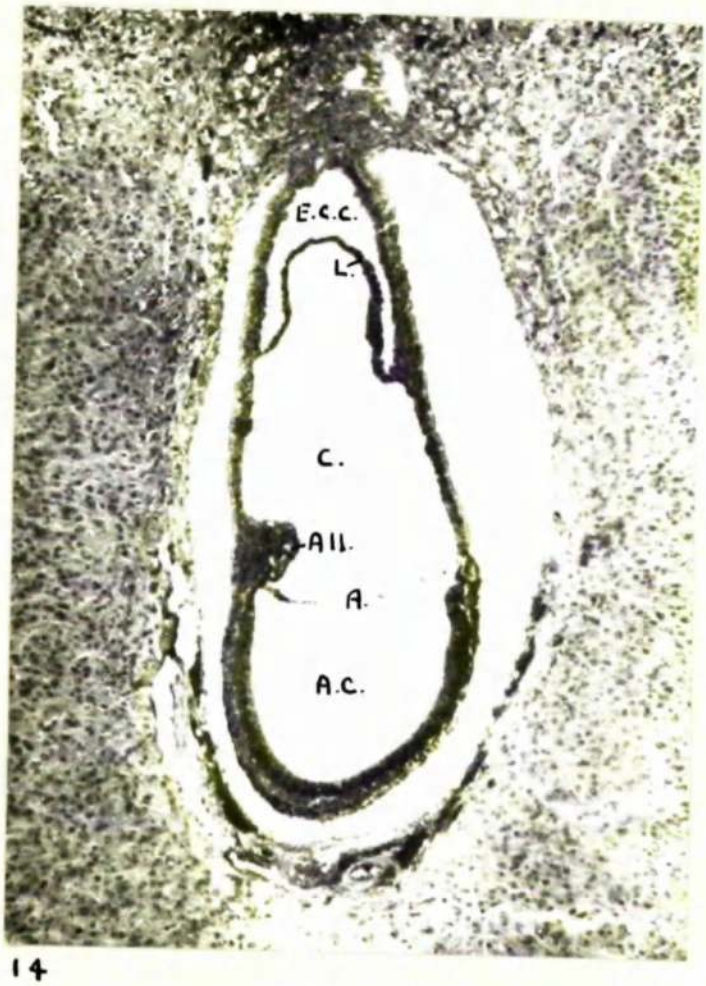

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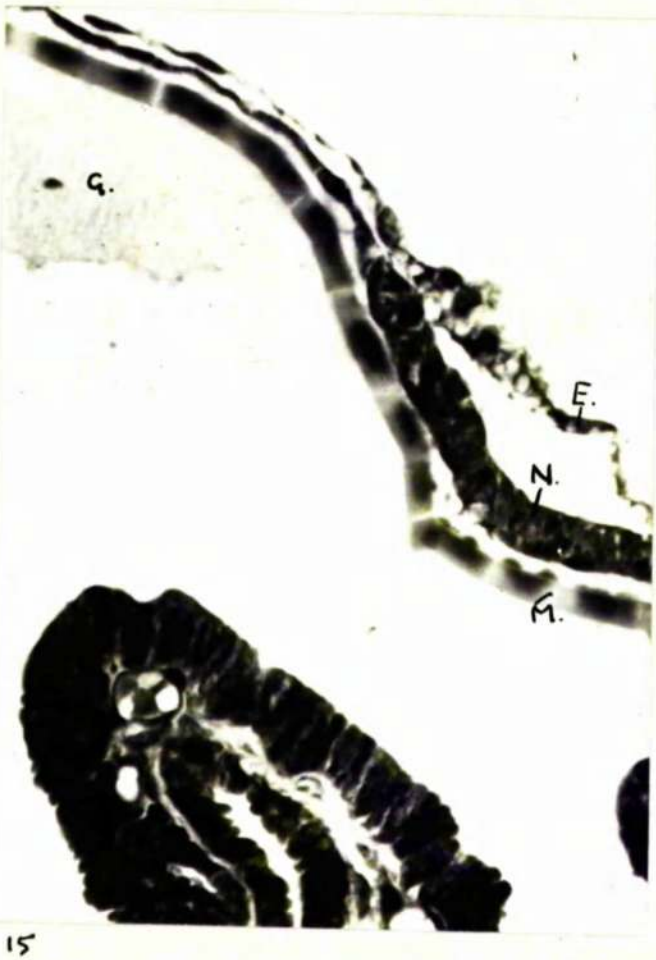
Fig. 15. 6 day pregnant rabbit, showing the flattened neural plate (N.) with, on its interior, a layer of endoderm (E.). The outside of the blastocyst is covered by a hyaline mucolemma (M.) and a gliolemma (G.). Masson. X 375.

Fig. 16. 7 day pregnant rabbit blastocyst, showing the thinning of the mucolemma (M.), almost disappearance of the gliolemma (G.), the appearance of the antimesometrial trophoblastic knobs (K.), and the spread of the endoderm (E.) antimesometrially, to form a bilaminar omphalopleure. Masson. X 800.

Fig. 17. 7 day pregnant rabbit embryo, showing the primitive streak (S.) and intra-embryonic mesoderm (M.). Masson. X 400.

Fig. 18. 7 day and 18 hour pregnant rabbit, showing the insinuation of a trophoblastic process (T.) between the uterine epithelial cells (Ep.) to contact a sub-epithelial capillary. Masson. X 800.



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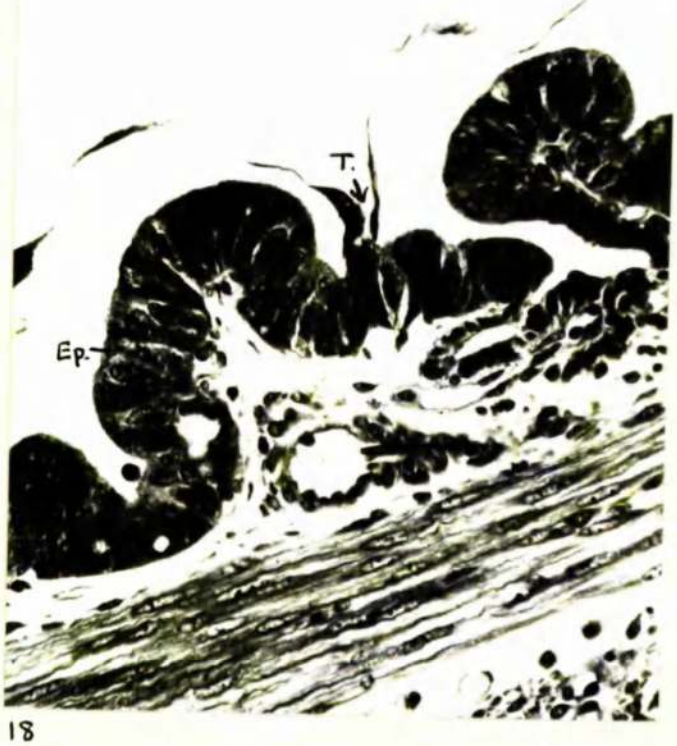

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Fig. 19. 7 day and 22 hour pregnant rabbit, showing the enlargement of the trophoblastic processes (T.) in the plane of the uterine epithelium and subepithelially, the conversion of the epithelium into symplasma (S.), and the accumulation of yolk in the yolk-sac (Y.) which is enclosed by the non-vascular endoderm (E.). Masson. X 150.

Fig. 20. 9 day pregnant rabbit, showing the antimosometrial yolk-sac placenta, the obplacental giant cells (G.C.), degenerating maternal epithelium (Ep.), unaffected epithelium at the bases of the uterine glands (U.G.), and maternal blood (B.) circulating in contact with the trophoblastic wall. Masson. X 150.

Fig. 21. 7 day and 12 hour pregnant rabbit, showing the thickening of the endothelium of the maternal blood vessels in the placental folds. Masson. X 375.

Fig. 22. 7 day and 22 hour pregnant rabbit, showing the trophectoderm (T.) in contact with the uterine epithelium (Ep.). The mesoderm(M.) and thickened endoderm (E.) on its interior are visible. Masson. X 500.

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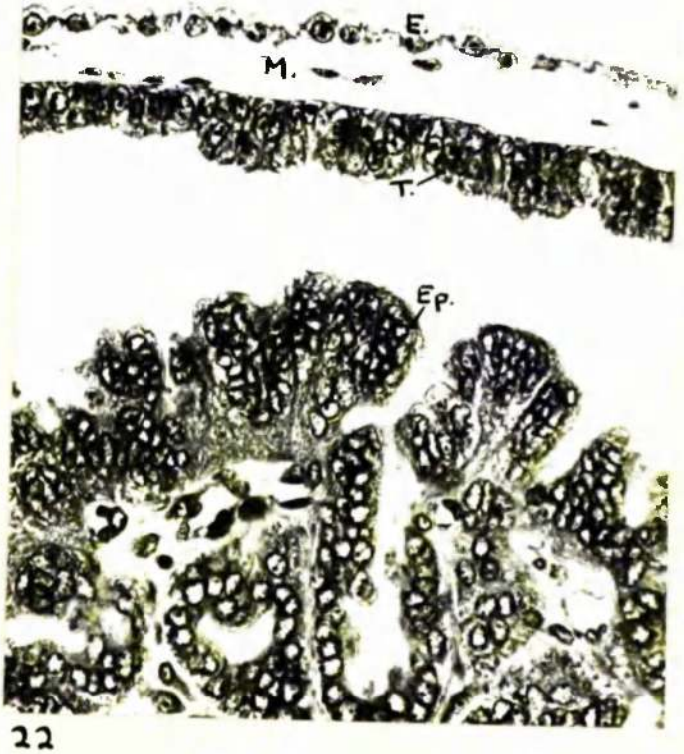

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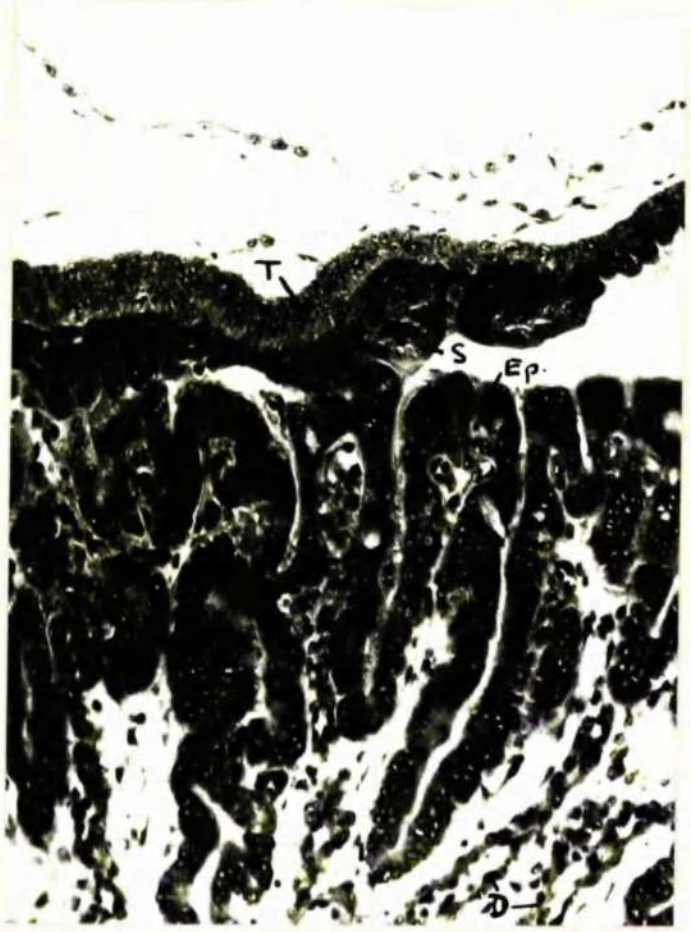
Fig. 23. 8 day pregnant rabbit, showing the trophoectoderm (T.), the syncytiotrophoblast (S.) fusing with the maternal symplasma (Ep.), and deeper in the mucosa decidual development around the maternal blood vessels (D.). Masson. X 500.

Fig. 24. 9 day pregnant rabbit, showing the degeneration of the deeper parts of the uterine glands (U.G.), although not, as yet, their bases (G.), and the decidual development (D.). Masson. X 150.

Fig. 25. 8 day and 6 hour pregnant rabbit, showing a narrow band of connective tissue separating the syncytiotrophoblast (S.) from a maternal capillary (C.). Masson. X 375.

Fig. 26. 9 day pregnant rabbit showing the endoderm of the placental region, with many cytoplasmic inclusions. Masson. X 600.



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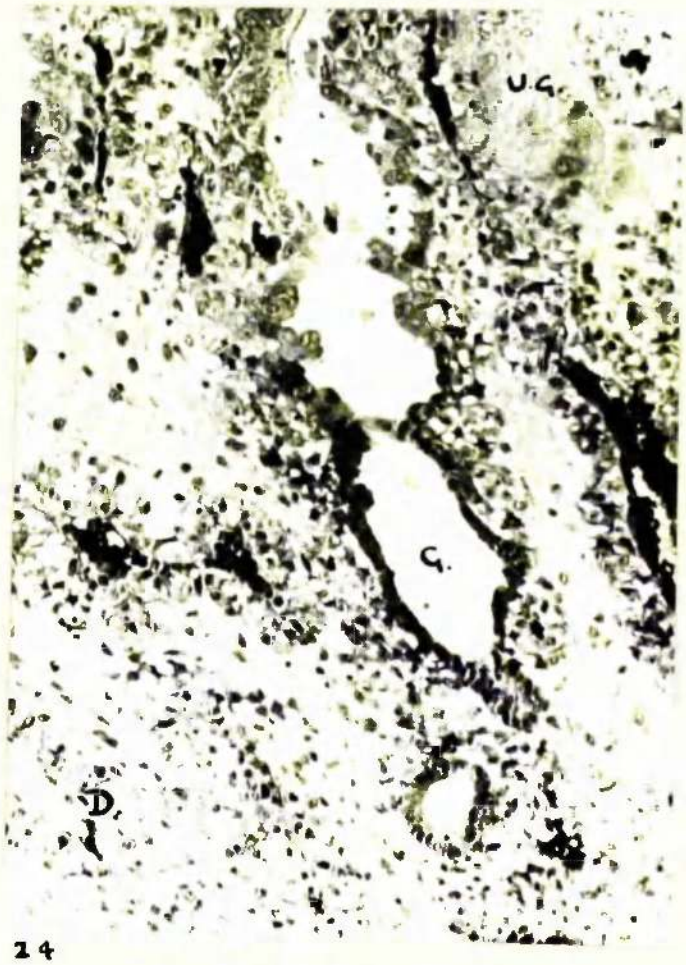

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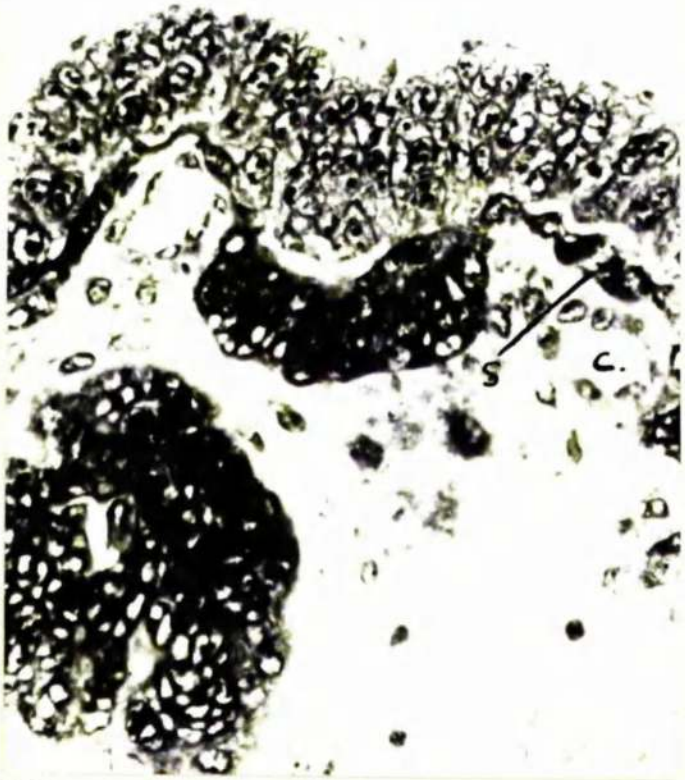

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Fig. 27. 5 day rat embryo to show glycogen granules in the trophoblast (T.). PAS-dimedone.

Fig. 28. 5½ day rat embryo at the time of implantation, showing the accumulation of glycogen in the trophoblastic giant cells (G.C.), and commencing accumulation in the primary decidua (P.D.) PAS-dimedone.

Fig. 29. 6 day rat embryo, showing the presence of glycogen in the ectoplacental cone (E.C.), forming visceral endoderm (E.), trophoblast (T.), uterine epithelium (Ep.), and adjacent decidua (D.). PAS-dimedone.

Fig. 30. 8½ day rat embryo, showing the distribution of glycogen in the ectoplacental cone (E.C.), extra-embryonic (X.M.) and intra-embryonic (M.) mesoderms, ectoderm (E.), and parietal endoderm (P.E.) but not in the yolk-sac endoderm (Y.S.). PAS-dimedone.

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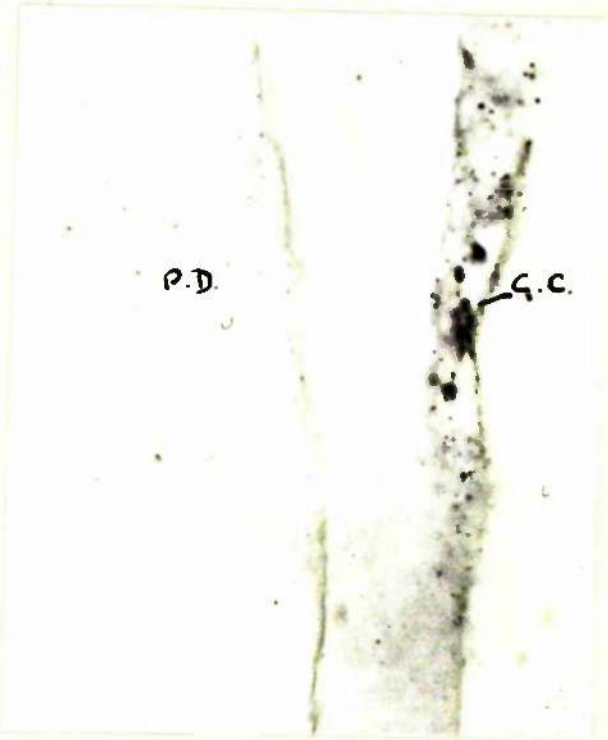

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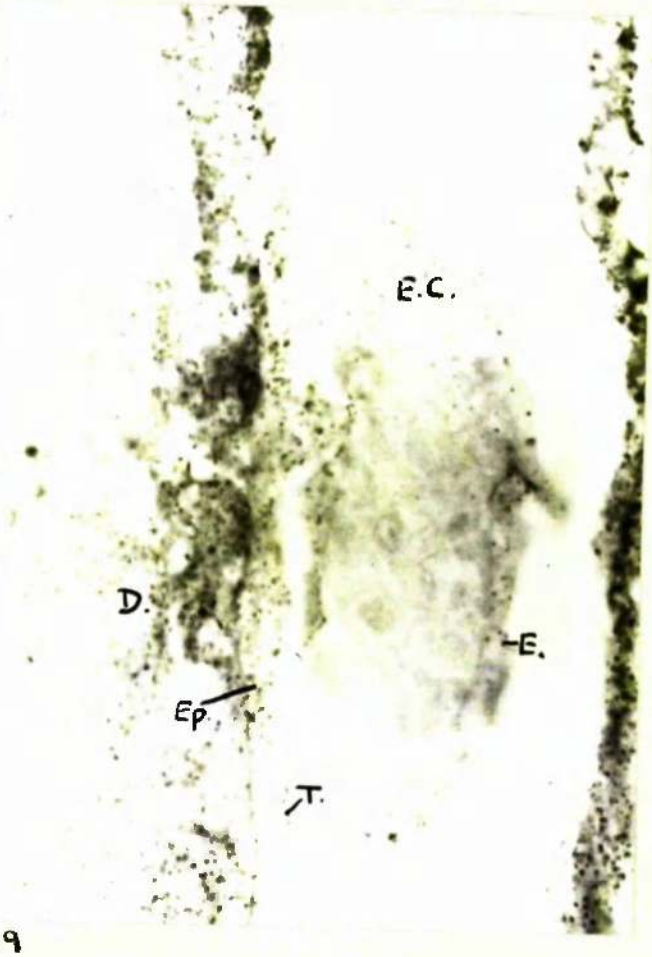

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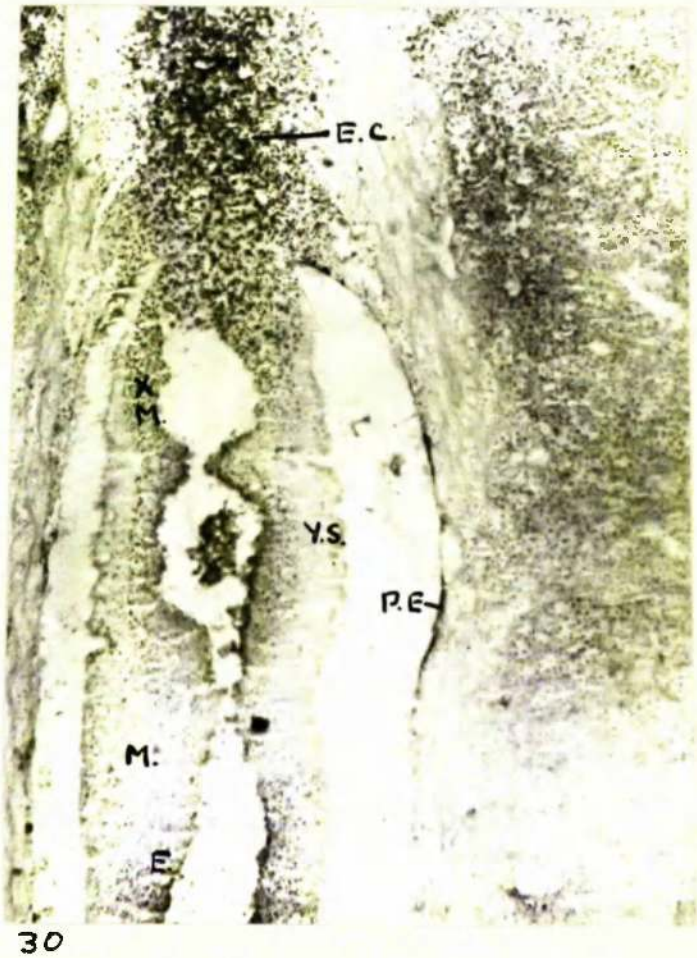

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Fig. 27. 5 day rat embryo to show glycogen granules in the trophoblast (T.). PAS-dimedone.

Fig. 28. 5½ day rat embryo at the time of implantation, showing the accumulation of glycogen in the trophoblastic giant cells (G.C.), and commencing accumulation in the primary decidua (P.D.) PAS-dimedone.

Fig. 29. 6 day rat embryo, showing the presence of glycogen in the ectoplacental cone (E.C.), forming visceral endoderm (E.), trophoblast (T.), uterine epithelium (Ep.), and adjacent decidua (D.). PAS-dimedone.

Fig. 30. 8½ day rat embryo, showing the distribution of glycogen in the ectoplacental cone (E.C.), extra-embryonic (X.M.) and intra-embryonic (M.) mesoderms, ectoderm (E.), and parietal endoderm (P.E.) but not in the yolk-sac endoderm (Y.S.). PAS-dimedone.

Fig. 31. 7½ day pregnant rat uterus, showing the intense staining for glycogen in the glycogen wings (G.W.) and in the antimesometrial stroma (S.). PAS-dimedone.

Fig. 32. 9½ day pregnant rat uterus, showing the further accumulation of glycogen in the wings (G.W.), and its absence from the antimesometrial region (S.). PAS-dimedone.

Fig. 33. High power view of a similar specimen to Fig. 32, showing glycogen in the parietal endoderm (P.E.) and yolk-sac cavity (Y.). PAS-dimedone.

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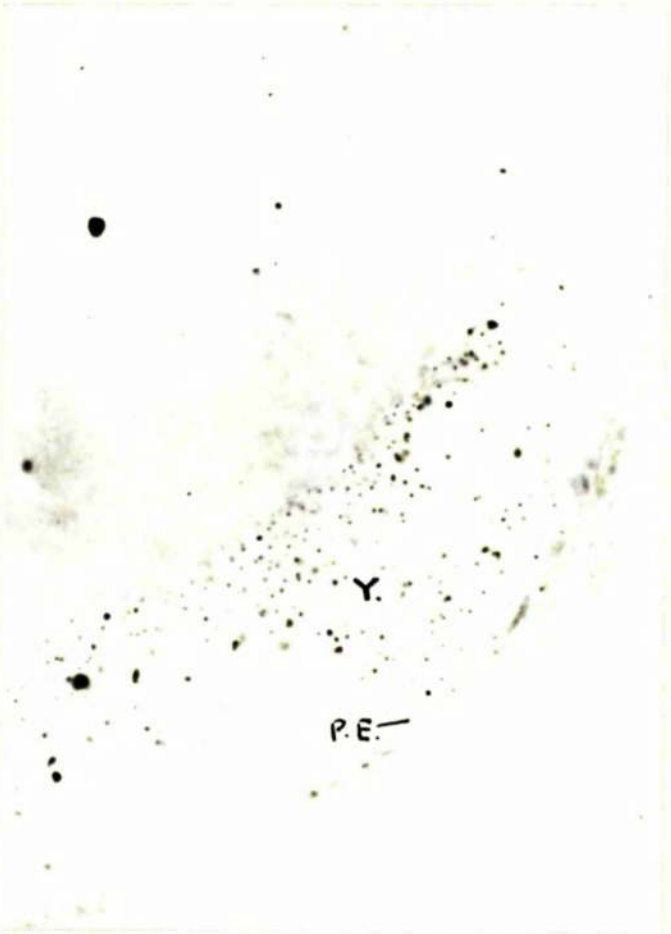

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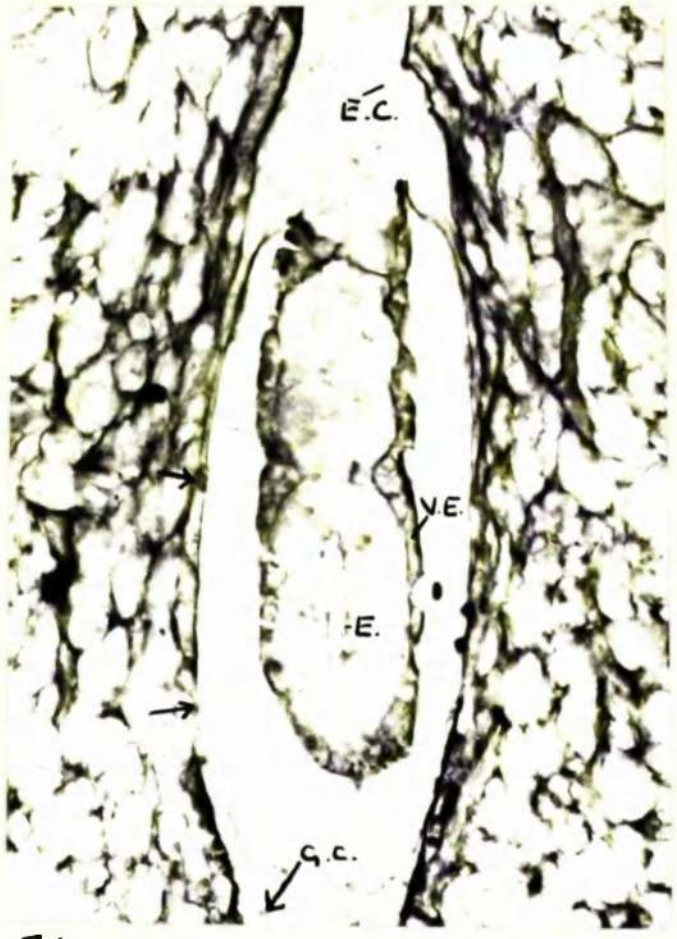
Fig. 34. 7 day rat embryo, showing the PAS-positive brush border and basement membrane of the visceral endoderm (V.E.), the positive reaction of the free edge of the ectoderm (E.), the replacement of the epithelial basement membrane by Reichert's membrane (arrowed), and the inclusions in the abembryonic giant cells (G. C.), and cells of the ectoplacental cone (E.C.). PAS-diastase.

Fig. 35. 9½ day rat embryo - lateral giant cell, to show the inclusions (arrowed). PAS-diastase.

Fig. 36. Ectoplacental cone giant cell from the same embryo, to show similar diastase-fast PAS-positive inclusions.

Fig. 37. 9½ day rat embryo - visceral (yolk-sac) endoderm, to show cytoplasmic inclusions (V.E.). PAS-diastase.



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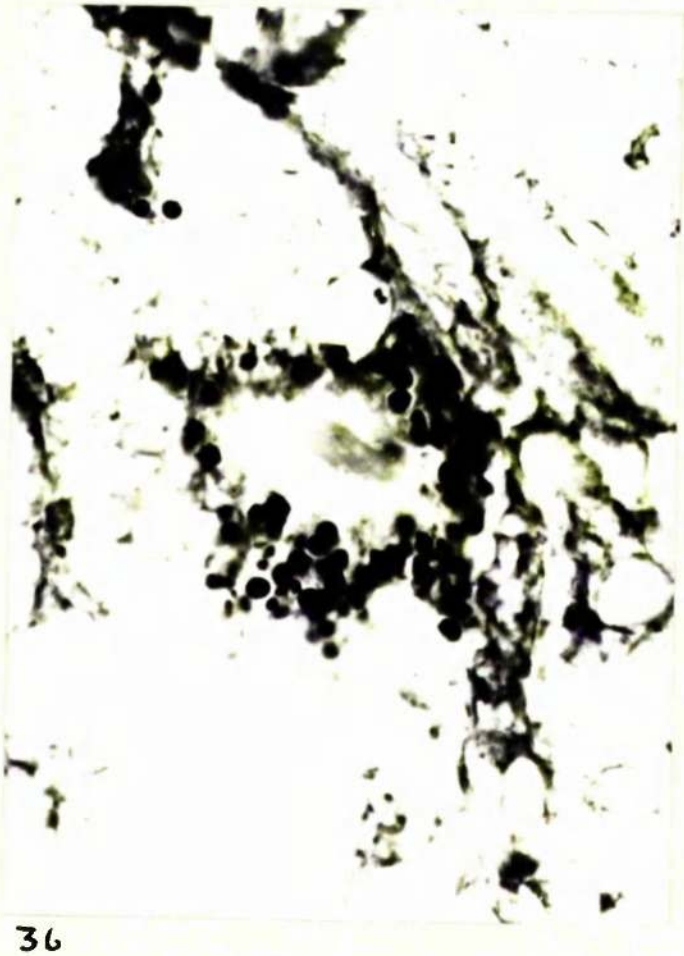

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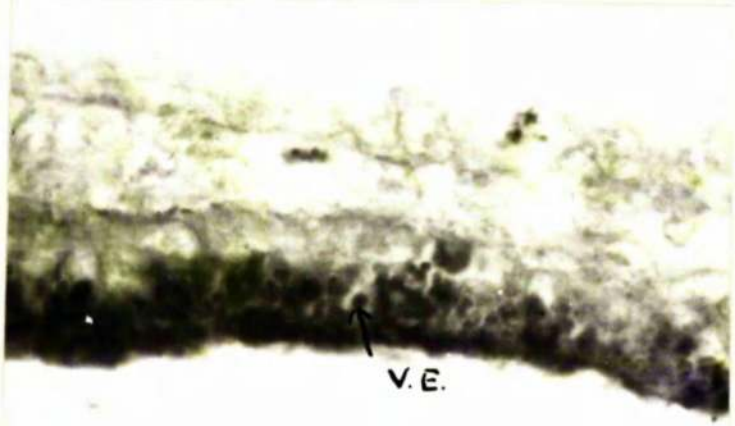

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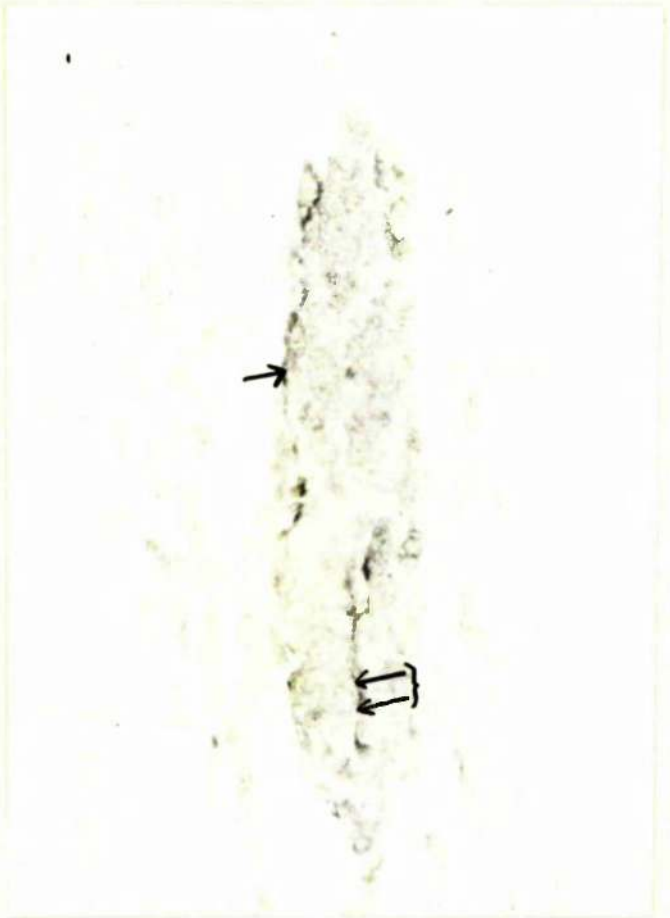
Fig. 38. 7 day rat embryo, showing acid mucopolysaccharide in the yolk sac cavity (single arrow), and in the commencing cavitation of the inner cell mass (double arrow). Dialyzed iron.

Fig. 39. 8½ day rat embryo, showing acid mucopolysaccharide in the yolk sac cavity (single arrow) and cavity of the inner cell mass (double arrow) in relation to the chorio-amniotic folds (F.). Dialyzed iron.

Fig. 40. 5½ day rat embryo and adjacent structures, showing the acid mucopolysaccharide staining in the stroma (S.), with less in the primary decidua (D.). Dialyzed iron.

Fig 41. 9½ day rat implantation site, showing the localization of acid mucopolysaccharide staining to the mesometrial stroma (S.). The fibrinoid capsule (F.) and decidua (D.) are negative. Some probably non-specific nuclear staining is also observed. Dialyzed iron.



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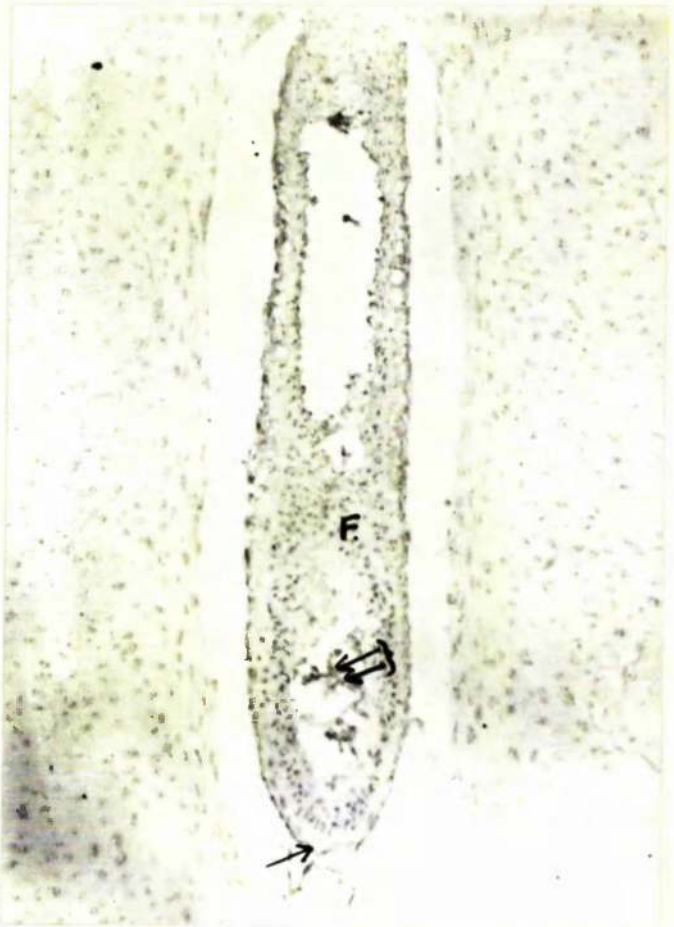

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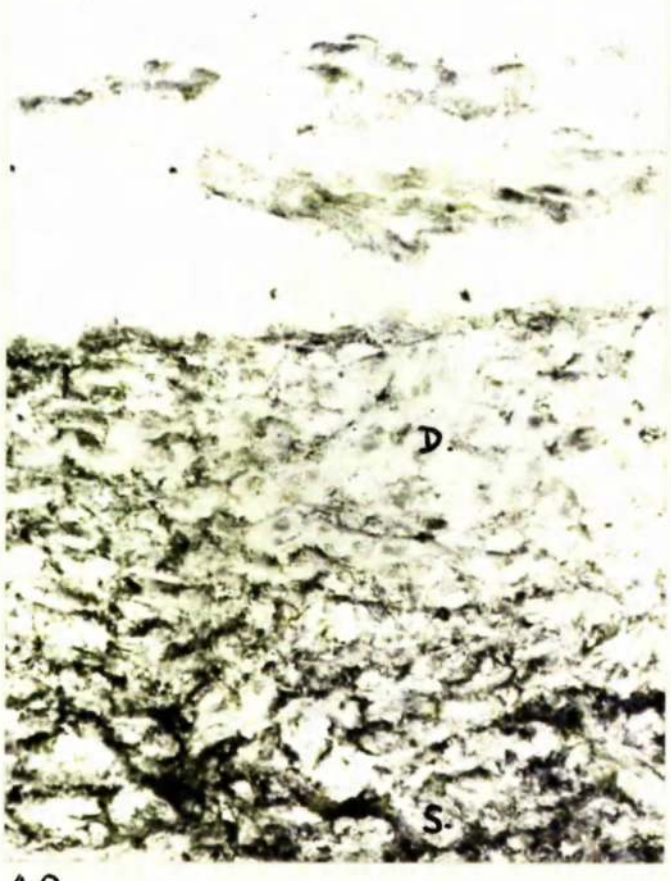

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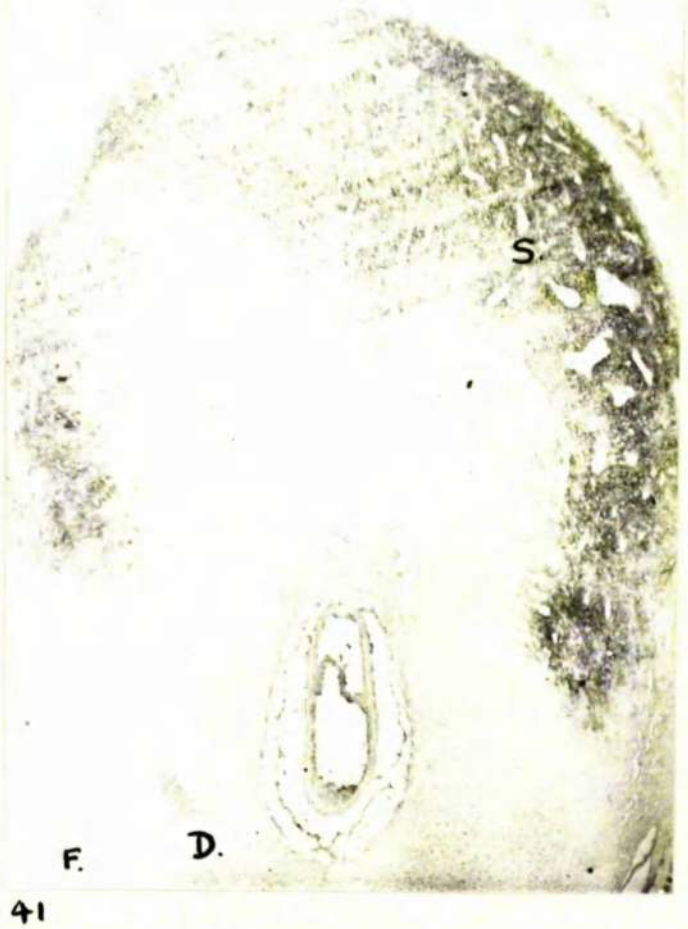

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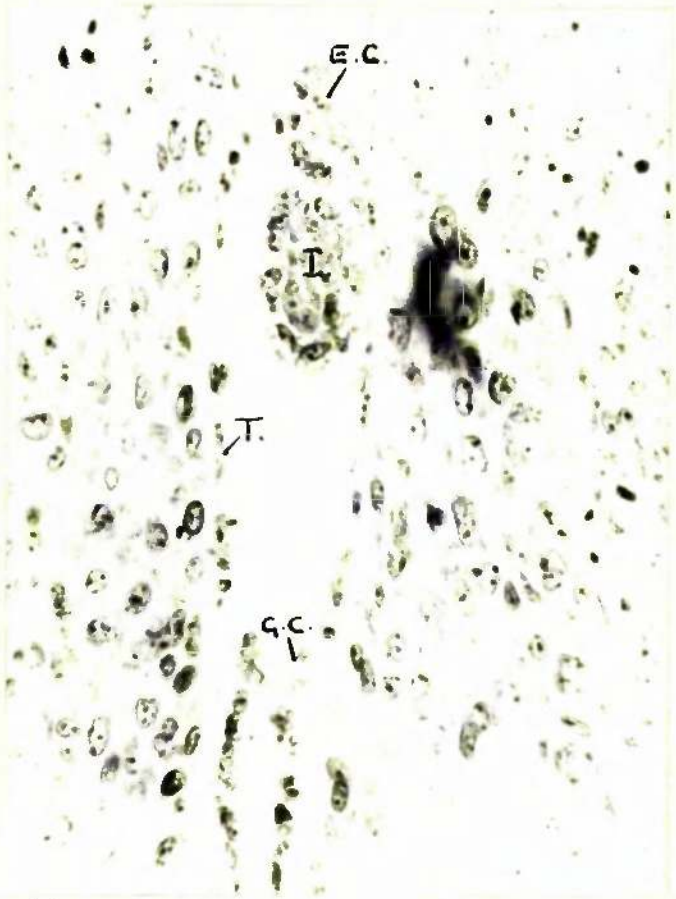
Fig. 42. 6½ day rat embryo, showing RNA in the trophoblast (T.), inner cell mass (I.), abembryonic giant cells (G.C.), and ectoplacental cone (E.C.). Cytoplasmic staining in this, and the next three figures is due to RNA. Chrome-alum-gallocyanine.

Fig. 43. 7½ day rat embryo showing considerable RNA staining in the ectoderm (Ec.), less in the mesoderm (M.), visceral endoderm (V.E.), and abembryonic giant cells (G.C.) which also contain some ribonuclease-resistant inclusions, and less still in the secondary decidua (D.). Chrome-alum-gallocyanine.

Fig. 44. 9½ day rat embryo, showing decrease in RNA content of the yolk sac endoderm (Y.S.) as compared to the mesoderm (M.), and ectoplacental cone (E.C.). The glycogen wing region (G.W.) does not show very marked staining. Chrome-alum-gallocyanine.

Fig. 45. 5½ day rat embryo, showing marked RNA concentration in the primary decidua (P.D.), and faint staining in the uterine epithelium (Ep.). Chrome-alum-gallocyanine.



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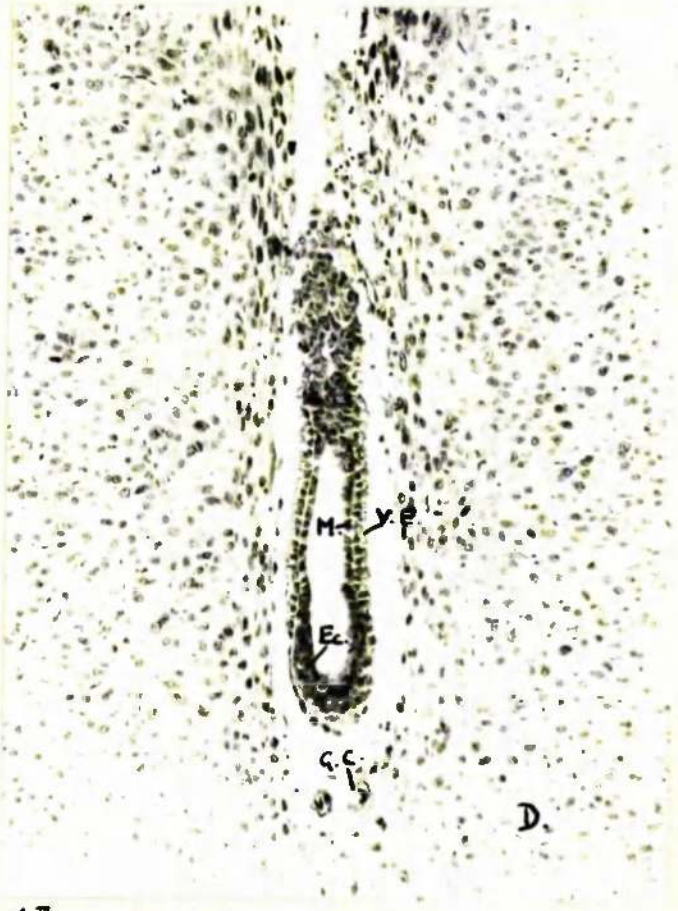

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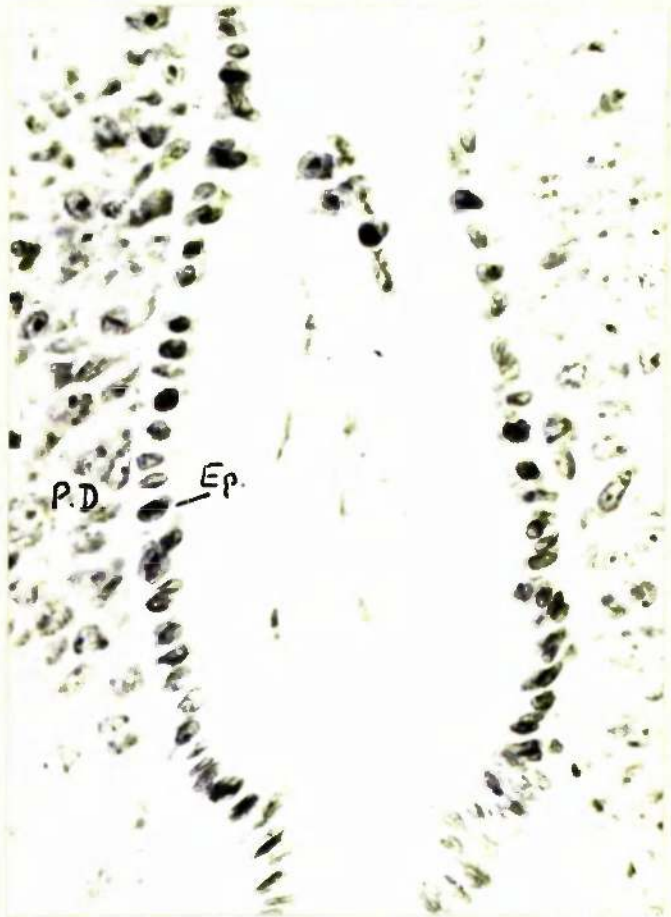

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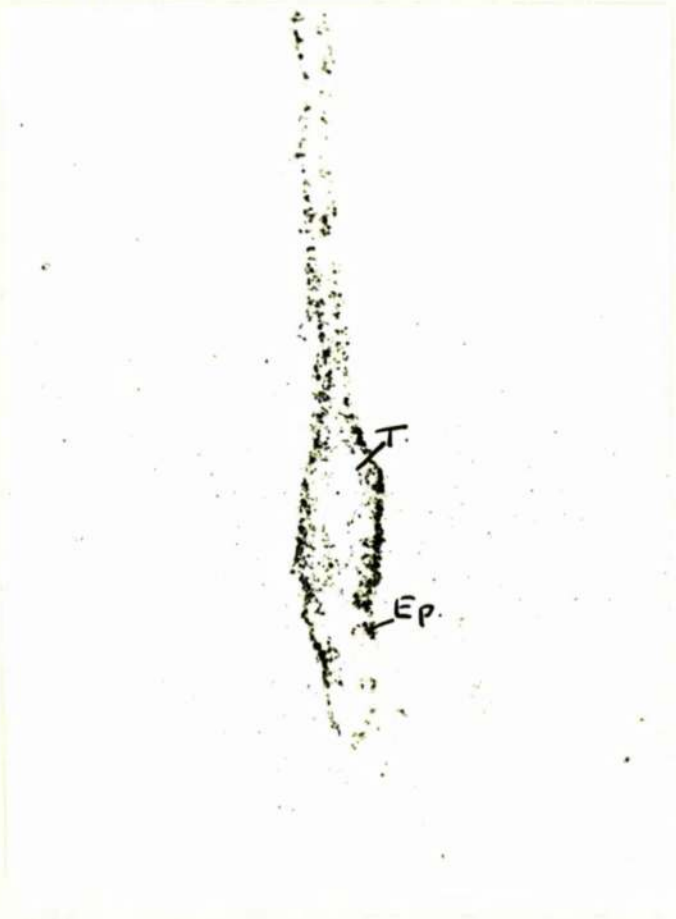
Fig. 46. 5½ day rat embryo, showing lipid in the uterine epithelium (Ep.), and trophoblast (T.). Sudan Black B.

Fig. 47. 6½ day rat embryo, showing an increase in the lipid content of the antimesometrial epithelium (Ep.) compared with Fig. 46, and its presence in the ectoplacental cone (E.C.), inner cell mass (I.), and abembryonic giant cells (G.C.). Some is also seen in the decidua (D.) and stroma (S.). Sudan Black B.

Fig. 48. 7½ day rat embryo, showing lipid in the implantation zone (I.Z.) and secondary decidua (D.). Sudan Black B.



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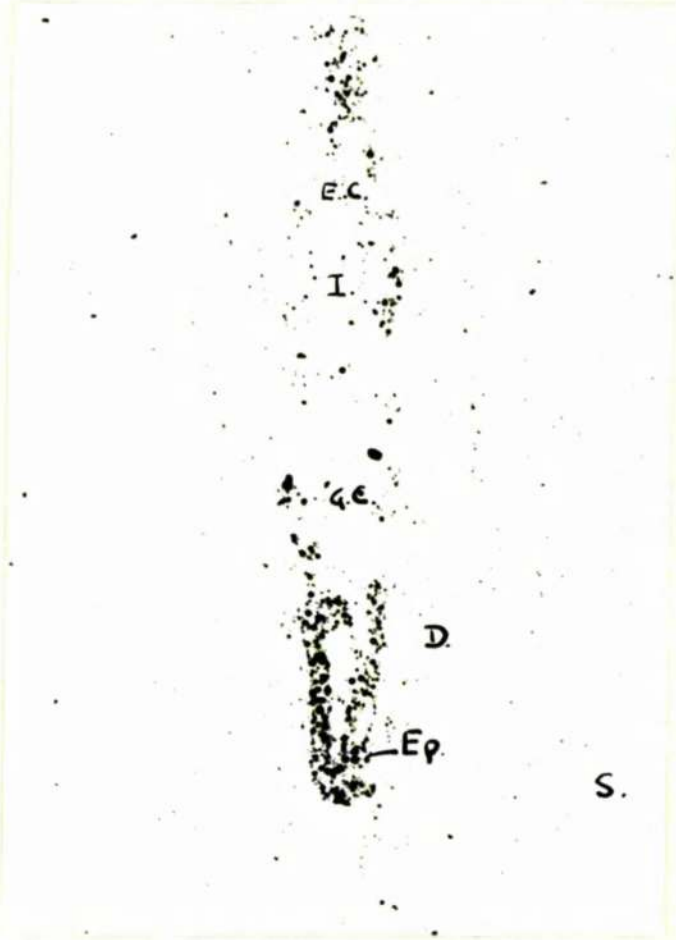

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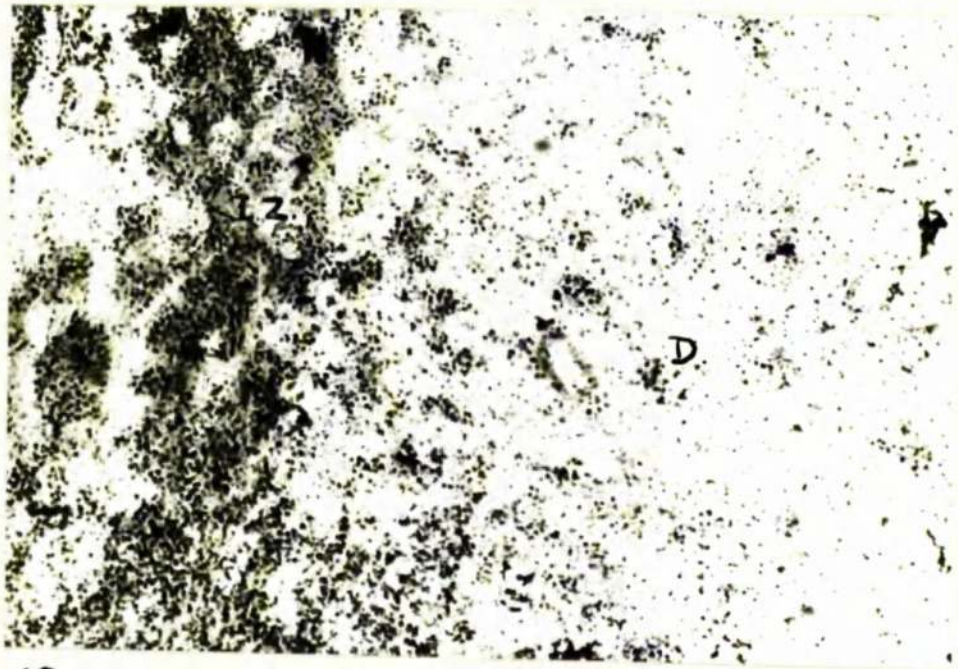

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Fig. 49. 5 day rat embryo, showing acid phosphatase activity in the trophoblast (T.), uterine epithelium (E.), and primary decidua (D.).

Fig. 50. 9½ day rat embryo, showing acid phosphatase activity in the yolk sac endoderm (Y.), mesoderm (M.), parietal endoderm (P.), and a lateral giant cell (G.C.).

Fig. 51. 6 day rat embryo, showing acid phosphatase in the trophoblast (T.), inner cell mass (I.), epithelium surrounding the embryo (E.), and abembryonic giant cells (G.C.). Enzyme activity is also seen in the decidua (D.).

Fig. 52. 8½ day rat embryo, showing acid phosphatase in the yolk sac endoderm (Y.), but decreasing in the embryonic endoderm (E.), and in the abembryonic giant cells (G.C.), ectoderm (Ec.), and mesoderm (M.).

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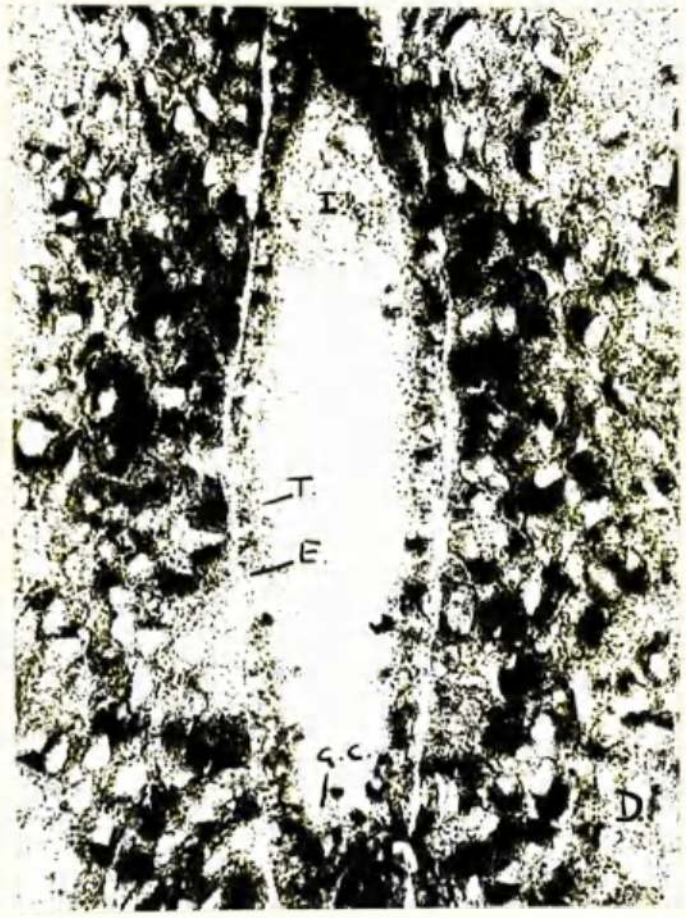

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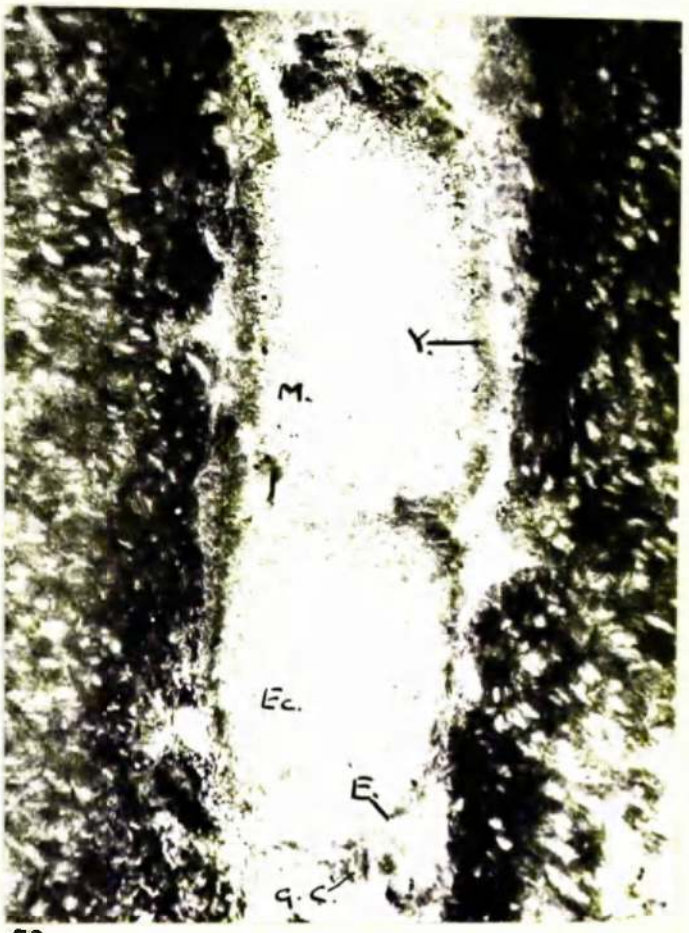

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Fig. 53. 6 day rat implantation site, showing acid phosphatase in the decidua (D.), stroma (S.), and uterine epithelium (E.), in which a gradient of activity decreasing from mesometrial to antimesometrial is visible.

Fig. 54. 9½ day rat implantation site, showing acid phosphatase in the glycogen wings (G.W.), and increasing in the antimesometrial decidua (D.). The lack of staining in the embryonic endoderm (arrowed) is visible, as is the heavy reaction of the parietal endoderm (adjacent) which has here shrunk away from the giant cells (G.C.).

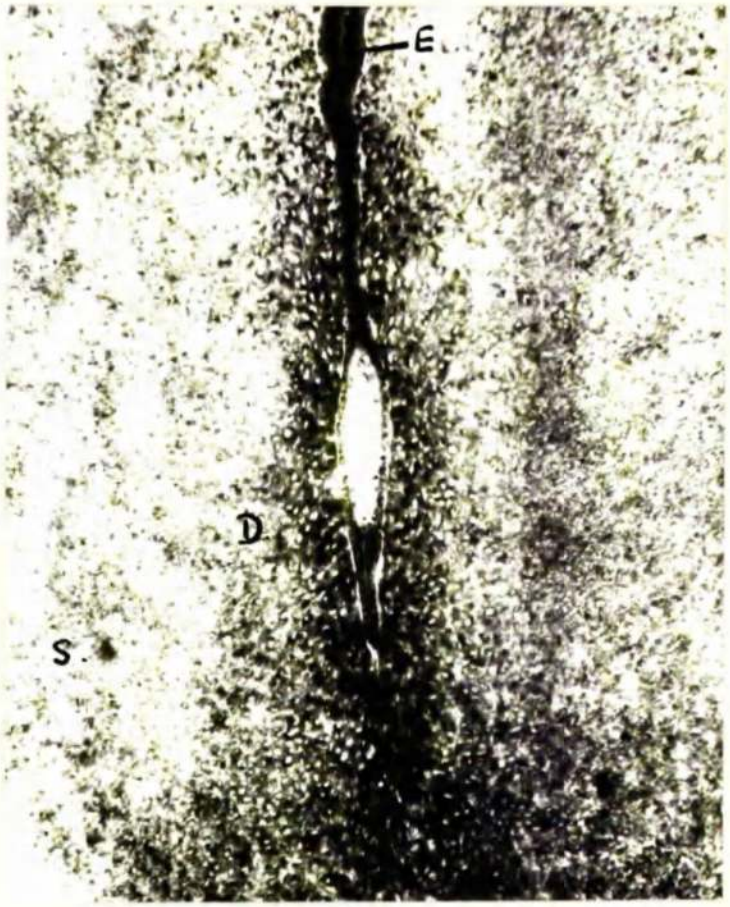

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Fig. 55. 7½ day rat embryo, showing non-specific esterase in the uterine epithelium (Ep.), visceral endoderm (E.), and decidua (D.). Naphthol-AS-acetate.

Fig. 56. 6 day rat implantation site, showing non-specific esterase in the trophoblast (T.), uterine epithelium (Ep.), inner cell mass (I.), and decidua (D.). α-naphthyl acetate.

Fig. 57. 9½ day rat implantation site, showing non-specific esterase in the uterine epithelium (Ep.), and antimesometrial decidua (D.), but not in the glycogen wings.(G.W.). α-naphthyl acetate.

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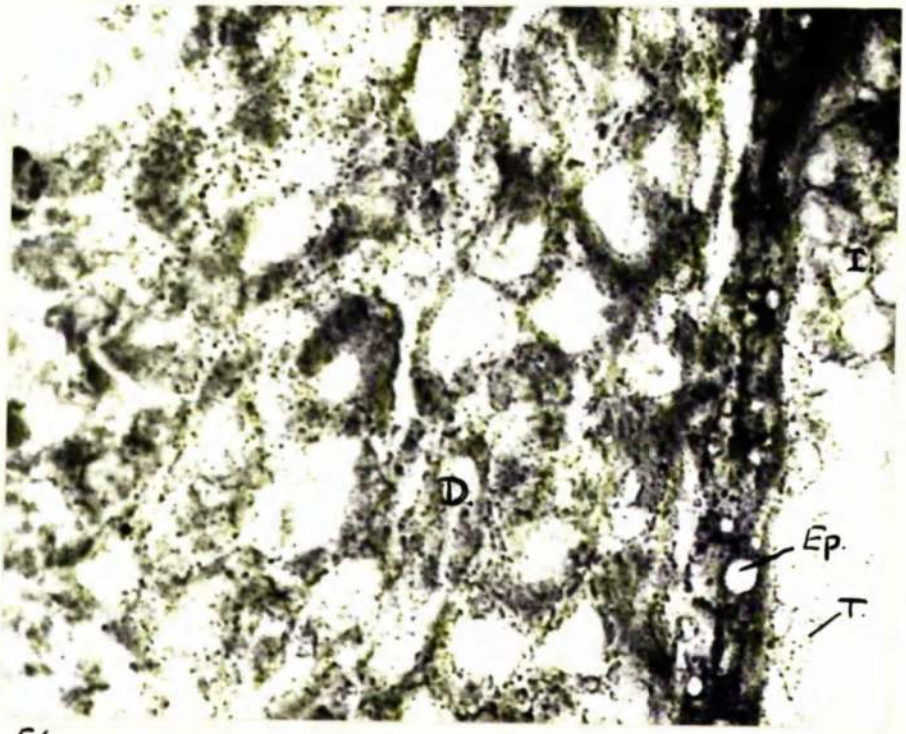

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Fig. 58. β-glycerophosphatase (pH 7.2) in the uterus of pregnant rats at:-

A) $4\frac{1}{2}$ days

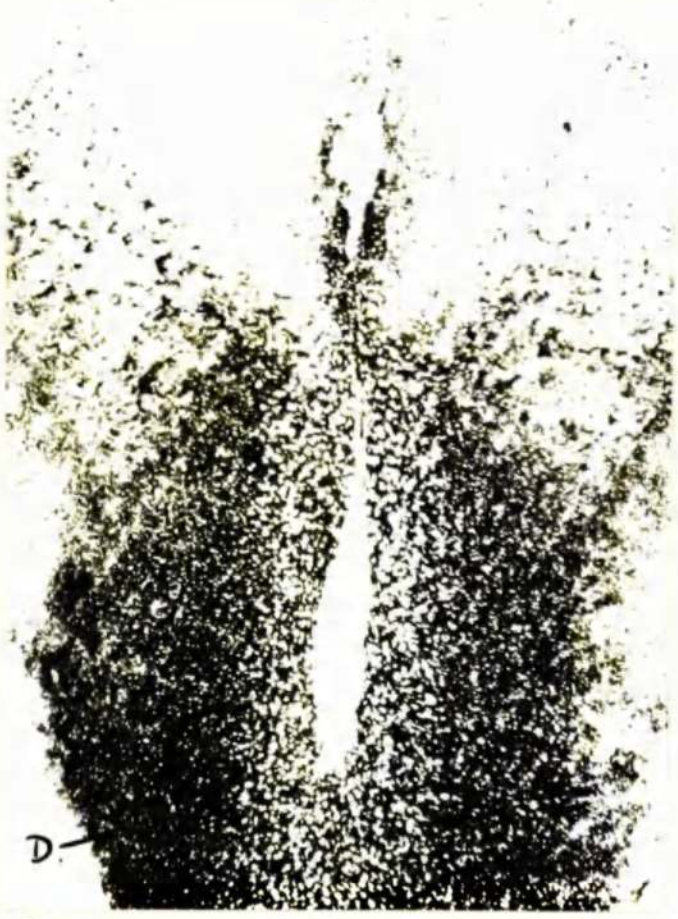
B) $5\frac{1}{2}$ days when it appears in the primary decidua (D.)

C) $7\frac{1}{2}$ days when activity is maximal in the secondary decidua (D.)

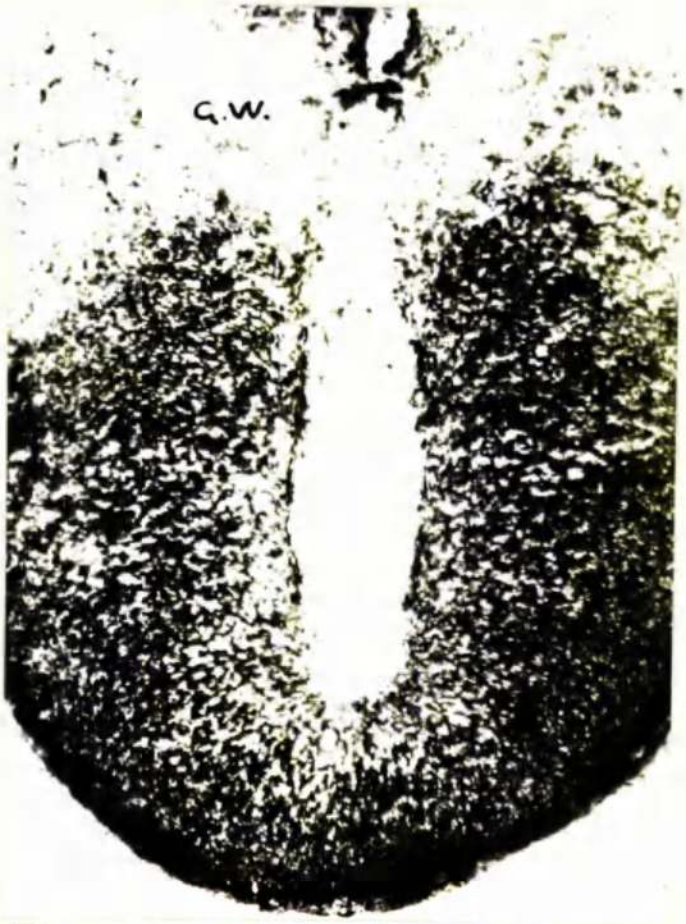
D) $9\frac{1}{2}$ days when the activity in the decidua has decreased markedly. The glycogen wings (G.W.) are negative with this method.

A

B



C



D

Fig. 59. AMPase activity in the uterus of pregnant rats at:-

A) 4½ days

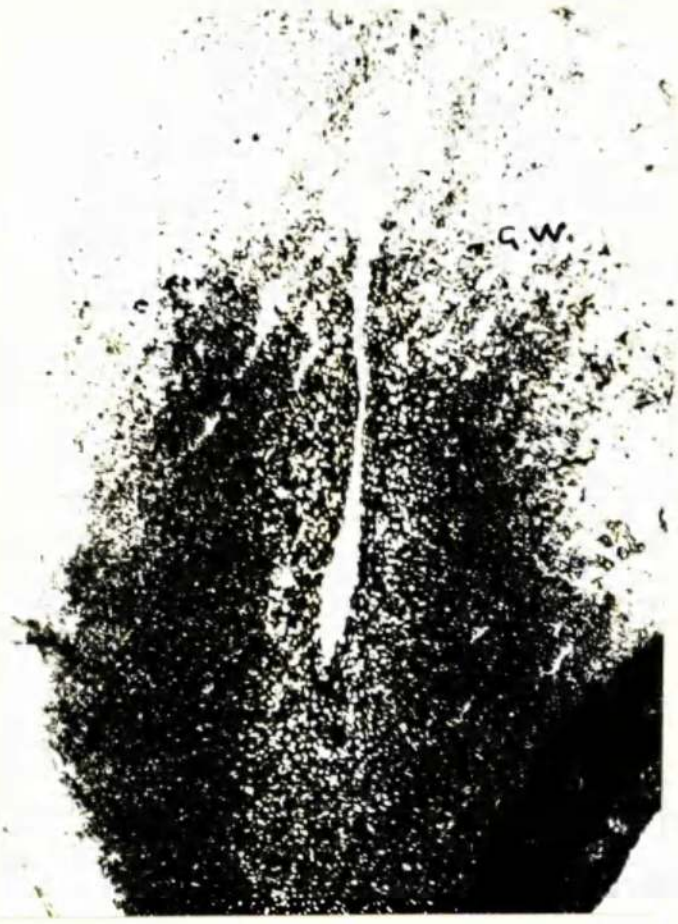
B) 5½ days showing enzyme accumulation in the decidua (D.)

C) 7½ days showing spread of enzyme activity, maximal in the decidua, into the glycogen wings (G.W.).

D) 9½ days when activity has increased considerably in the glycogen wings with no alteration in the decidua (D.).

A

B



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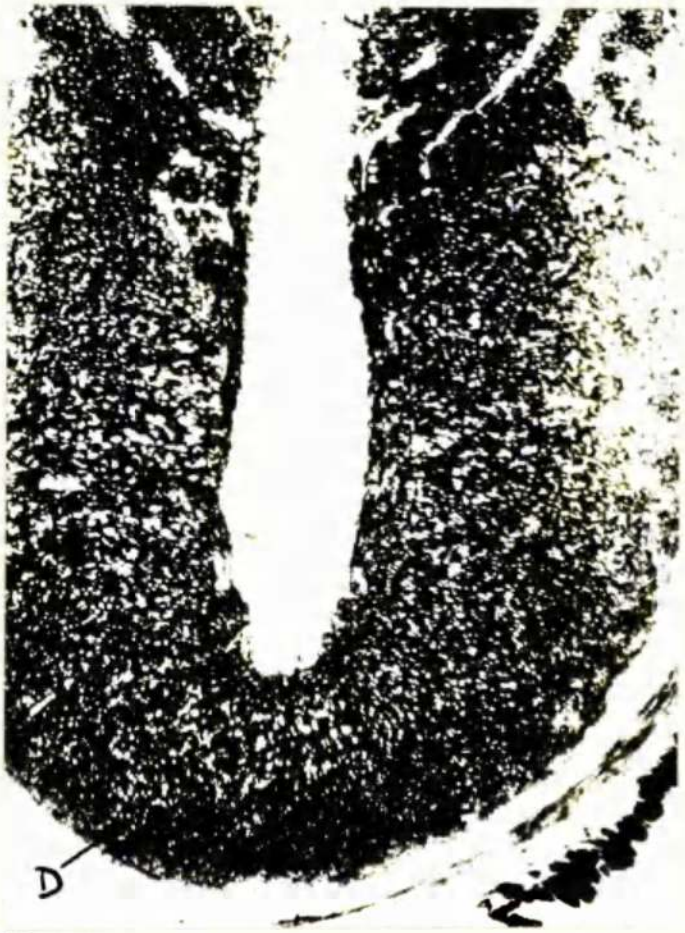


D

Fig. 60. ATPase activity in the uterus of pregnant rats at:-

A) 4½ days showing the increase in activity in the antimesometrial stroma (S.)

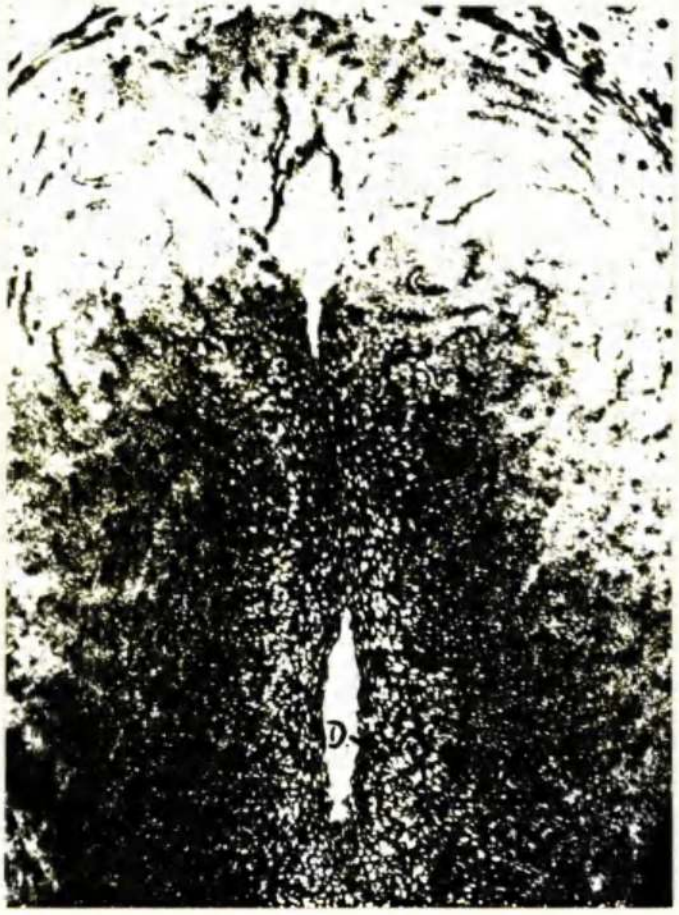
B) 5½ days showing enzyme accumulation in the decidua (D.)

C) 7½ days showing the increased reaction in the decidua (D.)

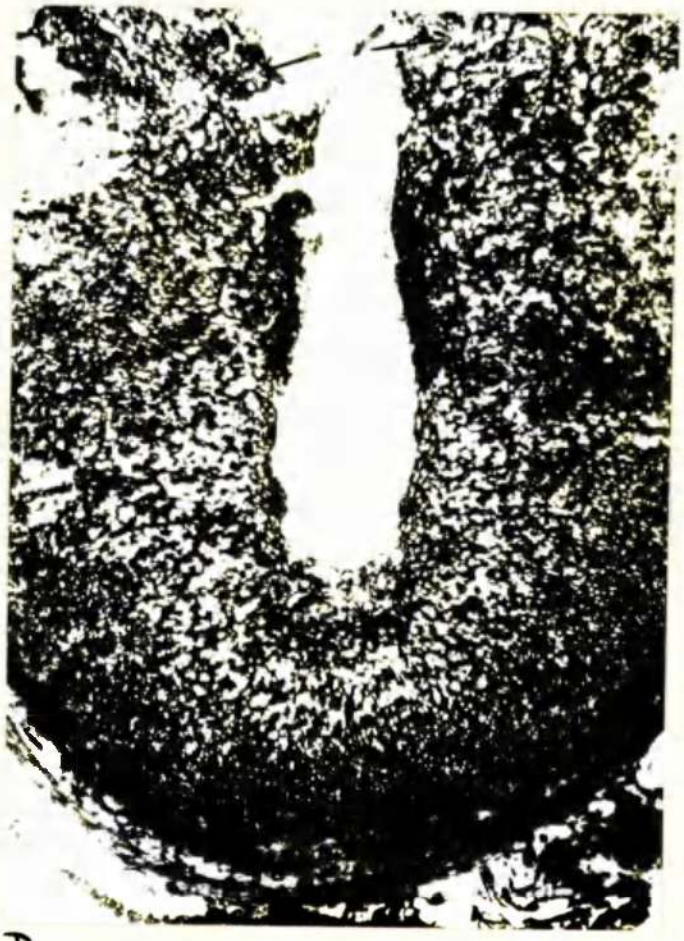
D) 9½ days showing slight fall off in decidual staining, and the accumulation of activity in the sinusoidal epithelium of the glycogen wings (arrowed).

A

B



C



D

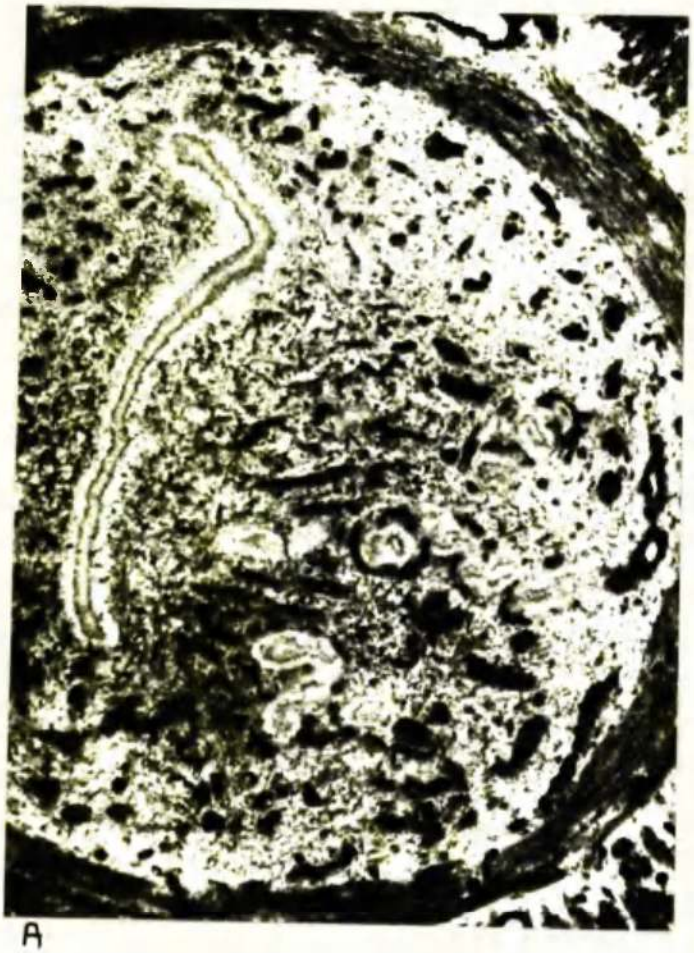
Fig. 61. TPPase activity in the uterus of pregnant rats at:-

A) $4\frac{1}{2}$ days

B) $5\frac{1}{2}$ days showing activity in the decidua (D.)

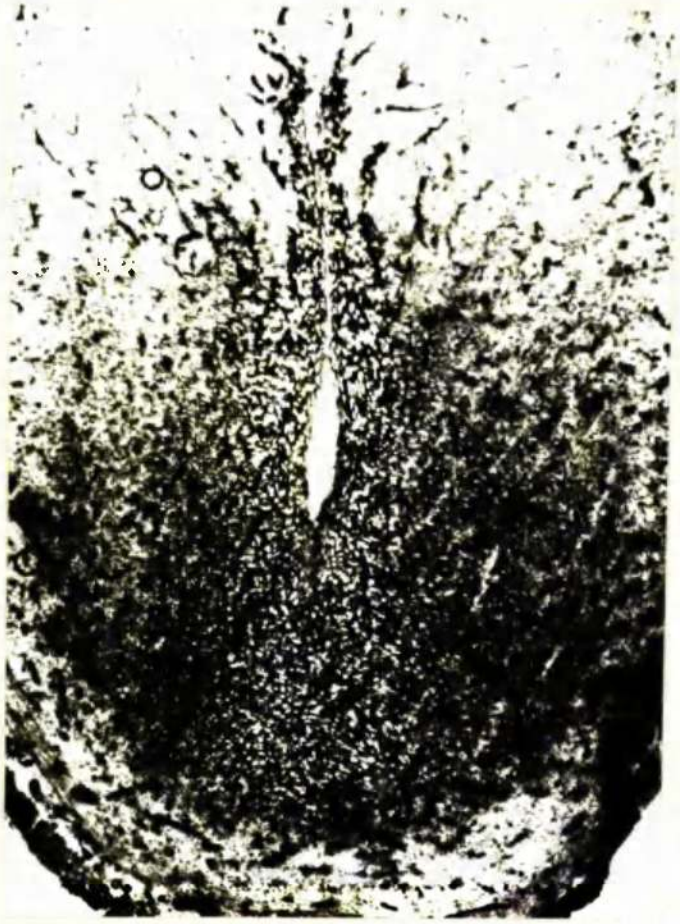
C) $7\frac{1}{2}$ days showing some increase in activity in the decidua

D) $9\frac{1}{2}$ days showing marked increase in activity in the sinusoids of the glycogen wings (arrowed).



A

B



C

D

Fig 62. UDPase activity in the uterus of pregnant rats at:-

A) $4\frac{1}{2}$ days

B) $5\frac{1}{2}$ days showing increase in activity in the decidua (D.)

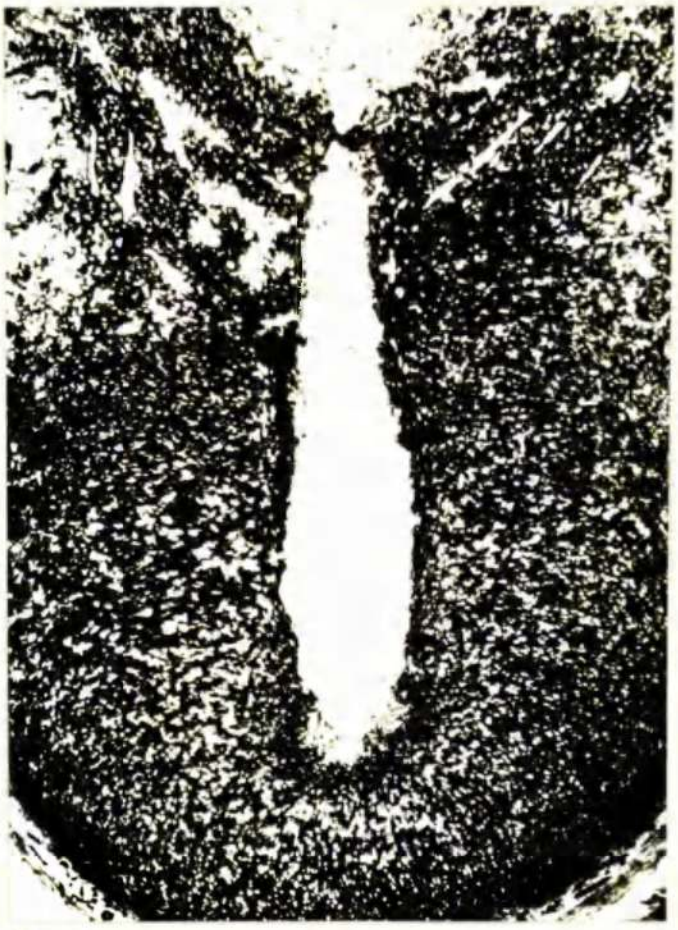
C) $7\frac{1}{2}$ days showing further accumulation of enzyme in the decidua and spread into the glycogen wings (G.W.)

D) $9\frac{1}{2}$ days showing the decrease in activity in all areas

A

B

C

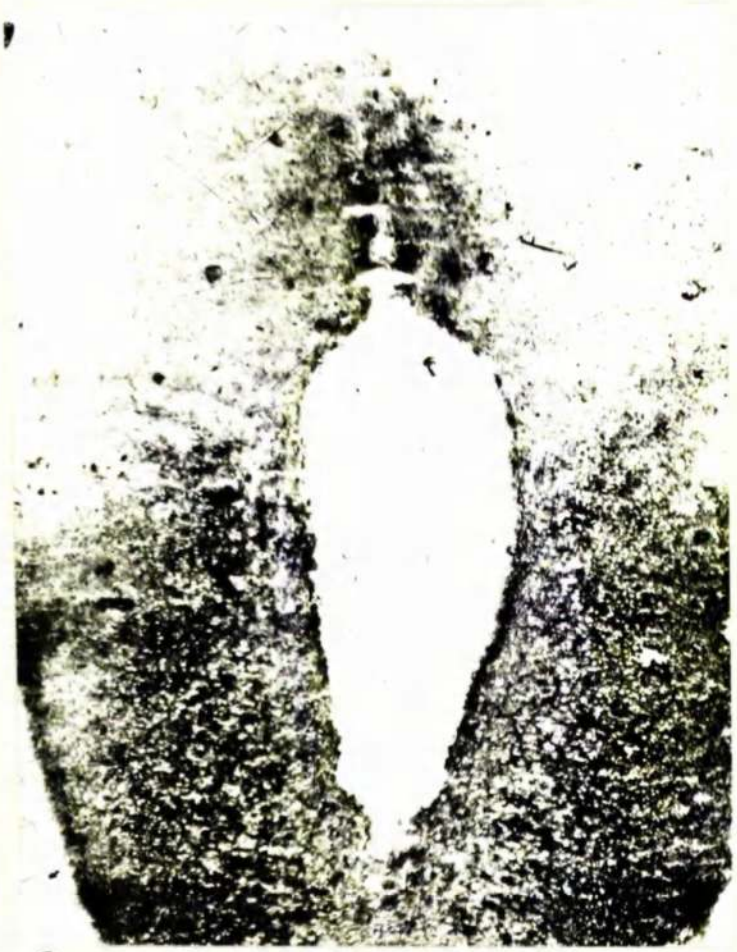


D

Fig. 63. 9½ day pregnant rat uterus showing phosphatase activity towards:-

A) β-glycerophosphate at pH 6.7, control for:-

B) glucose-6-phosphate, in an adjacent section, showing increased activity in the glycogen wings (G.W.). The activity seen in the decidua also may be due to non-specific phosphatase.



A

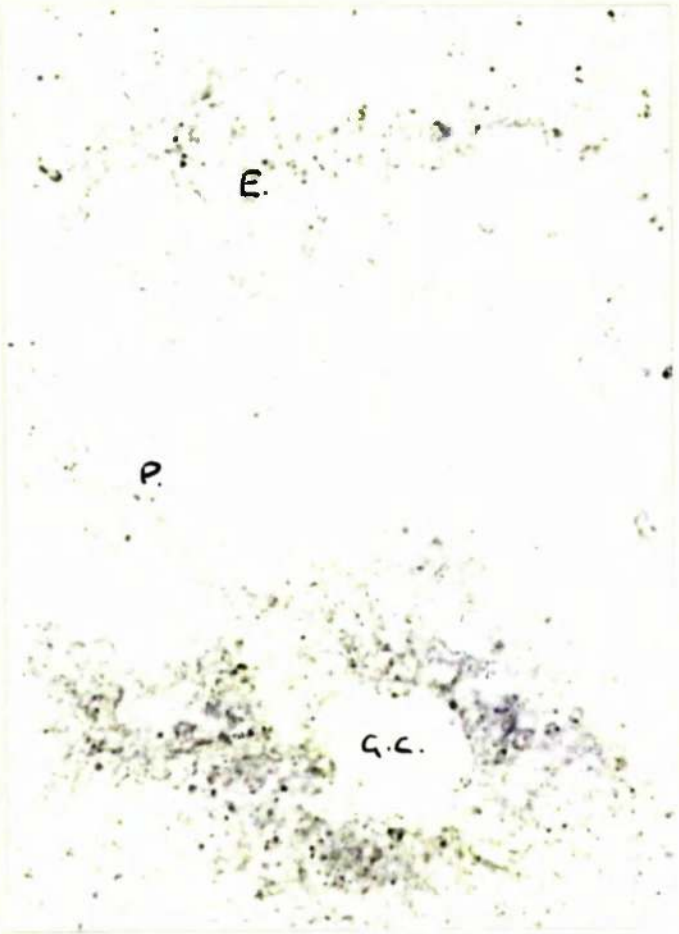
B

Fig. 64. α-glycerophosphate dehydrogenase in the embryonic endoderm (E.), parietal endoderm (P.), and abembryonic giant cells (G.C.) of a 10¼ day rat embryo.

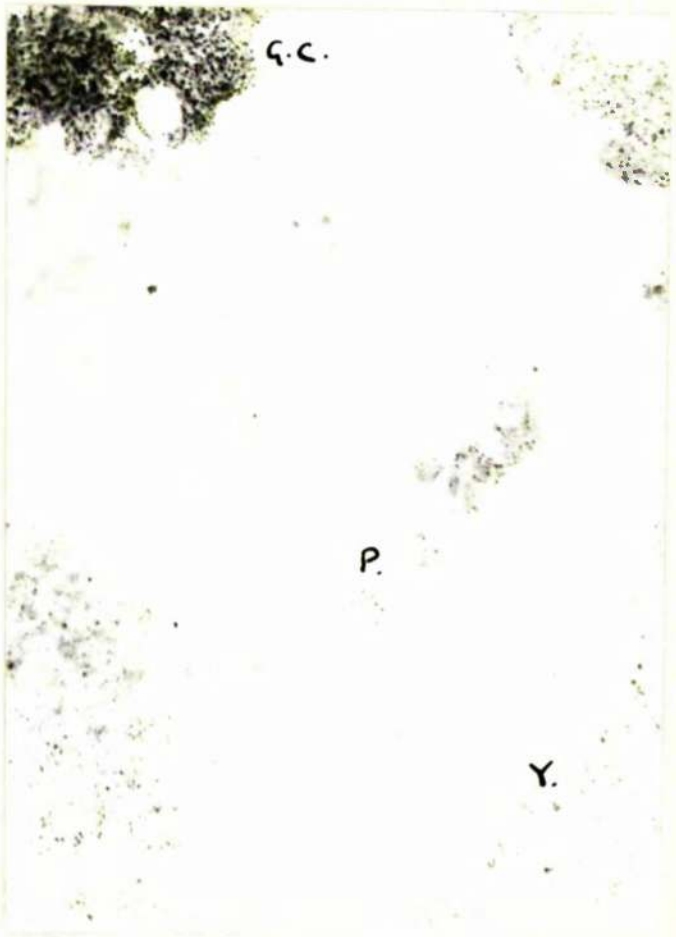
Fig. 65. Glucose-6-phosphate dehydrogenase in the yolk sac (Y.), parietal endoderm (P.), and abembryonic giant cells (G.C.) of a 10½ day rat embryo.

Fig. 66. Glucose-6-phosphate dehydrogenase at 8½ days of gestation in the rat, showing activity in the ectoderm (Ec.), mesoderm (M.), and visceral endoderm (E.), with slight reaction in the decidua (D.).

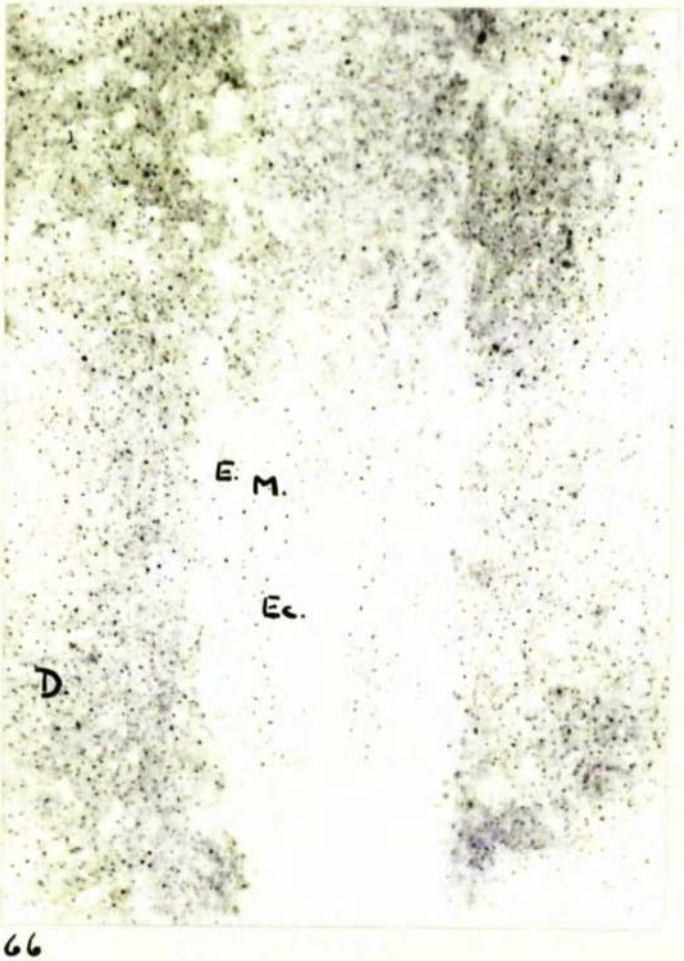
Fig. 67. Lactic dehydrogenase at 5¼ days of gestation in the rat. Activity is seen in the trophoblast (T.), primary decidua (D.), and uterine epithelium (Ep.).



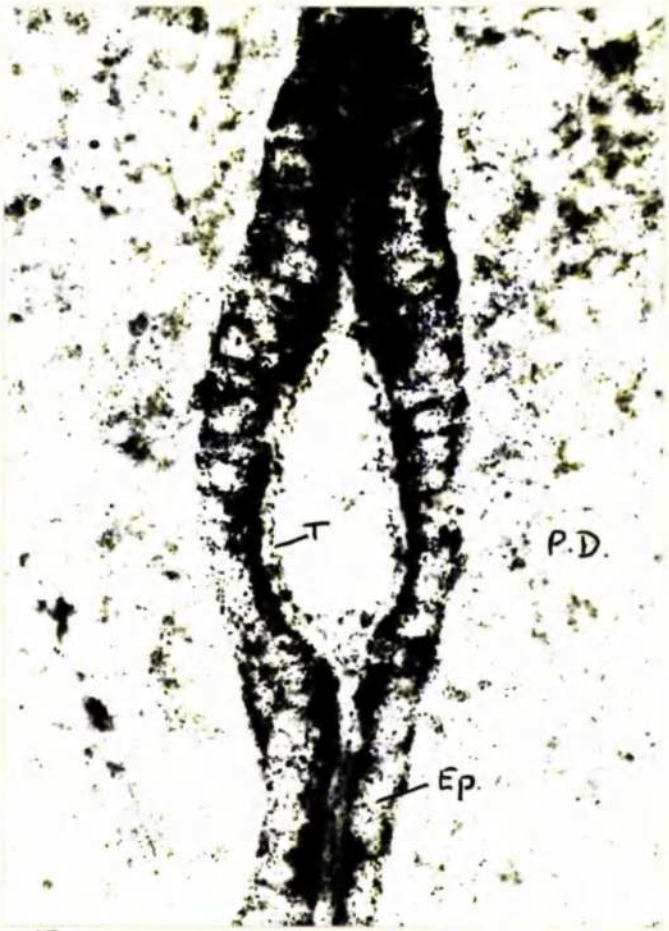
64



65



66

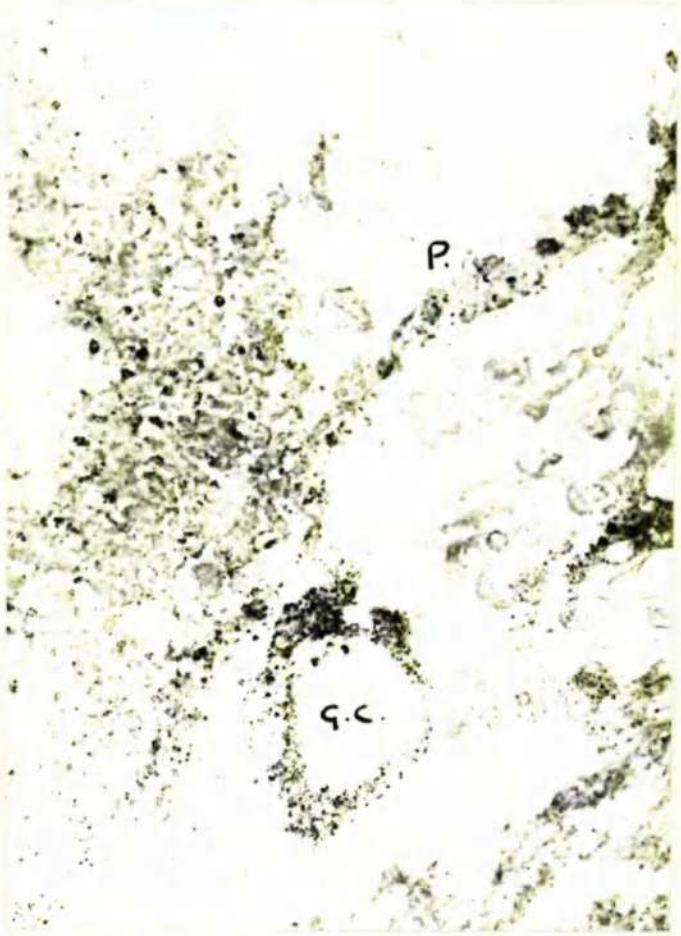


67

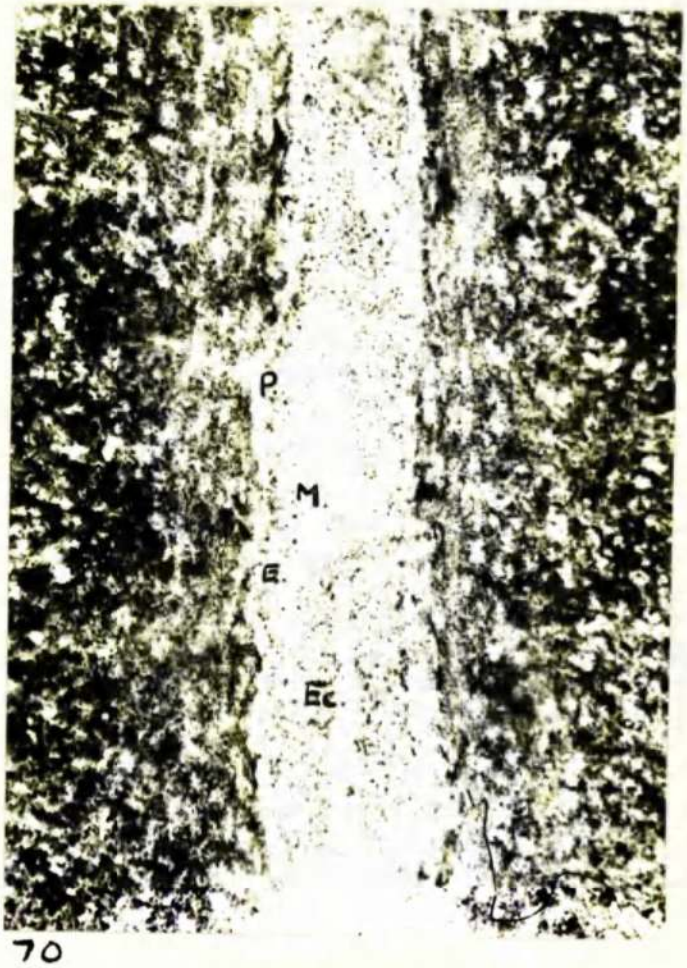
Fig. 68. 9½ day rat embryo, showing isocitric dehydrogenase in the abembryonic giant cells (G.C.), and parietal endoderm (P.).

Fig. 69. 9½ day rat embryo, showing malic dehydrogenase in the ectoplacental cone (E.C.), where activity is greater centrally, and in the glycogen wings (G.W.).

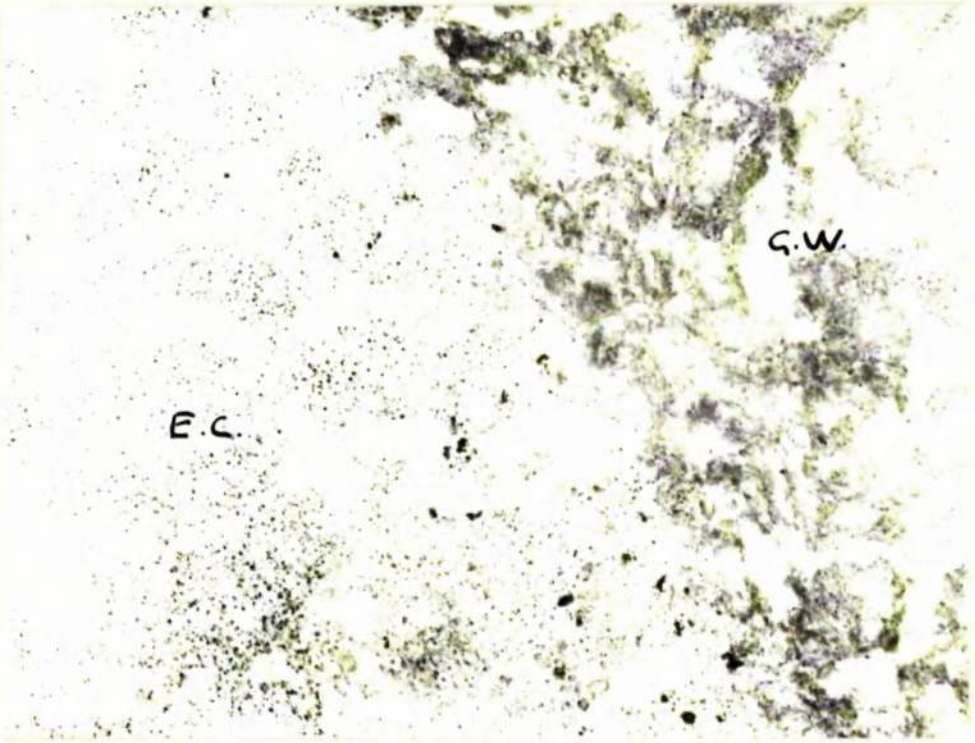
Fig.70. 8½ day rat embryo, showing succinic dehydrogenase in the ectoderm (Ec.), mesoderm (M.), and visceral endoderm (E.), and accumulating in the parietal endoderm (P.).



68



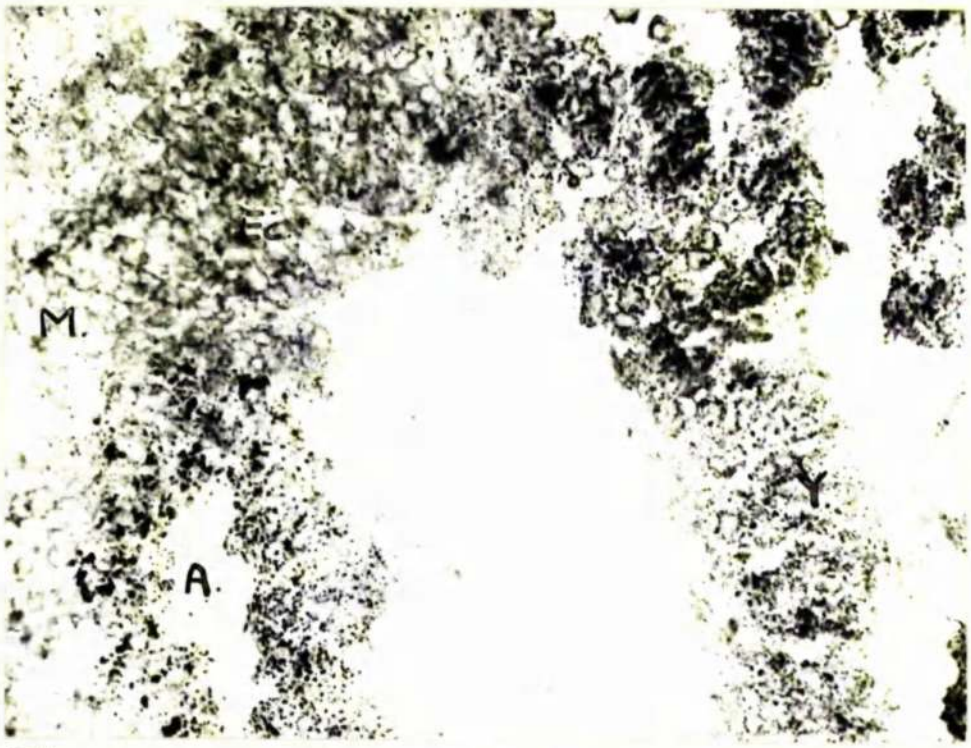
70



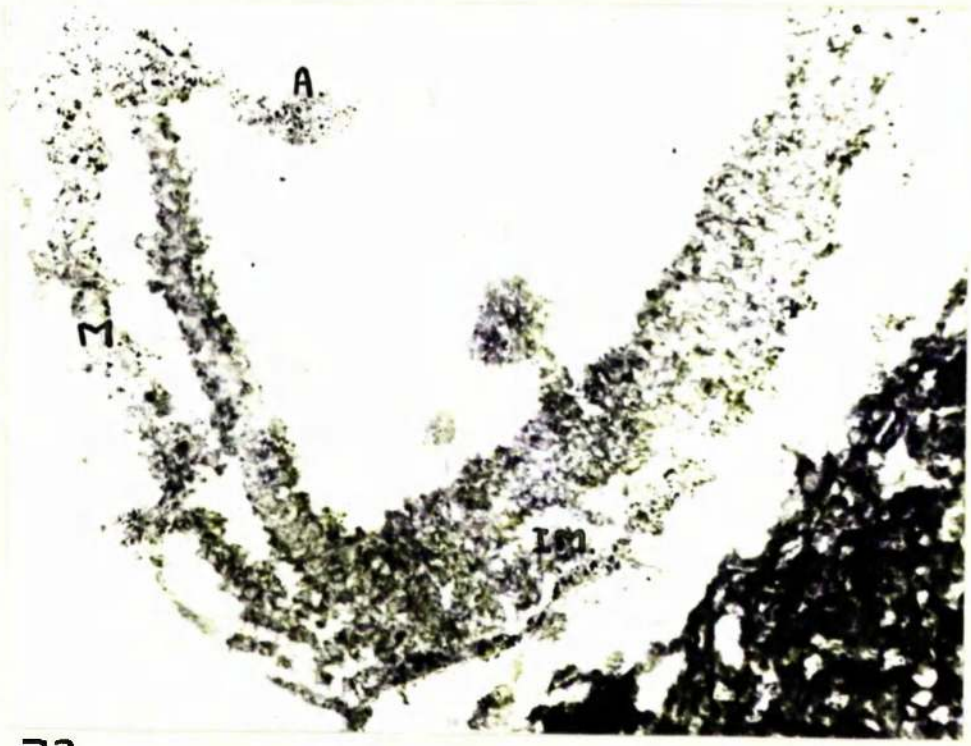
69

Fig. 71. 10½ day rat embryo, showing lactic dehydrogenase in the yolk sac (Y.), and anterior intestinal portal (A.). Less activity is seen in the ectoderm (Ec.), and parietal endoderm (P.).

Fig. 72. 9½ day rat embryo, showing malic dehydrogenase activity in the mesoderm (M.) budding off the primitive streak posteriorly, and in the allantoic outgrowth (A.). Less activity is seen in the remaining intra-embryonic mesoderm (IM.)



71



72

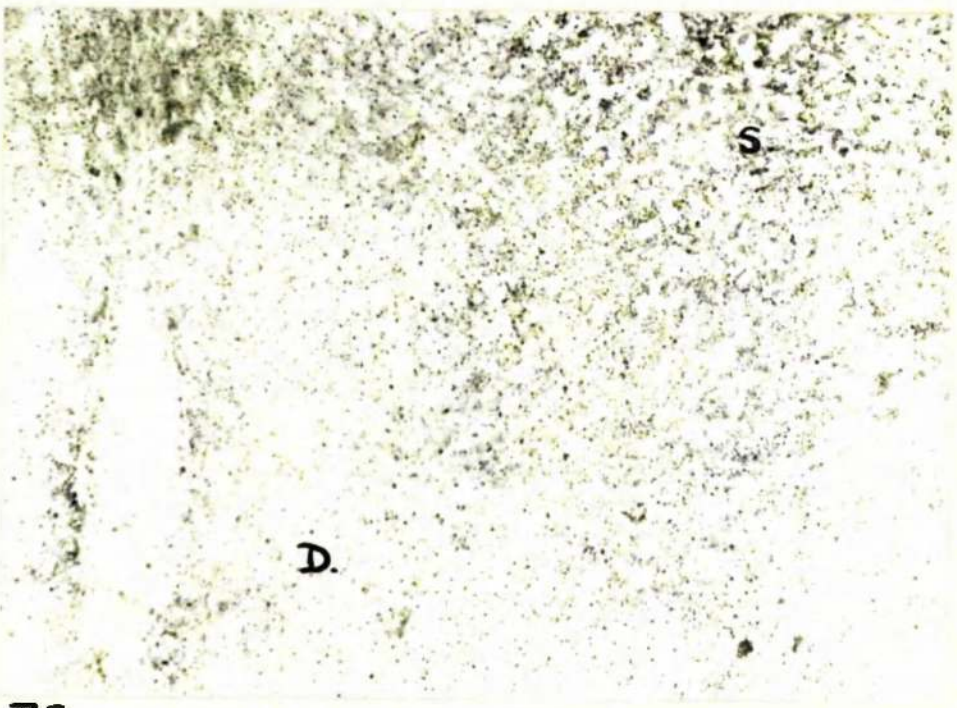
Fig. 73. 10½ day rat embryo, showing furfuryl dehydrogenase in the central part of the ectoplacental cone (E.C.), in the yolk sac (Y.) and parietal (P.) endoderms.

Fig. 74. 7 day rat implantation site - uterine epithelium. α-glycerophosphate dehydrogenase.

Fig. 75. 6½ day rat implantation site, showing glucose-6-phosphate dehydrogenase in the stroma (S.), mesometrial to the decidua (D.) which shows less activity.

73

74



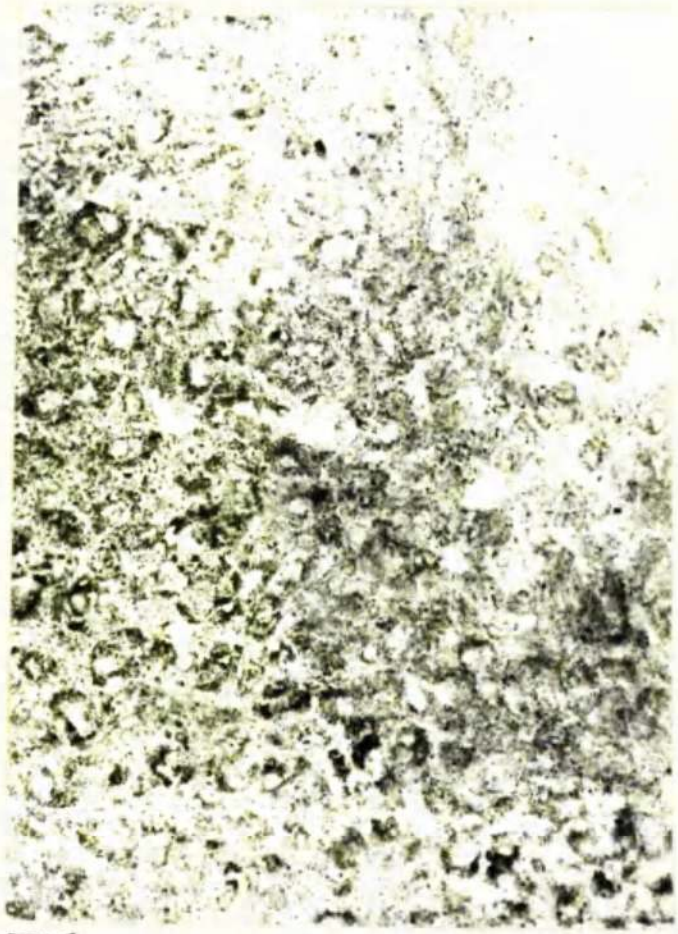
75

Fig. 76. 7½ day rat implantation site, showing intense staining in the decidua with:-

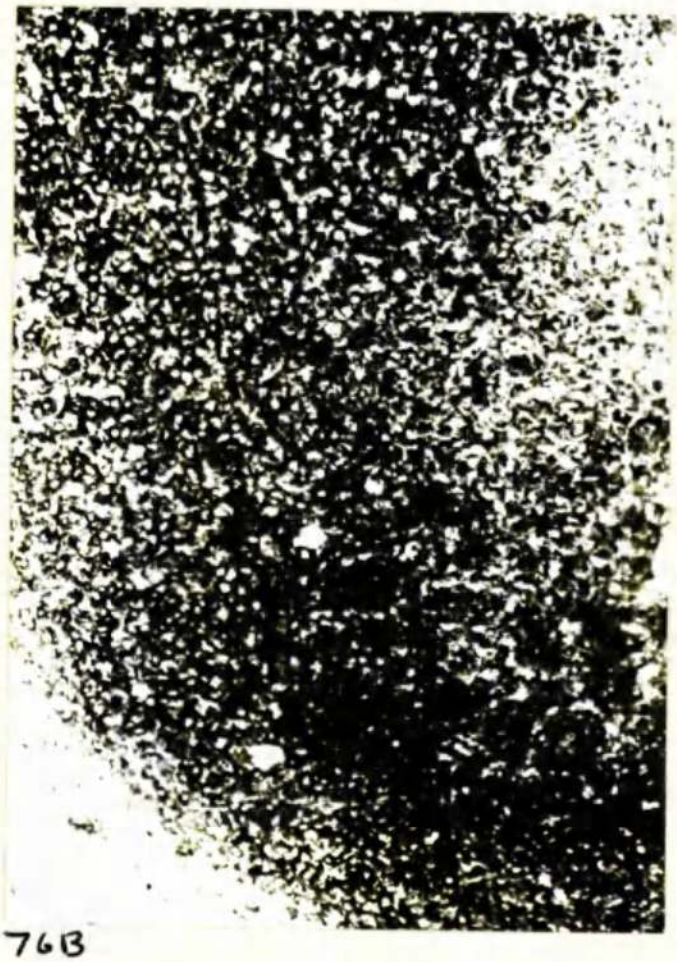
A) malic dehydrogenase

B) lactic dehydrogenase

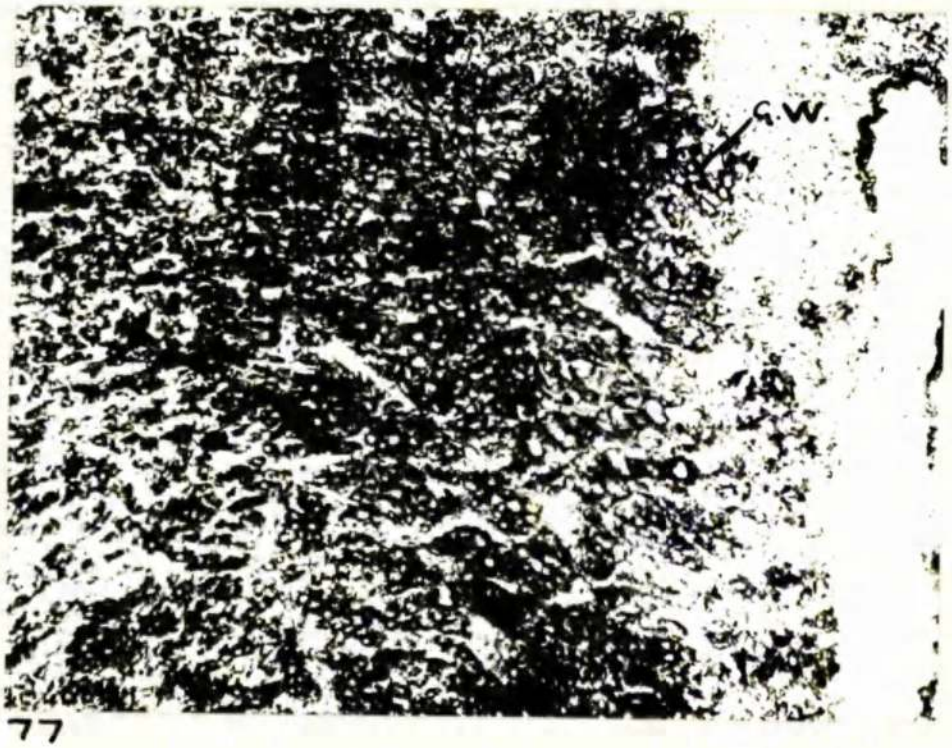
Fig. 77. 9½ day rat implantation site, showing lactic dehydrogenase in the glycogen wings (G.W.).



76A



76B

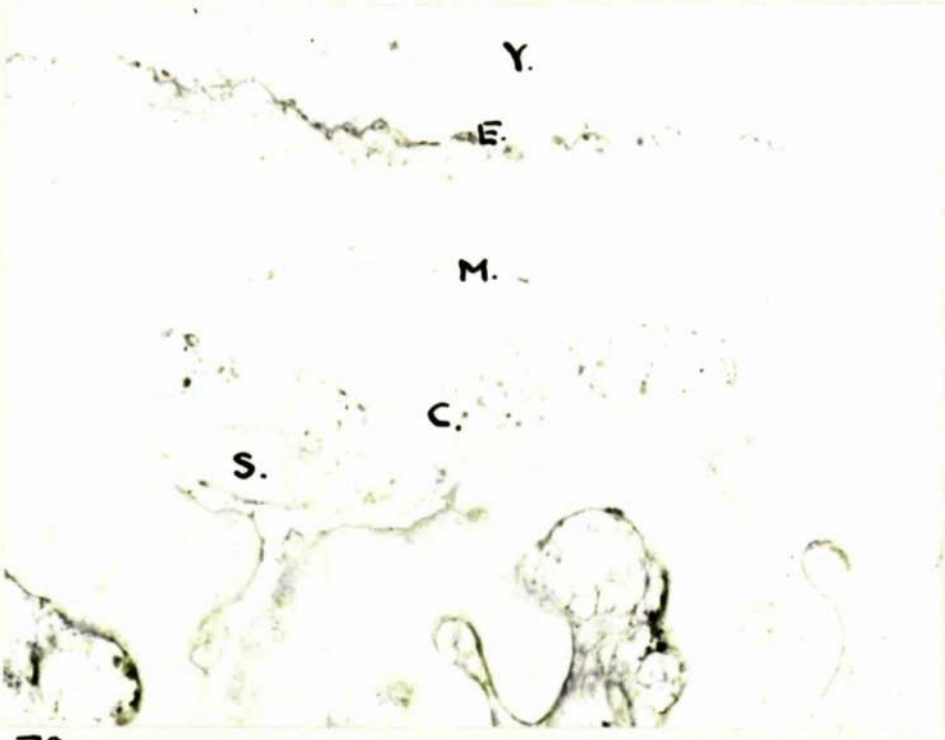


77

Fig. 78. Early 8 day rabbit embryo, showing glycogen in the yolk sac (Y.), endoderm (E.), mesoderm (M.), and ectoderm (Ec.). The epithelium of the placental fold is almost negative (Ep.), but the subjacent stroma shows some granules. PAS.

Fig. 79. Late 8 day rabbit embryo (placental region) showing glycogen still visible in the yolk sac (Y.), endoderm (E.), mesoderm (M.), and cytotrophoblast (C.), but not in the syncytiotrophoblast (S.). PAS.

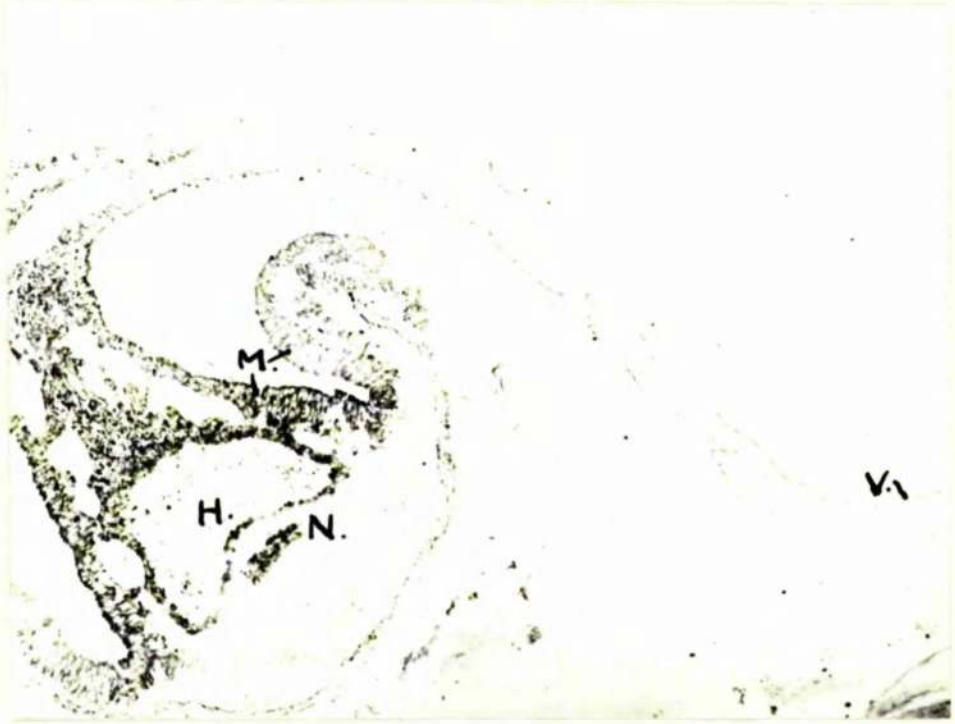
78



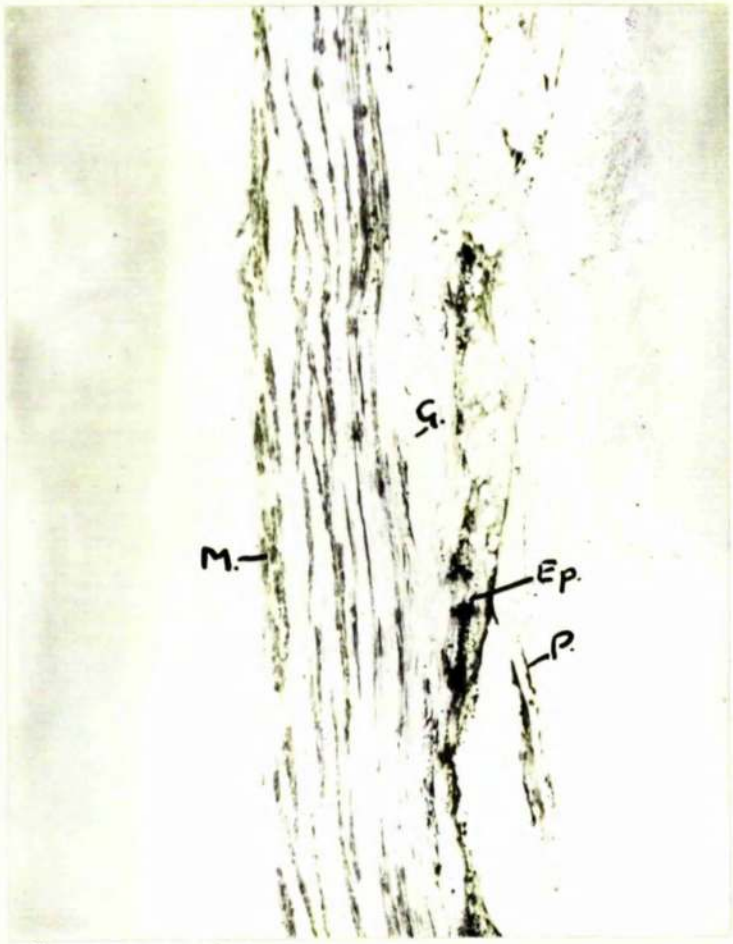
79

Fig. 80. 9 day rabbit embryo, showing glycogen in the intra-embryonic endoderm and hind-gut (H.), notochord (N.), and developing mesoderm (M.), but not in the endoderm (V.) related to the placental folds. PAS-dimedone.

Fig. 81. 9 day rabbit embryo, showing glycogen in the "parietal" endoderm (P.), degenerating uterine epithelium (Ep.), and subepithelial connective tissue, and muscle layers (M.). Traces only are present in surviving glandular epithelium (G.). PAS-dimedone.



80



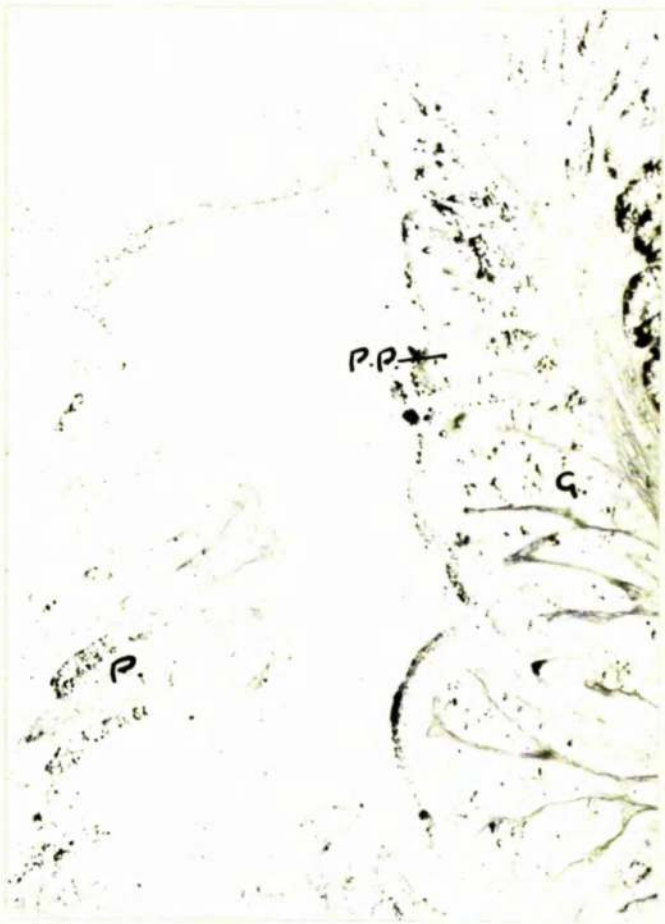
81

Fig. 82. 6 day rabbit uterus to show glycogen (arrowed) beneath the epithelium, and in the glands. PAS.

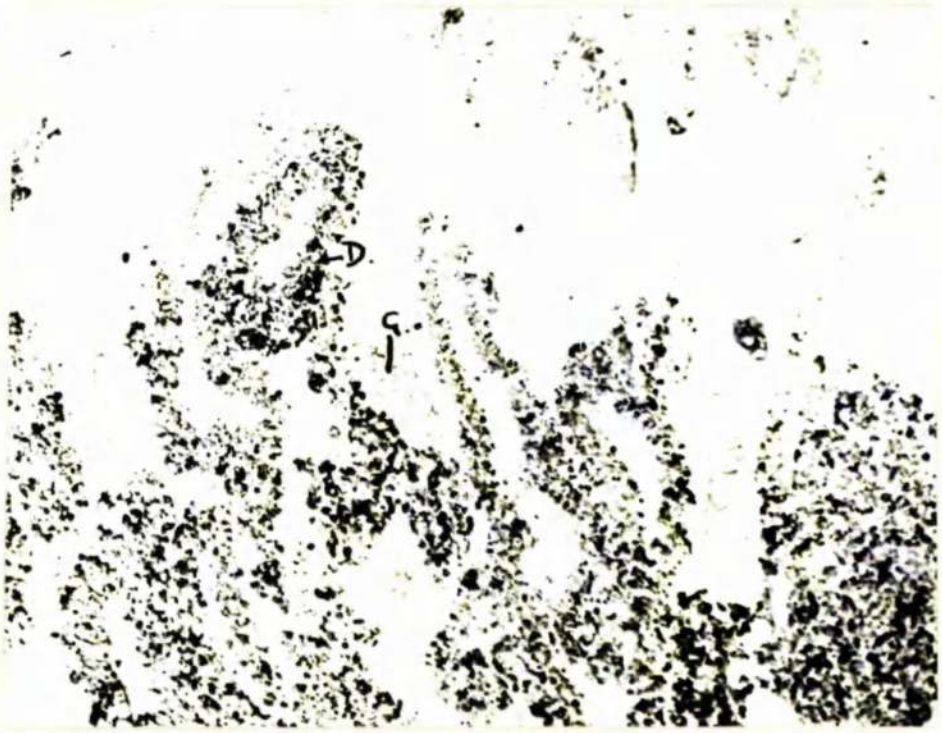
Fig. 83. Early 8 day rabbit placental site, showing glycogen in the epithelium and stroma of the placental (P.), and para-placental (P.P.) folds, and of the uterine glands (G.). PAS.

Fig. 84. 9 day rabbit placental site, showing glycogen accumulation in the decidua (D.), and in a subplacental gland (G.). PAS-dimedone.

82



83



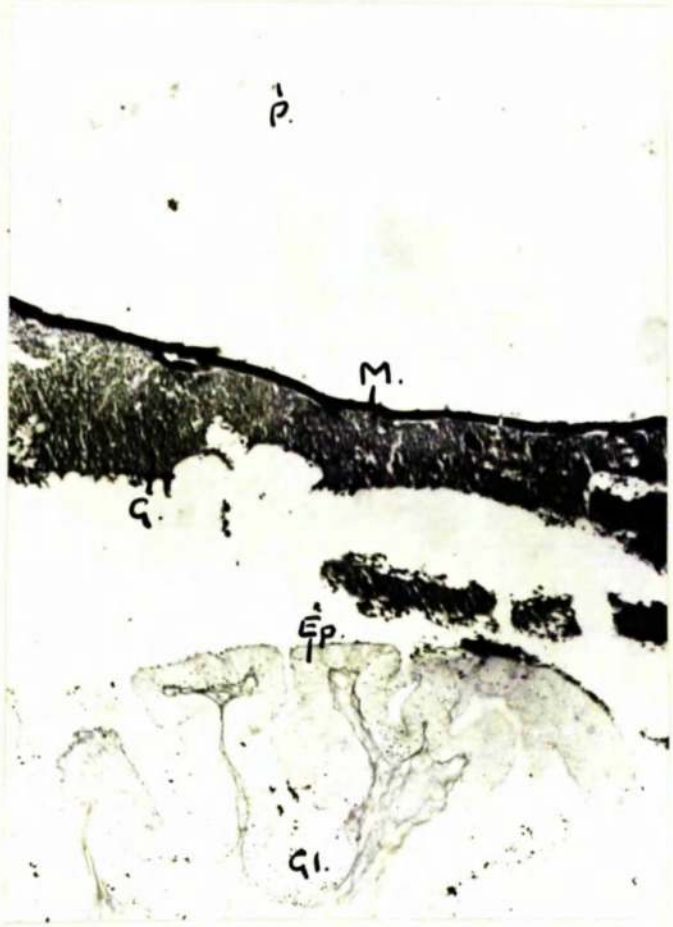
84

Fig. 85. 7 day rabbit embryo, showing intensely positive mucolemma (M.), granular gliolemma (G.), occasional granules related to the primitive streak (P.), and in the mesometrial epithelium (Ep.), and glands (Gl.). PAS-diastase.

Fig. 86. Same section of embryo as fig. 85, showing granules in the trophoblastic knobs (K.). PAS-diastase.

Fig. 87. Early 8 day rabbit embryo, showing the penetration of a trophoblastic invasion (I.) between the epithelial cells, which show a brush border. The cytoplasmic granules of the invading tissue are evident. PAS-diastase.

Fig. 88. Early 8 day rabbit embryo placental site, showing mucoprotein granules in the placental (P.), and para-placental (P.P.) folds, and in the lumen. PAS-diastase.



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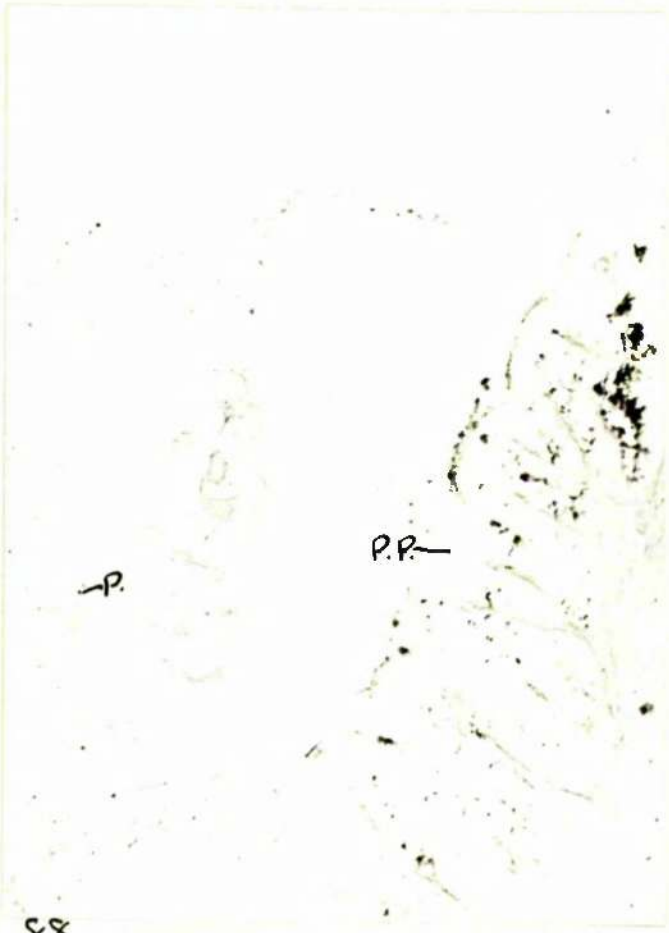
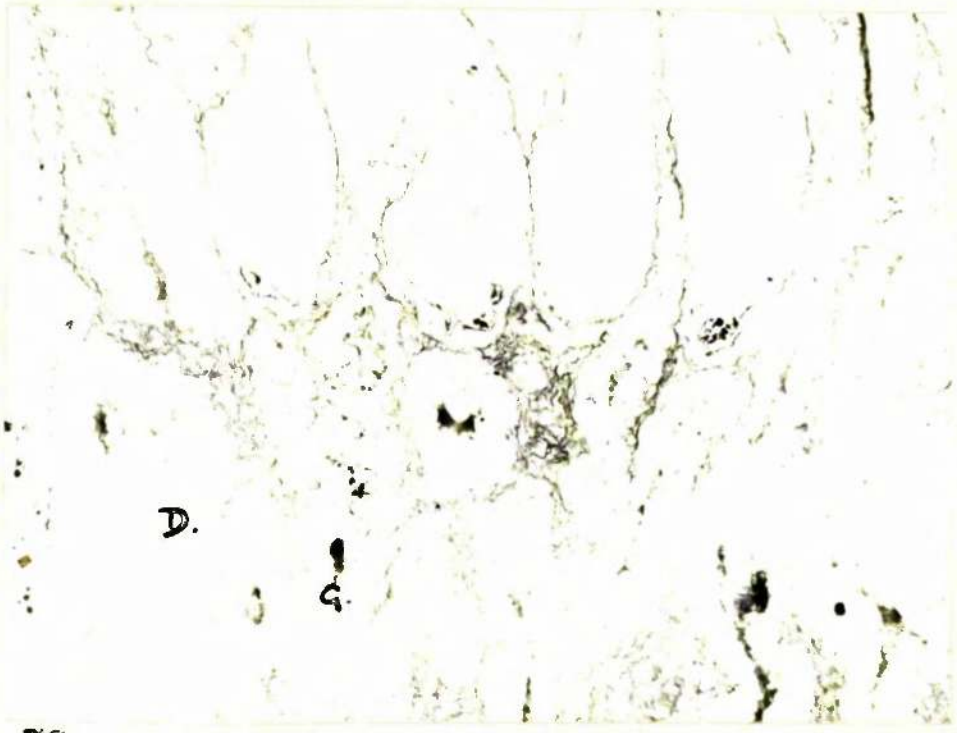


88

Fig. 89. 9 day placental site, showing the removal of glycogen from the decidua (D.) by diastase, and the presence of mucoprotein granules in the lumen of the deep parts of the subplacental glands (G.). Compare with fig. 84. PAS-diastase.

Fig. 90. Visceral endoderm of the same specimen as fig. 89, under higher magnification, showing the cytoplasmic inclusions (arrowed). PAS-diastase.



89

90

Fig. 91. 8 day rabbit placental site, showing intense acid mucopolysaccharide staining in the mucolemma (M.), in the glands of the placental (P.) and paraplacental (P.P.) folds, and in the uterine secretion. Dialyzed iron.

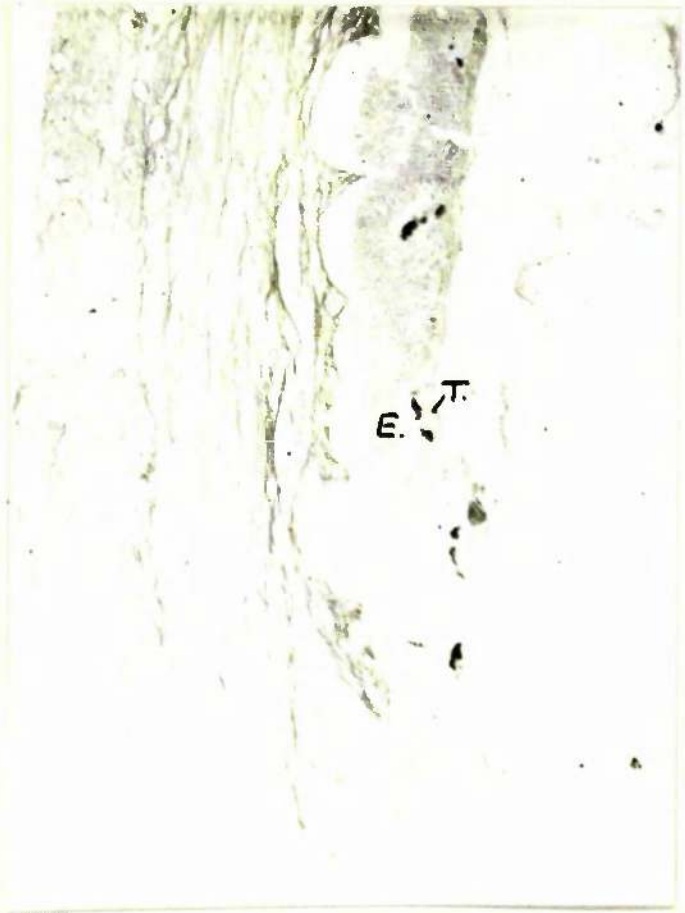
Fig. 92. Antimesometrial side of the same site, showing the considerable degree of secretion (intensely staining) of the glands between the invading trophoblastic knobs (K.). Dialyzed iron.

Fig. 93. 8 day rabbit implantation, slightly later than figs. 91, 92, showing acid mucopolysaccharide masses (? trapped secretion) between the trophoblast (T.), and symplasmic surface epithelium (E.). Dialyzed iron.

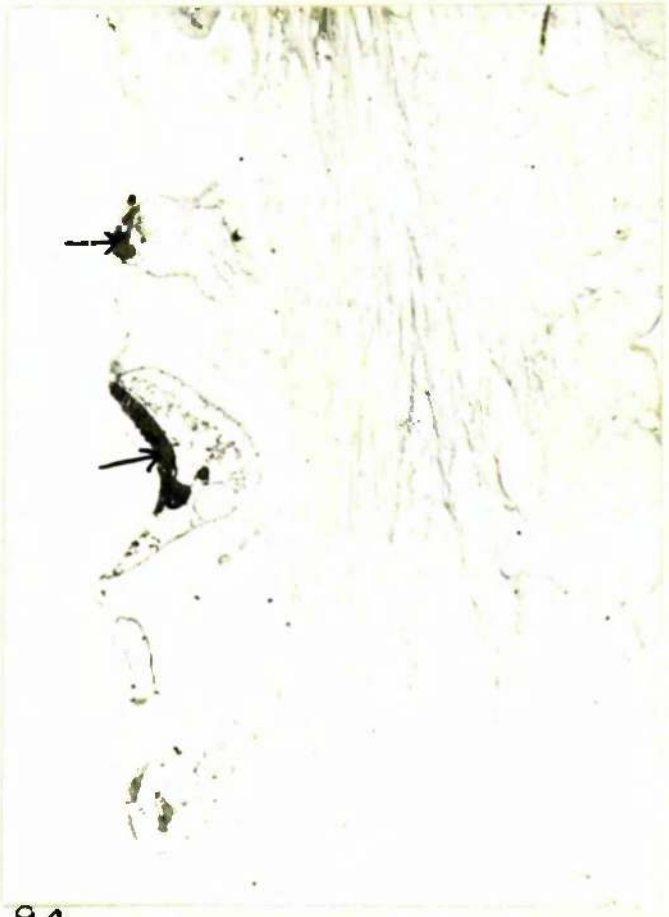
Fig. 94. 9 day rabbit implantation site, showing continued secretion by the surviving deep parts of the anti-mesometrial uterine glands, forming 'pockets' beneathe the trophoblast (arrowed). Dialyzed iron.

91

92



93



94

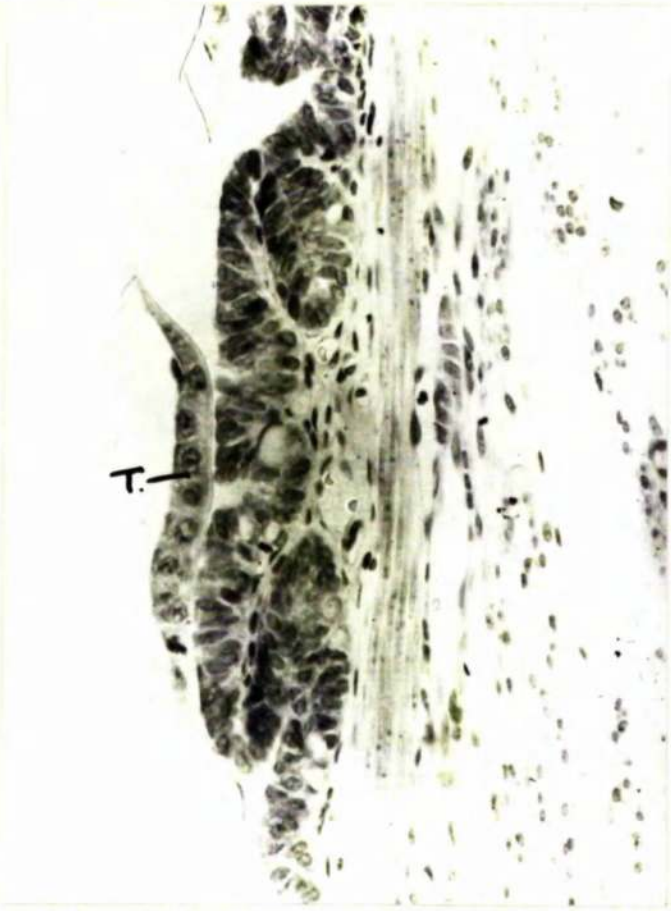
Fig. 95. Mesometrial side of the same site as fig. 94, showing acid mucopolysaccharide in the deepest parts (arrowed) of the subplacental glands (G.). The decidua (D.) is negative. Dialyzed iron.

95

Fig. 96. Late 7 day rabbit embryo, showing the accumulation of RNA in the trophoblast knobs (T.). Faint staining is also seen in the epithelium. Chrome-alum-gallocyanine.

Fig. 97. Early 8 day rabbit embryo, showing the increase in RNA content of the trophoblast surrounding an invasive process (P.) where it has fused to the maternal epithelium. Chrome-alum-gallocyanine.

Fig.98. Late 7 day rabbit embryo, showing increase in RNA content of the neural plate, particularly towards its mesometrial edge (arrowed). Chrome-alum-gallocyanine.



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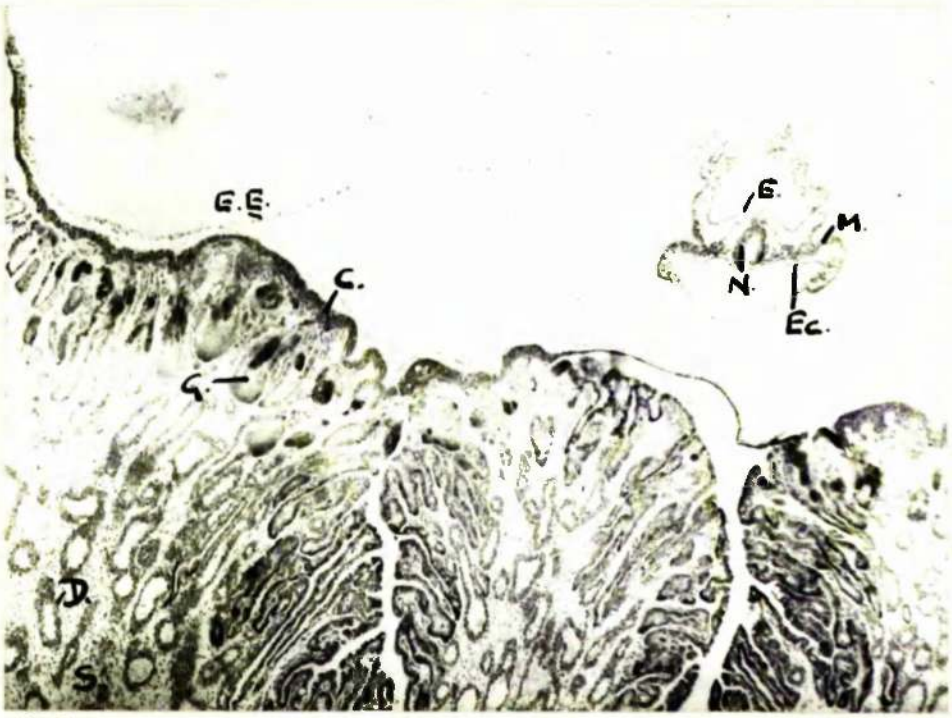
97

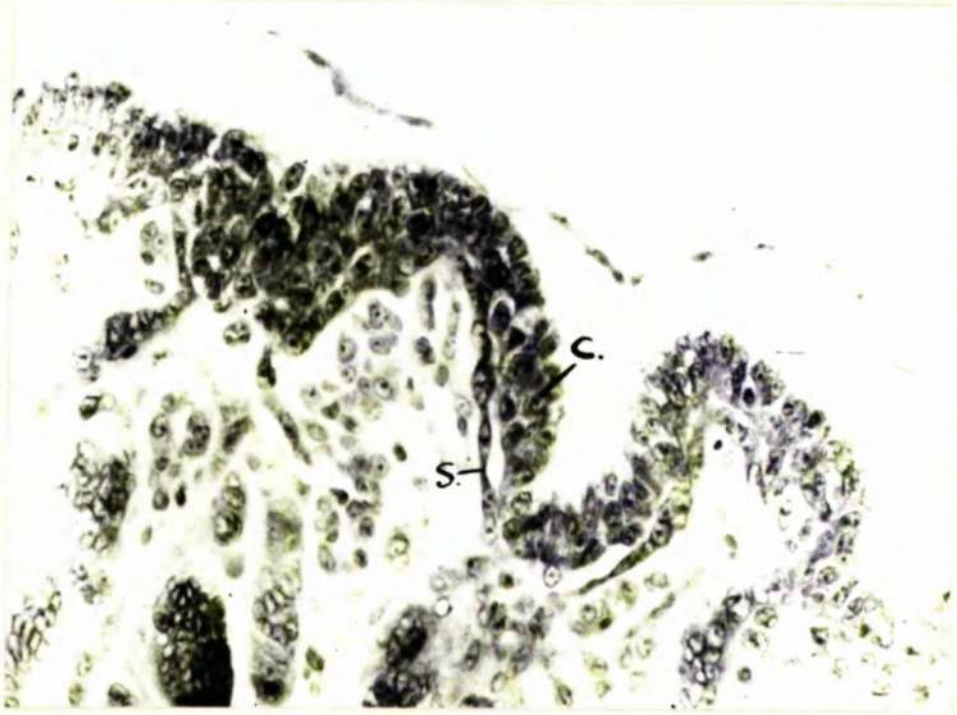
98

Fig. 99. Late 8 day rabbit implantation site, showing RNA in the decidua (D.), stroma (S.), syncytio- and cytotrophoblast (C.), uterine gland symplasma (G.), embryonic neural tube (N.), ectoderm (Ec.), mesoderm (M.), and endoderm (E.), but absence from the extra-embryonic endoderm (E.E.). Chrome-alum-gallocyanine.

Fig. 100. Higher power view of fig. 99, showing RNA in the syncytiotrophoblast (S.), and cytotrophoblast (C.). Chrome-alum-gallocyanine.



99

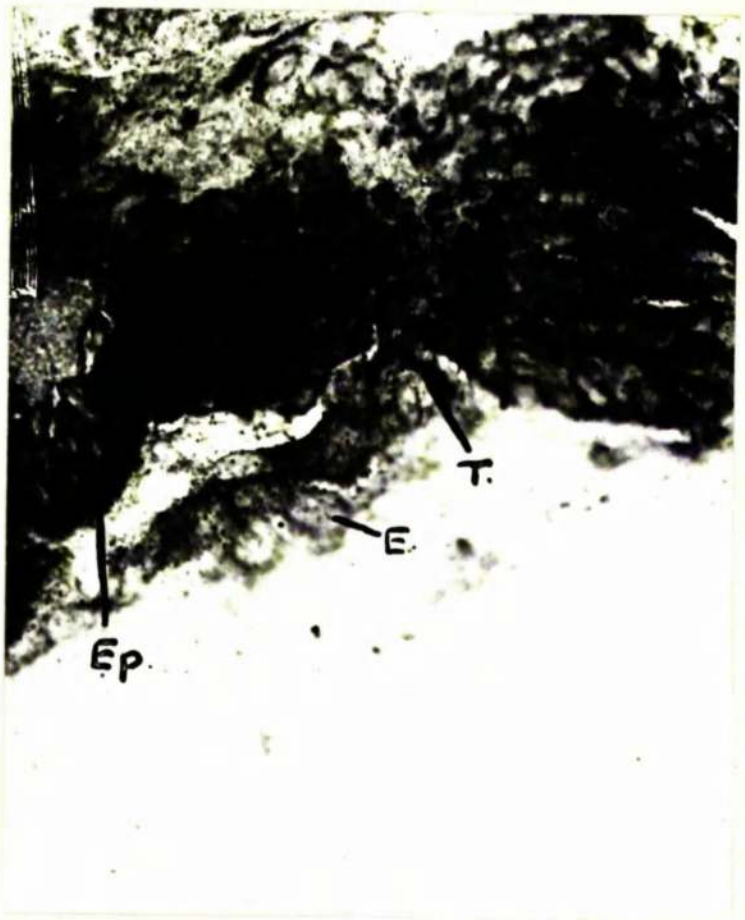


100

Fig. 101. 7 day rabbit implantation site, mesometrial region, showing acid phosphatase in the trophoblast (T.), and uterine epithelium (Ep.).

Fig. 102. Late 7 day rabbit implantation site, antimesometrial region, showing increase in acid phosphatase activity in the trophoblastic invasion (T.), and in the maternal epithelium (Ep.). Some staining is seen in the endoderm also (E.).

101



102

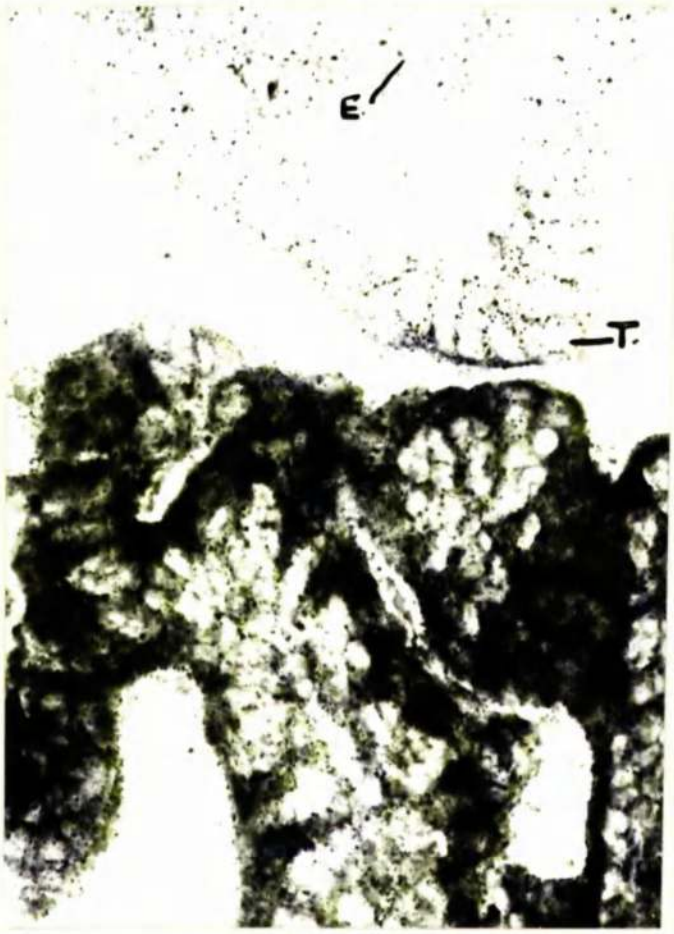
Fig. 103. Early 8 day rabbit implantation, mesometrial region, showing the increase in hydrolase activity in those areas of trophoblast (T.) contacting the maternal epithelium, compared to the inter-contact areas, and the activity in the endoderm (E.).

A) acid phosphatase

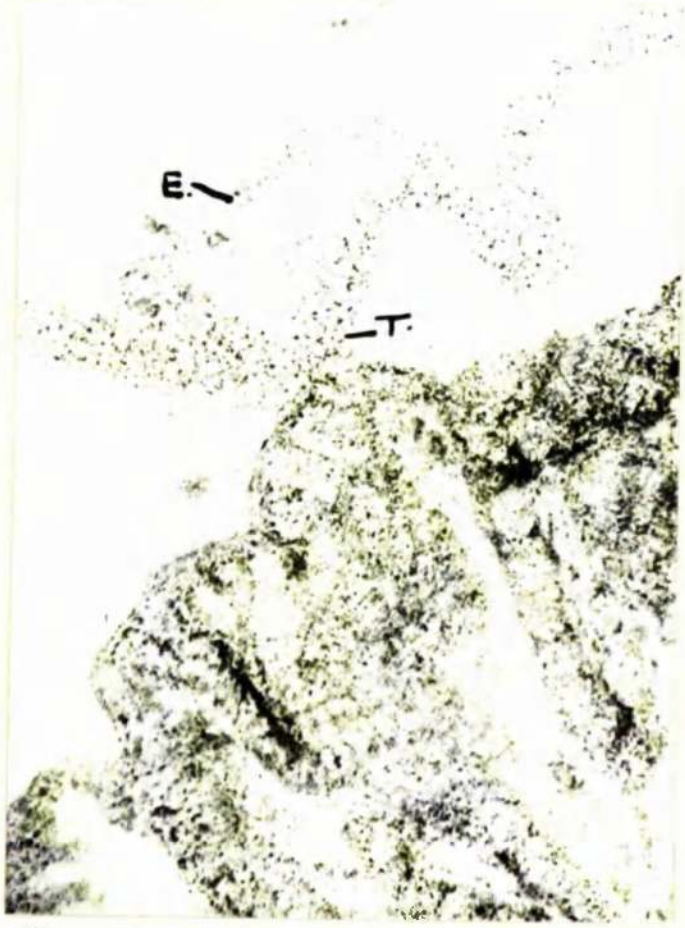
B) Naphthol-AS-acetate esterase.

Some increase in epithelial staining with acid phosphatase, as compared to fig. 101, is also visible.

Fig. 104. Mid-8 day rabbit implantation, mesometrial side, to show further acid phosphatase accumulation in the syncytiotrophoblast (S.), cytotrophoblast (C.), endoderm (E.), and mesoderm (M.). Increased activity in the uterine epithelium (Ep.) is also seen.



103 A



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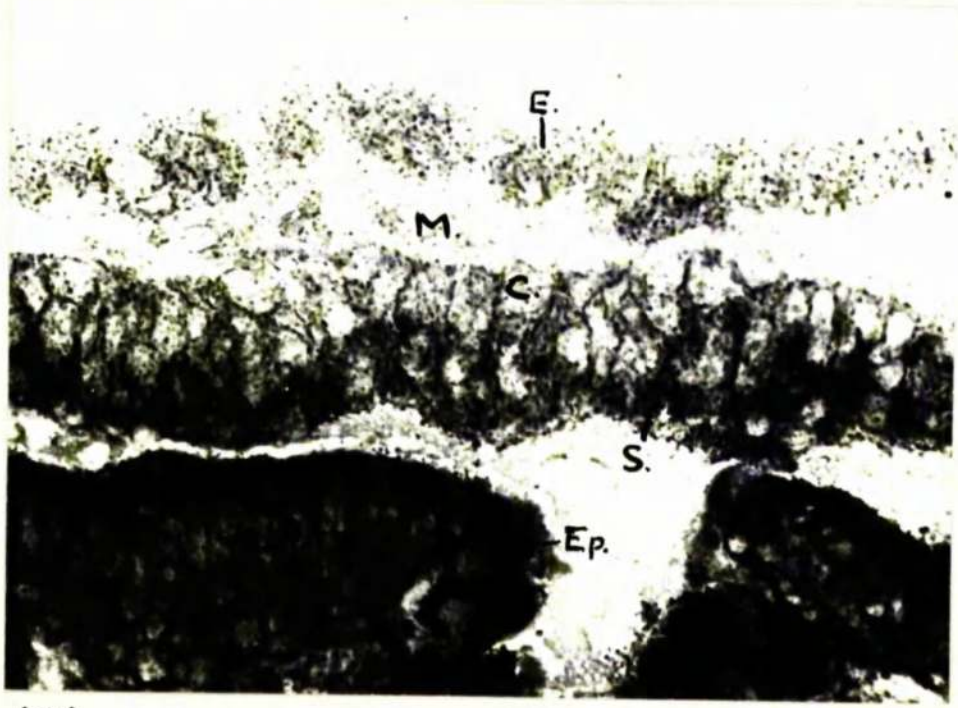


104

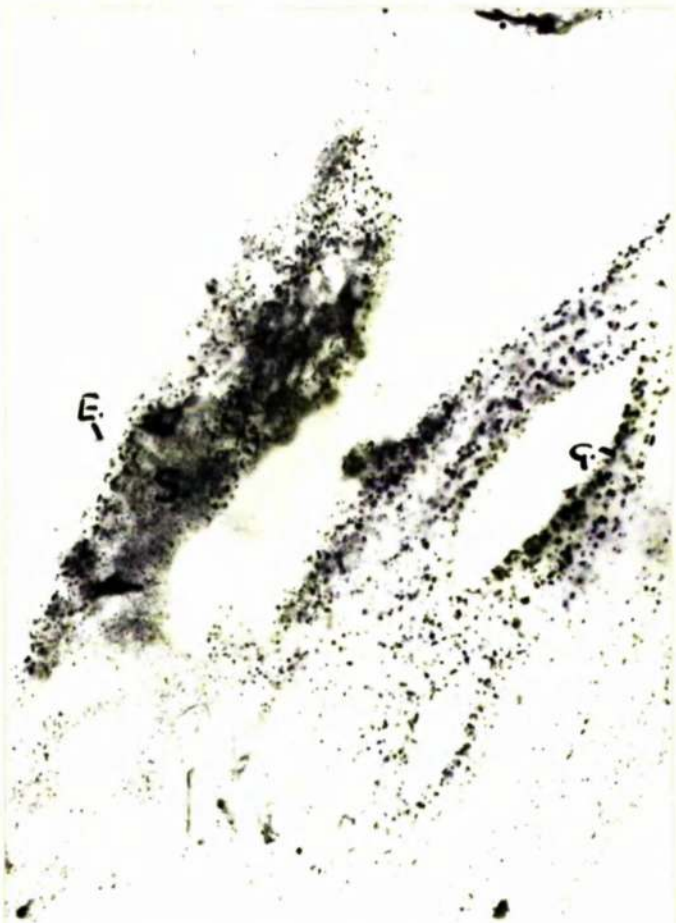
Fig. 105. 9 day rabbit implantation, showing increased acid phosphatase activity in the syncytiotrophoblast (S.), with less in the cytotrophoblast (C.).

Fig. 106. 9 day rabbit implantation site, showing intense acid phosphatase activity in the degenerating symplasma (S.) and trophoblast. Less activity is seen in the endoderm (E.), and surviving deep parts of the uterine glands (G.).

Fig. 107. 9 day rabbit implantation site, showing the same features as fig. 106.
Naphthol-AS-acetate esterase.

105

106



107

Fig. 108. 8 day rabbit implantation site, mesometrial region, showing increased epithelial (Ep.), and glandular (G.) hydrolase activity, in relation to the area of implantation. Symplasmic change (S.) in the subplacental glands is accompanied by increased activity, but the deeper parts (G.) are less affected.

A) acid phosphatase

B) Naphthol-AS-acetate esterase.

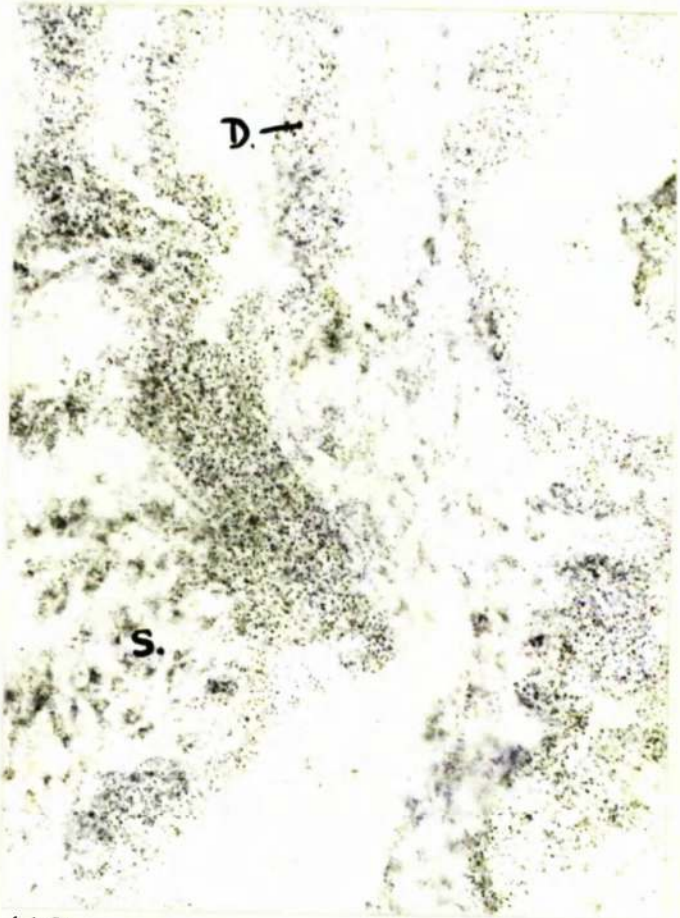
108A

B

Fig. 109. 9 day rabbit decidua, showing acid phosphatase in decidual (D.) and adjacent stromal (S.) cells.

Fig. 110. 8 day rabbit sub-implantation region, showing non-specific esterase in the decidua (D.) and stroma (S.). Naphthol-AS-acetate.

109



110

Fig. 111. 5 day rabbit embryo, showing ITPase in the trophoblast (T.) and uterine epithelium (E.).

Fig. 112. Early 8 day rabbit trophoblast (T.) invading the maternal epithelium (E.) over a blood vessel (V.). Inosine triphosphatase.

Fig. 113. Early 8 day rabbit implantation site showinh ITPase in the differentiating syncytiotrophoblast (S.), but not in the cytotrophoblast (C.). Some activity is present in the epithelium (Ep.) and stroma between the intensely positive blood vessels (V.).

Fig 114. Late 8 day rabbit implantation site, showing the accumulation of ITPase activity in the syncytiotrophoblast (S), in the centre of which a band of pale staining appears due to unstained nuclear ghosts. Activity is also seen in the cytotrophoblast (C.), and uterine epithelium (Ep.).

111

112

113

114

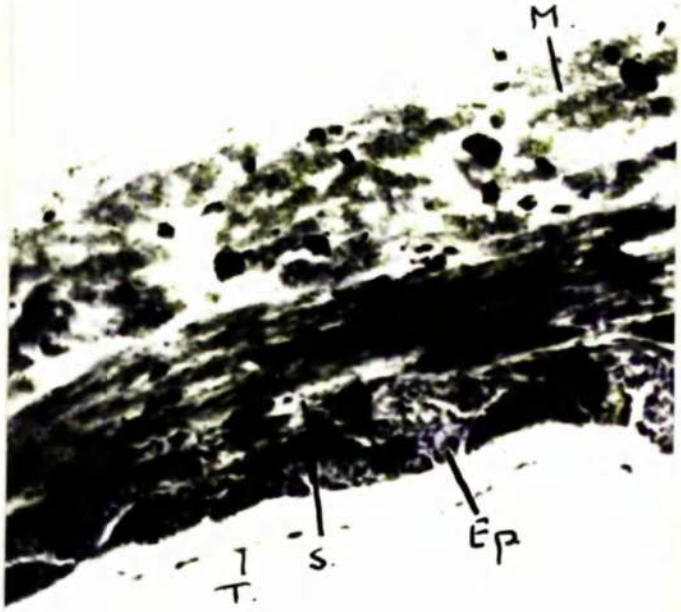
Fig. 115. Late 8 day rabbit implantation site, showing AMPase activity in the mesoderm (M.), particularly the somatopleuric layer applied to the inner aspect of the cytotrophoblast; in the syncytiotrophoblast (S.), the blood vessels (V.), and the decidua (D.).

Fig. 116. 7 day rabbit implantation site, showing ATPase activity in the epithelium (Ep.), particularly in its brush border, in the stroma (S.), and in the muscle layers (M.). Marked activity is present in the trophoblast (T.) also.

Fig. 117. Early 8 day rabbit implantation site, showing heavy non-specific alkaline phosphatase activity in the epithelium (Ep.) around an invading trophoblastic process (T.). The stroma (S.) is also intensely positive.

Fig. 118. 9 day rabbit implantation site, antimesometrial region, showing marked β-glycero-phosphatase activity in the degenerating tissue (D.), with less in the underlying glands (G.).

115



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118

Fig. 119. Late 7 day rabbit implantation, mesometrial side, showing prominent staining of the Golgi apparatus in the epithelium of the sub-placental glands. TPPase.

119

Fig. 120. AMPase activity in the decidua (D.) and stroma (S.) of the placental folds at:-

A) late 7 days

B) early 8 days

C) mid 8 days

D) late 8 days, when the decrease in staining of the outer zone of the decidua can be seen.

A

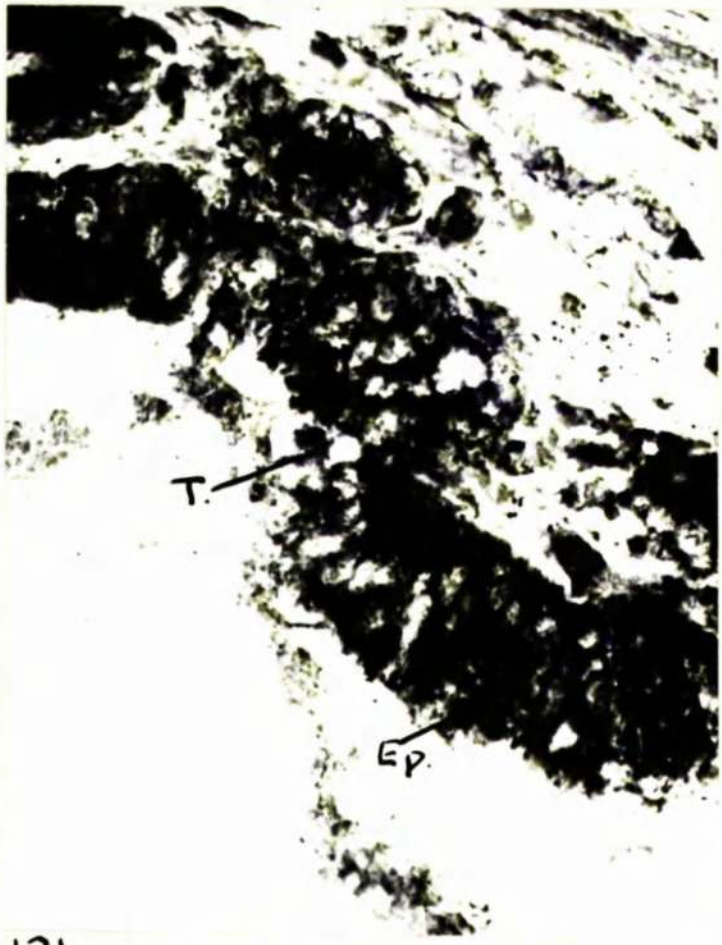
B

C

D

Fig. 121. Late 7 day rabbit implantation, antimesometrial region, showing MDH in a trophoblastic invasion (T.), and in the maternal epithelium (Ep.).

Fig. 122. Early 8 day rabbit implantation, mesometrial side, showing more MDH activity in the areas of trophoblast (T.) not contacting maternal epithelium than in areas of contact. Activity is also present in the endoderm (E.).



121

122

Fig. 123. Early 8 day rabbit implantation, mesometrial region, showing G-6-P in the syncytiotrophoblast (S.), cytotrophoblast (C.), and maternal epithelium (Ep.). Stromal cells (H.) and embryonic mesoderm (M.) also show some activity.

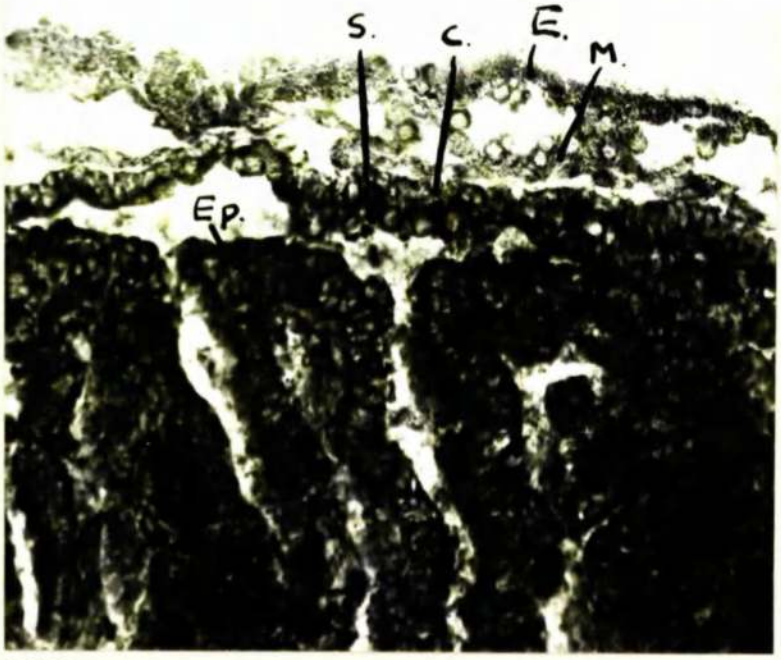
Fig. 124. Late 8 day rabbit implantation site, showing IDH in the syncytiotrophoblast (S.), and maternal epithelium, both on the surface (Ep.) and in the areas of glandular symplasma (G.). Less activity is seen in the cytotrophoblast (C.), but the mesoderm (M.) and endoderm (E.) are active.

Fig. 125. A similar site to fig. 124, showing MDH. The difference in activity between syncytiotrophoblast (S.) and cytotrophoblast (C.) is less clear. Mesoderm (M.), endoderm (E.), and maternal epithelium (Ep.) are again very active.

Fig. 126. GDH in the degenerating antimesometrial epithelium (Ep.) at the base of an area of fusion with trophoblast in a late 8 day rabbit embryo. Less activity is seen in the surviving deep parts of the glands (G.).

123

124



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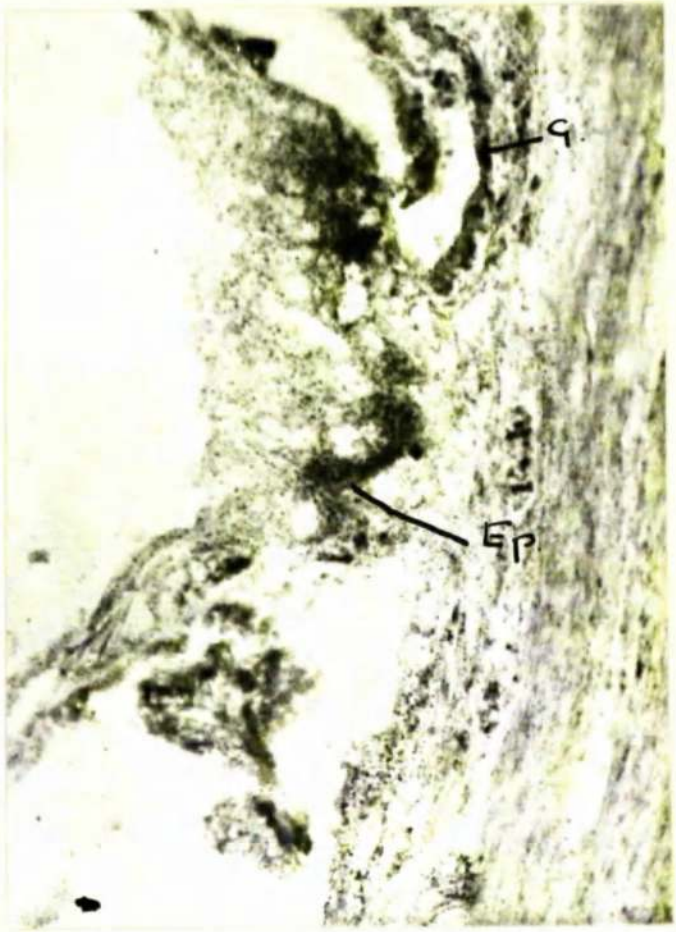


126

Fig. 127. G-6-P in the mesometrial epithelium (Ep.) at:-

A) 5 days

B) early 8 days.

A

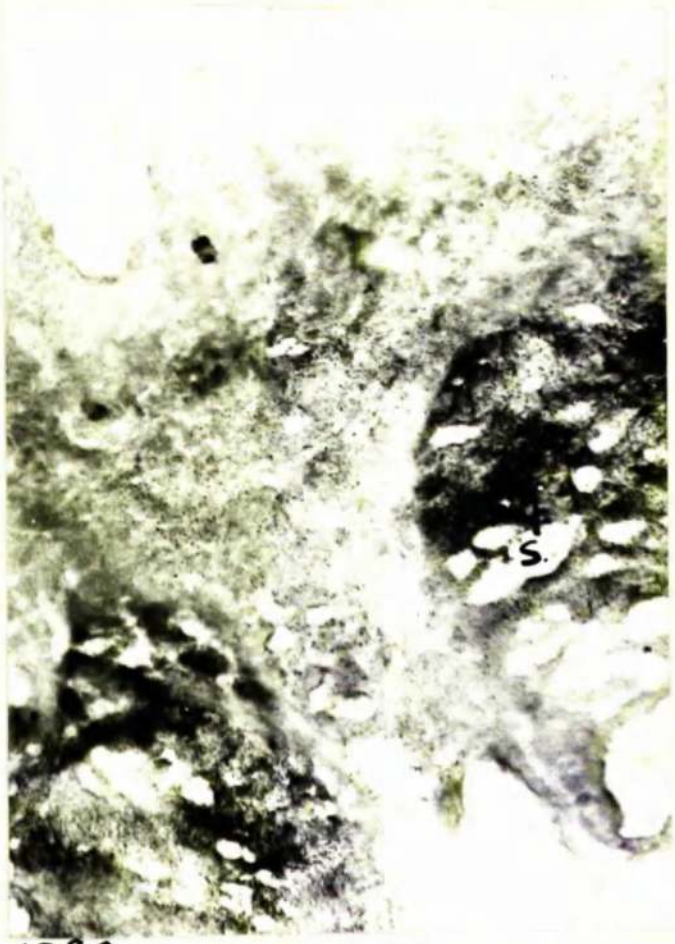
B

Fig. 128. Dehydrogenase activity in the symplasmic region of the mesometrial uterine glands (S.) at late 8 days with:-

A) β-hydroxy-butyrate

B) isocitrate

Fig. 129. Late 8 day rabbit placental fold, showing IDH in the decidua (D.).



128A

128B

129

Fig. 130. Term horse foetal placenta, shewing the allantoic endoderm (A.), the dense connective tissue of the chorionic plate (P.), containing the allantoic blood vessels, the primary villi (V_1), and the numerous secondary villi (V_2). Maternal secretion is present between the villi (S.). Masson. X 30.

Fig. 131. Higher power view of fig. 130, to show the columnar trophoblast of the chorionic plate (T.), and the thinned out trophoblast of the villi (C.), which shows some intra-epithelial capillaries (I.), and a vascular loose connective tissue core. Masson. X 300.

Fig. 132. The same specimen as fig. 131, showing the allantoic endoderm. Masson. X 300.

130

131

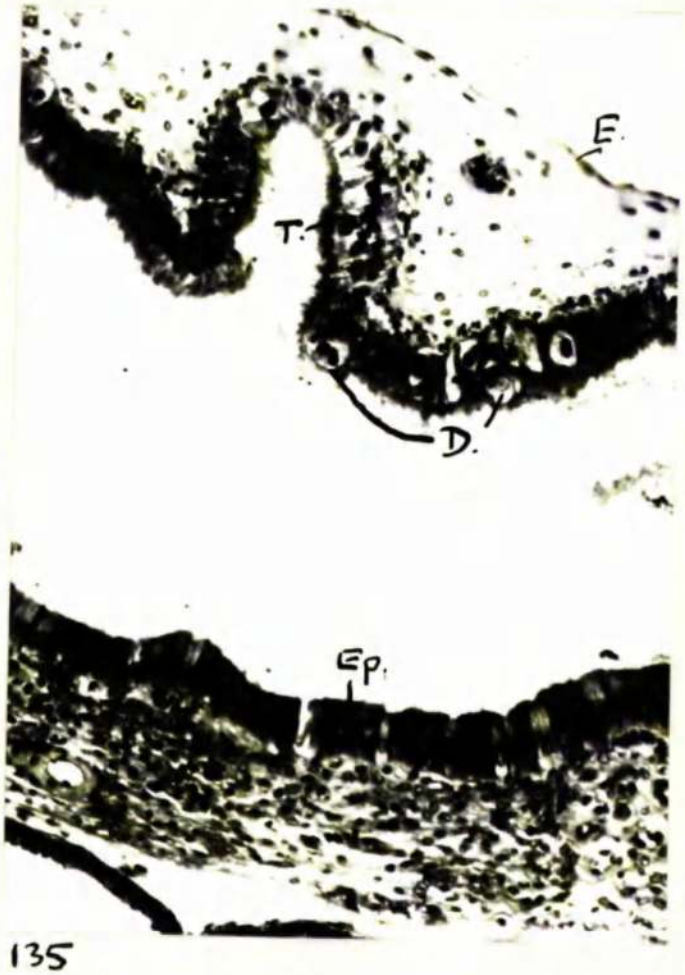
132

Fig. 133. 25 cm. sheep placenta, showing a cotyledon (C.), with foetal villi (V.), interdigitating with septa of maternal tissue (S.). the inter-cotyledonary chorion (T.) is in contact with intact maternal epithelium (Ep.) beneath which the many uterine glands are present (G.). Masson. X 10.

Fig. 134. Higher power view of the same section as fig. 133, showing cellular trophoblast (T.) covering the foetal villi, the syncytial trophoblast (S.) covering the maternal connective tissue (C.), (the space between is shrinkage artefact) and diplokaryocytes (D.) in the cellular trophoblast. Masson. X 400.

Fig. 135. Term sheep placenta, showing various stages of development of diplokaryocytes (D.), in the columnar epithelium of the extra-cotyledonary chorion (T.). The uterine epithelium (Ep.) and allantoic endoderm (E.) are also visible. Masson. X 160.

134



135

133

Fig. 136. Another area of the same placenta as fig. 135, showing the blood extravasated between the bases of the foetal villi (H.), and the accumulation of blood breakdown products in the trophoblastic cells (arrowed). Masson. X 600.

136

Fig. 137. Early stage of the chorio-allantoic placenta of the dog, showing the invading trophoblast (T.), the glandular symplasma (S.), the dilated mid-portion (D.), and the contracted bases (G.) of the uterine glands. Masson. X 45.

Fig. 138. Later stage of the chorio-allantoic placenta of the dog, the lettering being the same as in fig. 137. Masson. X 35.

Fig. 139. Higher power view of the same section as fig. 138, showing syncytiotrophoblast (S.), the cytotrophoblast at the apices of the placental villi (C.), and the slightly enlarged stromal cells of the decidua (D.). Masson. X 400.

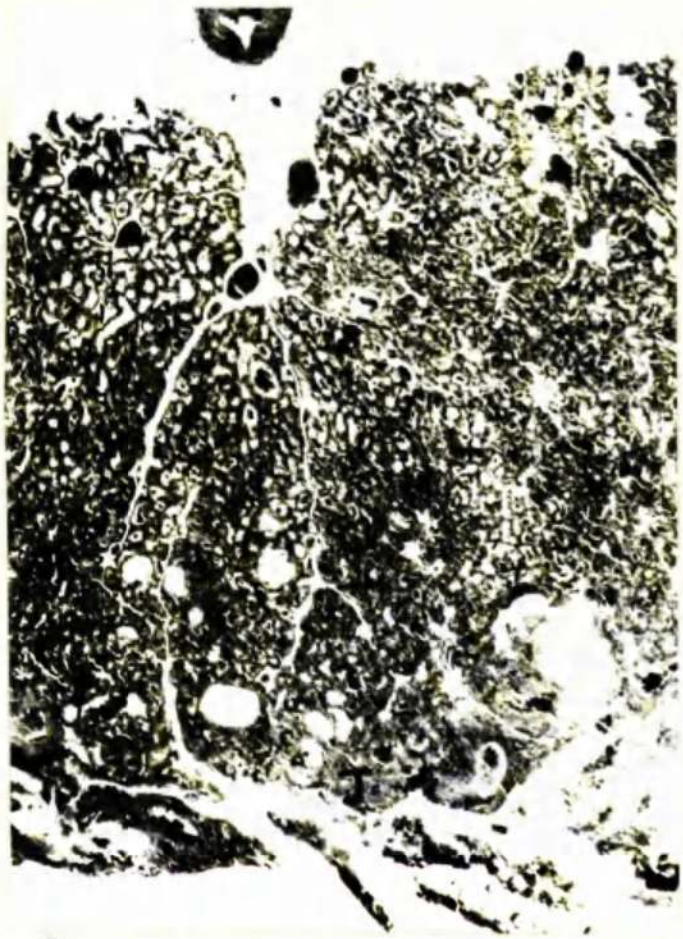
137

138

139

Fig. 140. Low power photomicrographs of the placenta in the cat (A), dog (B) - parturition specimen with part of the junctional zone and spongy zone missing - , and ferret (C), to show the labyrinth (L.), the junctional zone (J.), and the basal spongy zone (S.), next to the muscle layers (M.). Masson. X 40.

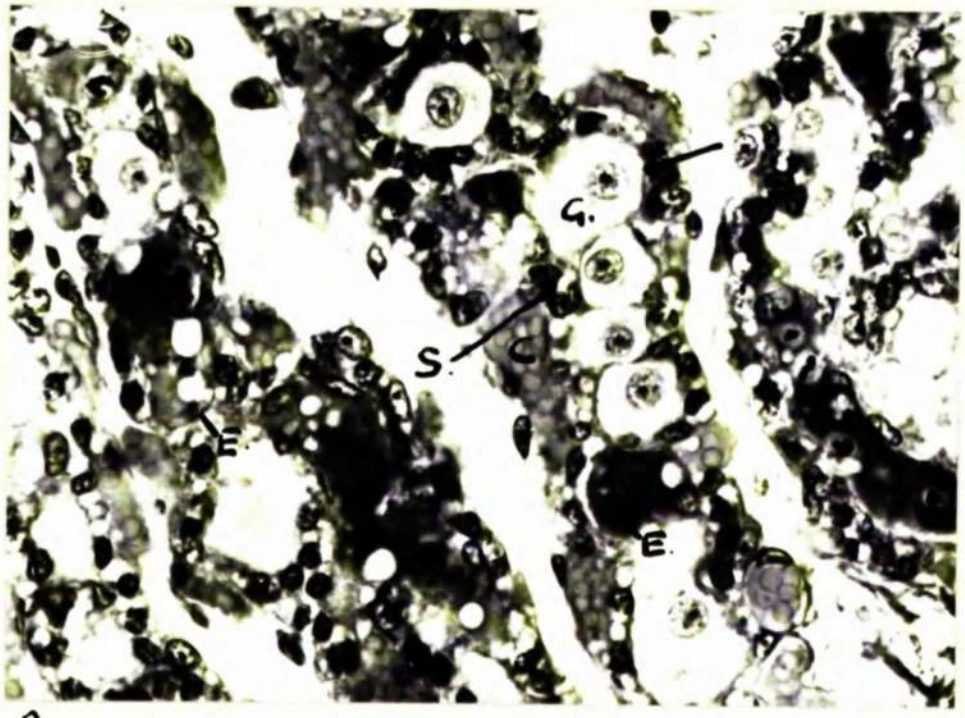
A



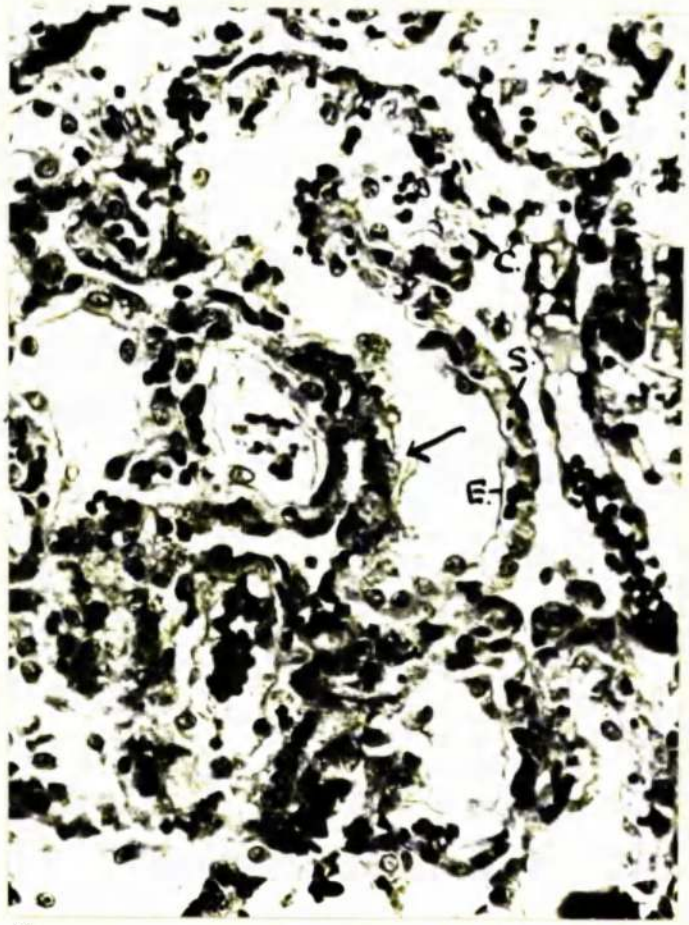
B

C

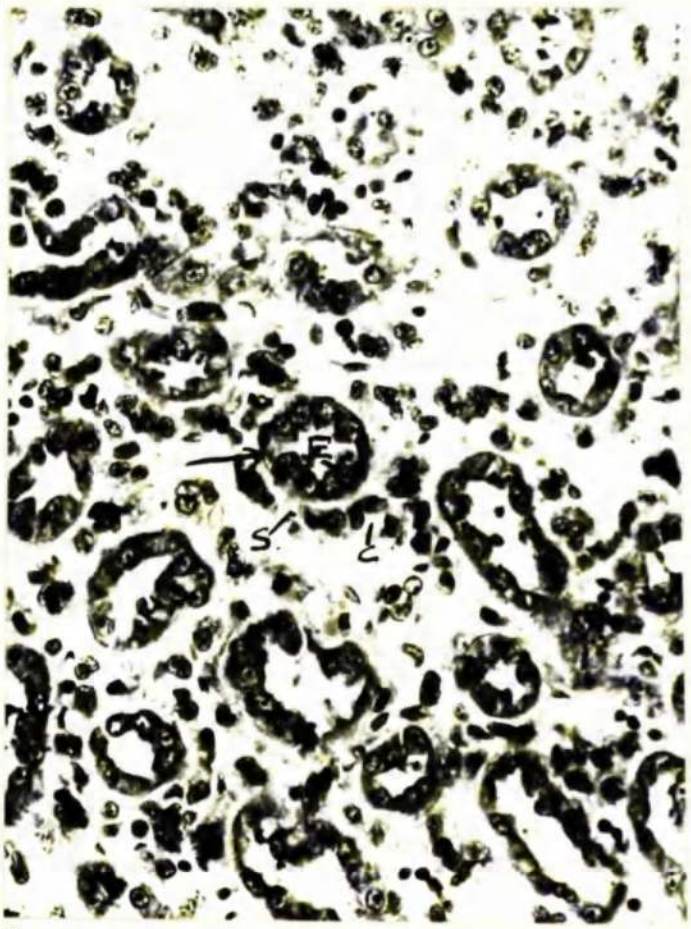
Fig. 141. High power photomicrographs of the labyrinth of the cat (A), dog (B), and ferret (C), showing syncytiotrophoblast (S.) with intra-epithelial capillaries (C.), the interstitial matrix (arrowed), the maternal endothelium (E.), and, in the cat, decidual giant cells (G.). Masson. X 600.



A



B



C

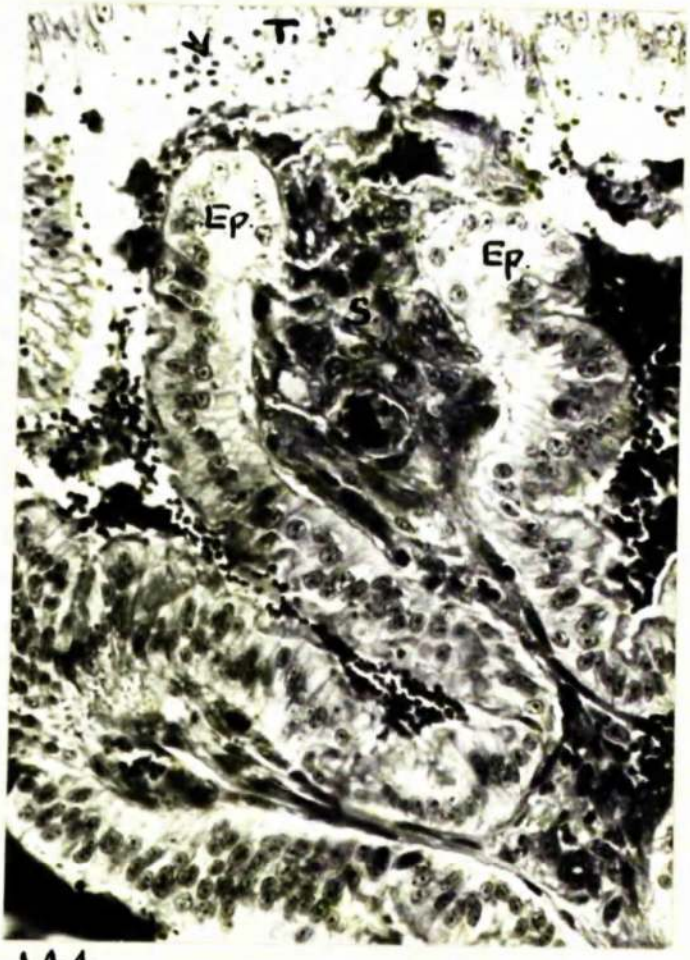
Fig. 142. Term cat placenta, showing the junctional and spongy zones. The cytotrophoblast (C.), and dilated uterine glands containing histiotrophe (H.) are seen, and the change from stromal (S.) to decidual (D.) cells. Masson. X 350.

Fig. 143. The same region of the ferret placenta showing the hypertrophied cells of the uterine glands (G.). Masson. X 250.

Fig. 144. The brown border of the cat placenta at term, showing the columnar trophoblast (T.), whose cells contain some inclusions (arrowed), and which is attached to a mass of symplasma (S.), lying between and beneath the epithelium (Ep.). Masson. X 300.

142

143



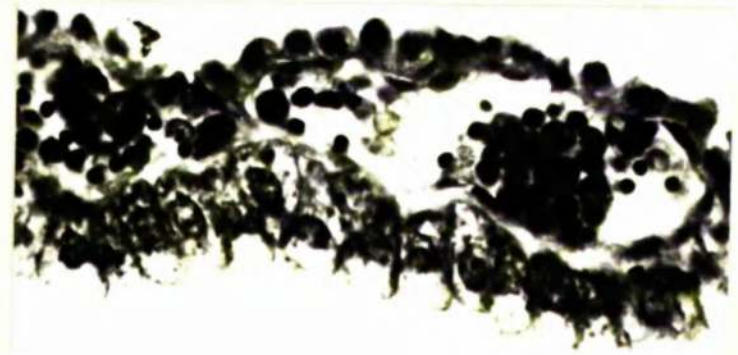
144

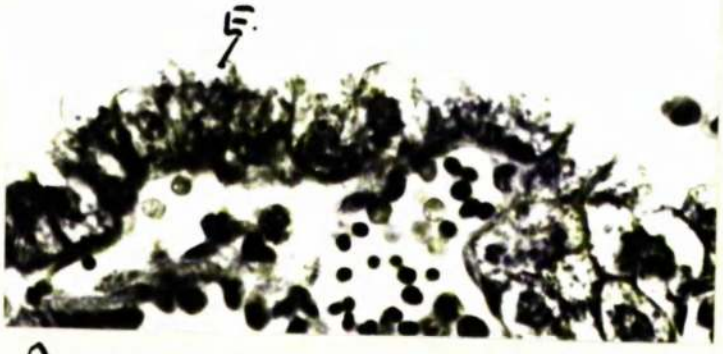
Fig. 145. The yolk sac of:-

A) the ferret, showing apical vacuolation of the endoderm (E.).

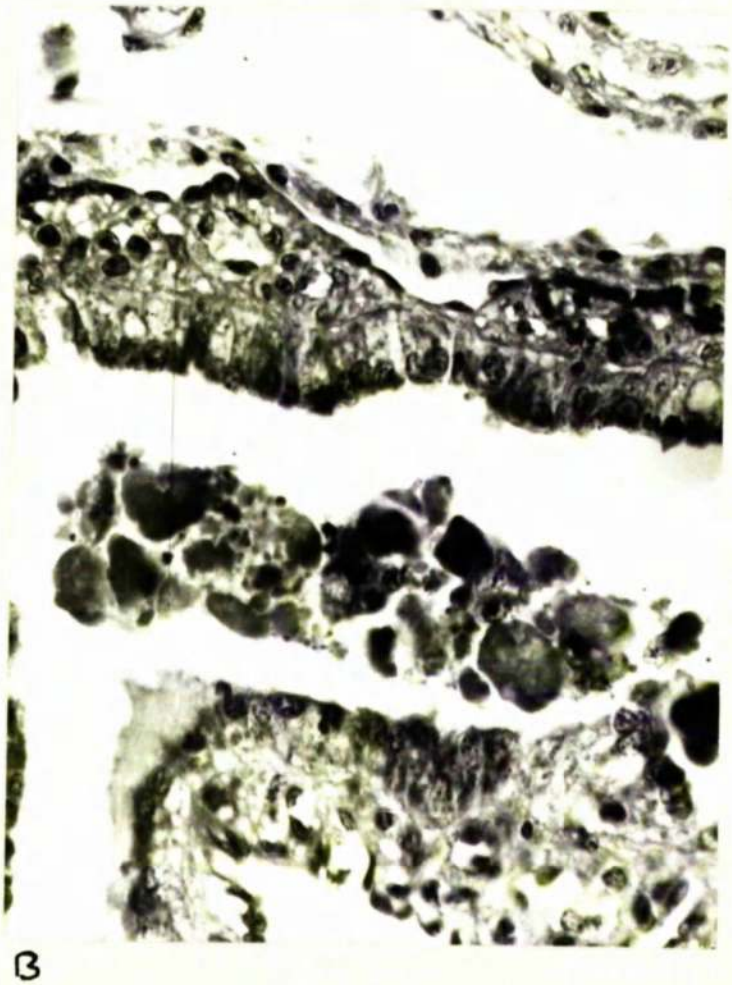
B) the cat at term, showing apical nuclei, and secretion in the lumen.

Masson. X 600.





A



B

Fig. 146. Placenta of:-

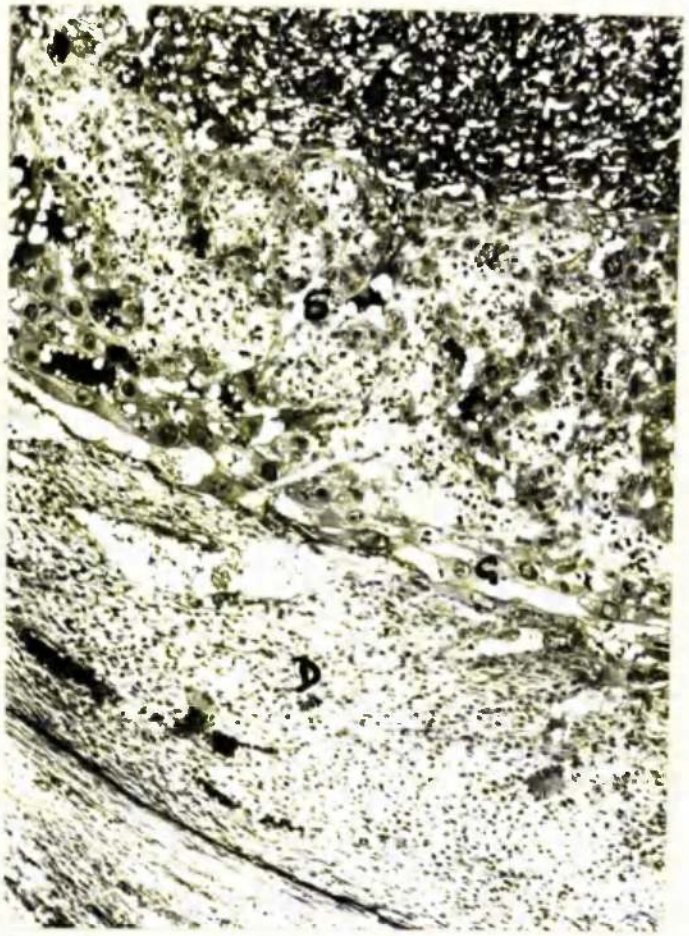
A) the rat at 14½ days

B) the guinea-pig at 20 days,

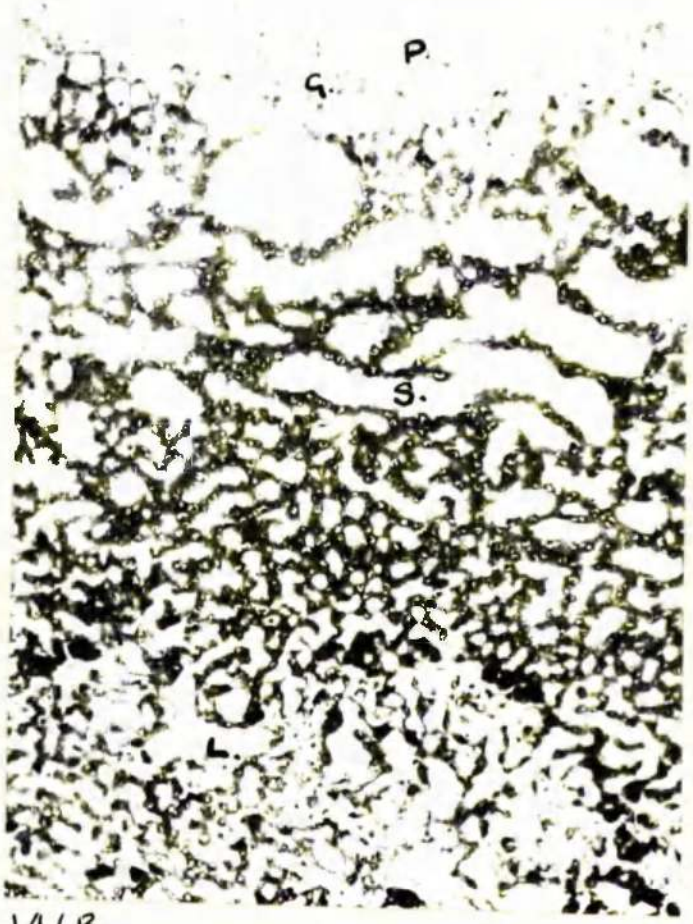
showing the labyrinth (L.), spongy zone (S.), and giant cells (G.), and, in the rat, decidua basalis (D.), in the guinea-pig, placental endoderm (E.). Masson. X 75.

Fig. 147. Rat placental spongy zone at 17½ days, showing the vacuolated cells (V.), and the cytotrophoblast cells (C.). Masson. X 375.

Fig. 148. 14½ day rat placenta - antimesometrial region - showing the visceral endoderm (V.), parietal endoderm (P.), Reichert's membrane (R.), the giant cells (G.), and the degenerating decidua capsularis (C.). Masson. X 300.



146A



146B

147

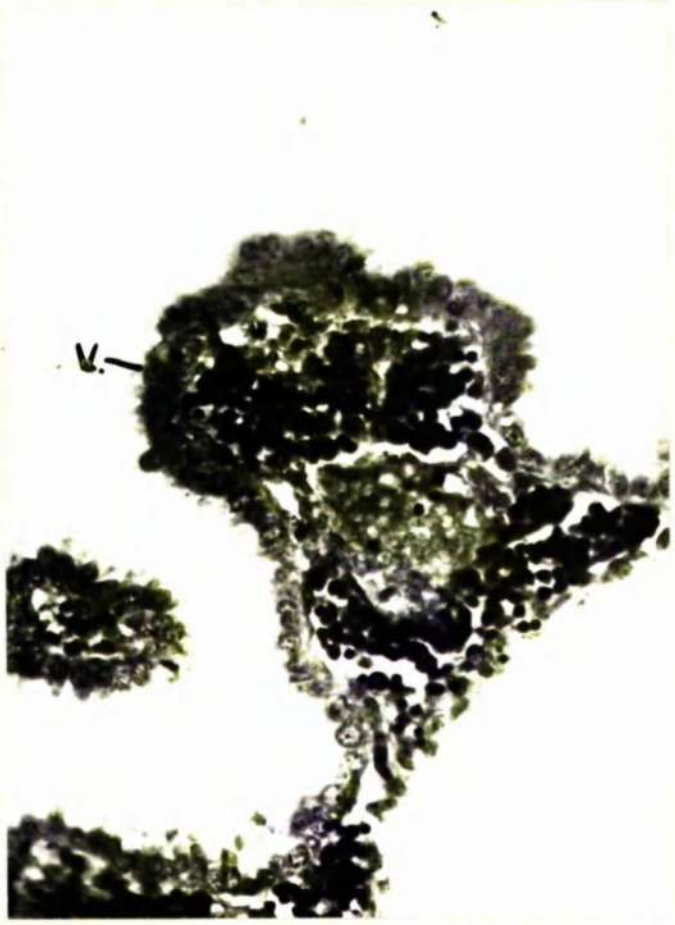
148

Fig. 149. Term rat placenta, showing the endodermal sinus, with the visceral endoderm (V.), parietal endoderm resting on Reichert's membrane (P.), and placental labyrinth (L.). Masson. X 130.

Fig. 150. 20 day guinea-pig yolk-sac, showing the 'peg' appearance of the visceral endodermal cells (V.). Masson. X 350.

Fig. 151. Another part of the same section as fig. 150, showing the subplacenta (S.), and necrotic junctional zone (J.). Masson. X 150.

149



150.

151

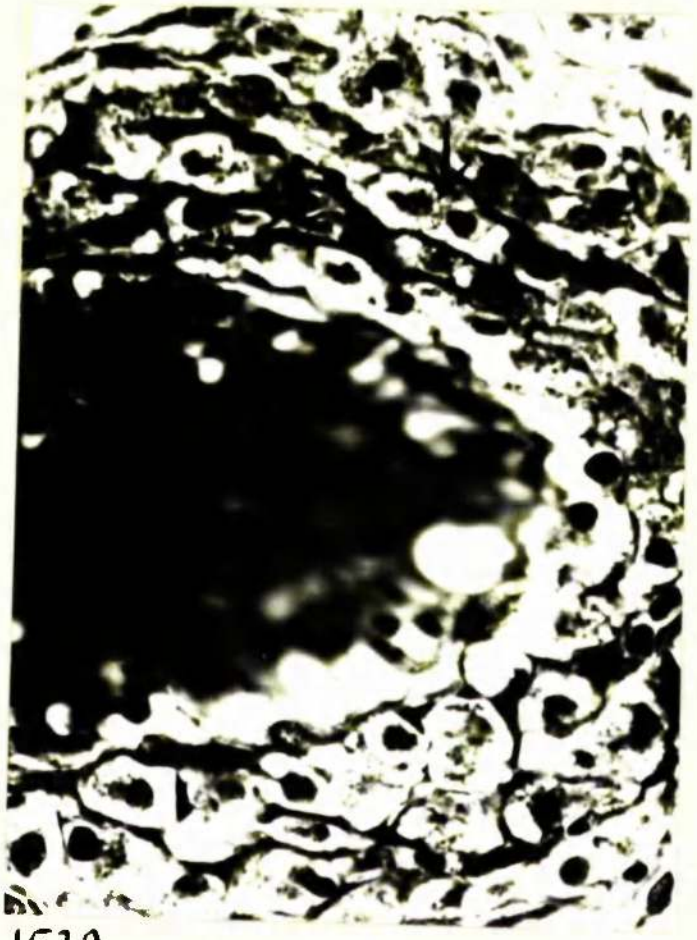
Fig. 152. 18½ day rat mesometrial triangle, showing:-

A) metrial gland cells, binucleate, with centrally placed granules and a rim of clear cytoplasm.

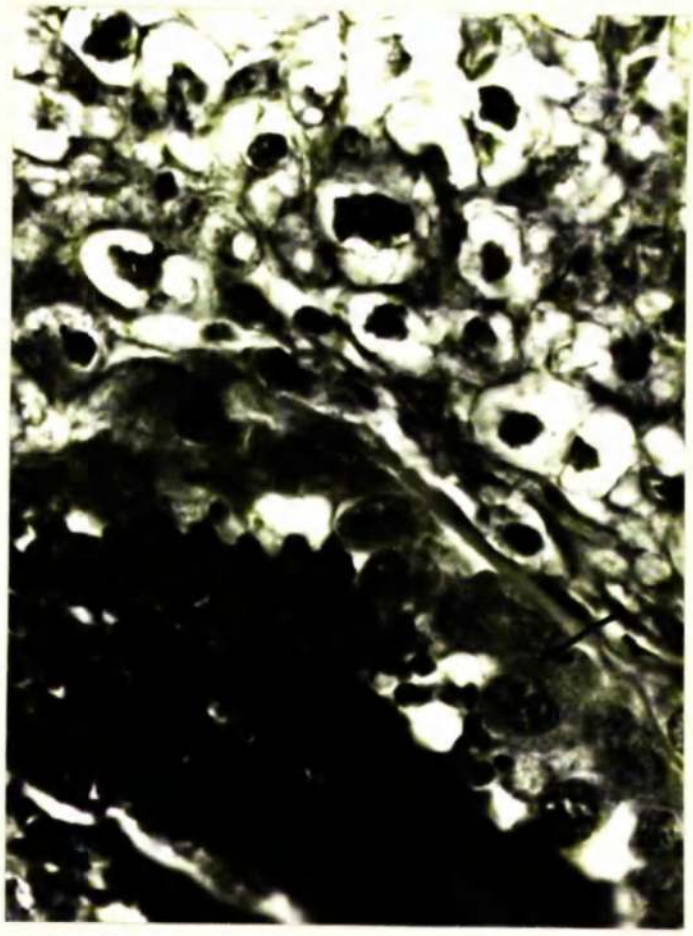
B) endovascular plasmodium, forming an incomplete lining to maternal blood vessels. Masson. X 400.

Fig. 153. 13 day rabbit placenta, showing trophoblast (T.) separating the foetal (F.) and maternal (M.) blood streams. E - foetal endothelium. Masson. X 400.

Fig. 154. Deeper part of the same placenta showing the transition between uninucleate (U.), and multinucleate (M.) decidual cells, and an area of degenerating gland symplasma (S.). Masson. X 150.



152A



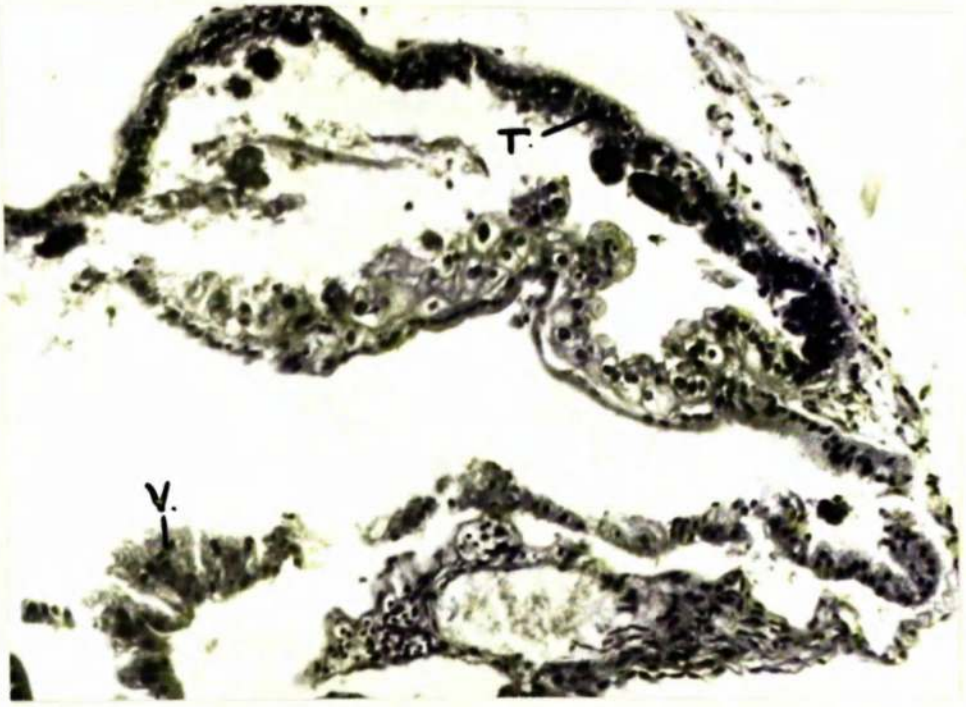
152B

153

154

Fig. 155. Trophoblastic fringe of a 20 day rabbit placenta, showing the trophoblast (T.) and the visceral endoderm (V.) with its brush border. Masson. X 300.

Fig. 156. 13 day rabbit placenta, showing the degenerating parietal endoderm (P.), the regenerating uterine epitheliun (Ep.), and the obplacental trophoblastic giant cells (G.). Masson. X 160.



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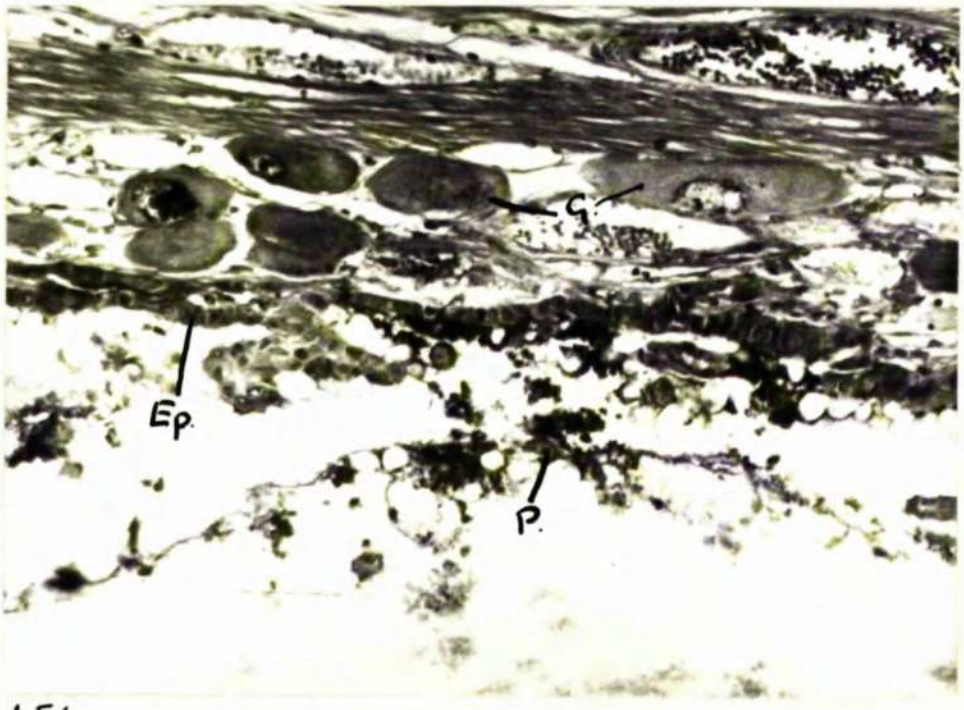


156

Fig. 157. 75 m.m. human placenta, showing the cytotrophoblastic cell columns (C.) anchoring the tips of the anchoring villi to the maternal tissues (M.). Some basal syncytiotrophoblast (S.) is still present between the villi, and it forms a continuous covering for them. Masson. X 250.

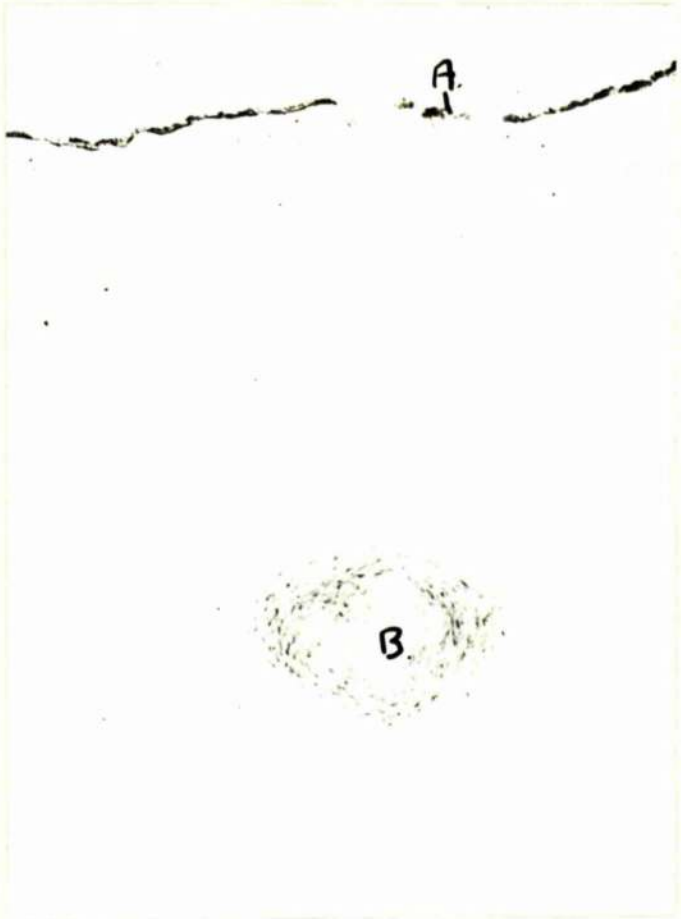
157

Fig. 158. Term horse placenta, showing glycogen in the allantois (A.), and its blood vessels (B.). PAS-dimedone.

Fig. 159. Sheep placenta showing glycogen:-

A) in the diplokaryocytes at 2.5 cm.

B) in the maternal (M.) and foetal (F.) stroma at 15 cm. PAS-dimedone.



158

159A

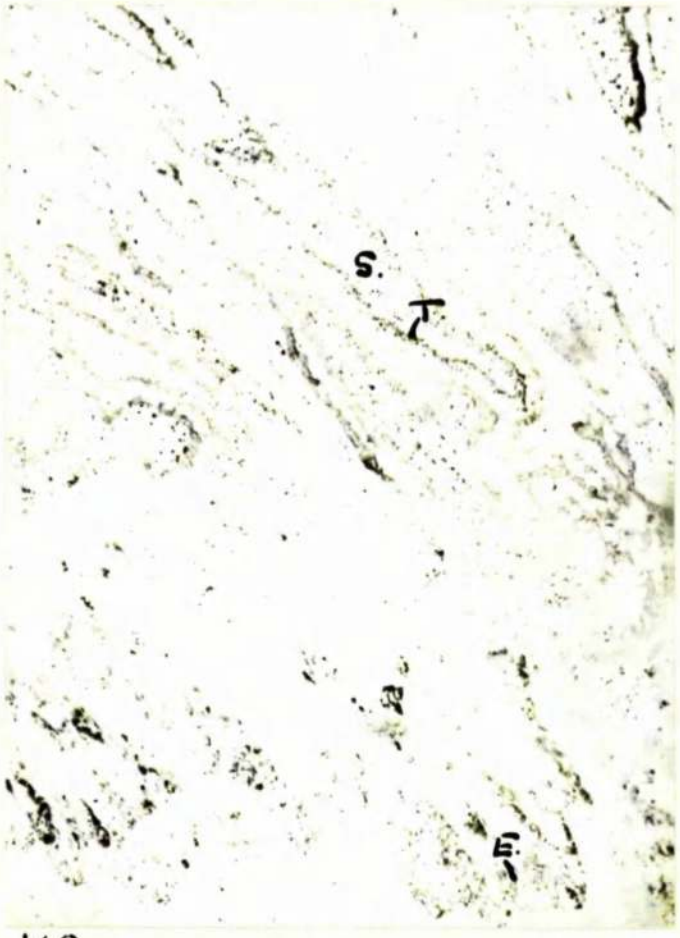
159B

Fig. 160. 7 cm. cat placenta, showing glycogen in the foetal stroma (S.), the syncytiotrophoblast (T.), and the spongy zone epithelium (E.). PAS-dimedone.

Fig. 161. Brown border of the same specimen, showing glycogen in the maternal epithelium (E.), but not in the trophoblast (T.). Much staining is seen in the sub-chorial connective tissue (C.). PAS-dimedone.

Fig. 162. Ferret placenta, showing glycogen in the yolksac endoderm (E.), mesoderm (M.), and in the allantoic endoderm (V.). PAS-dimedone.

Fig. 163. 18½ day rat placenta, showing glycogen in the labyrinth (L.), the vacuolated cells of the spongy zone (S.) which are spilling over into the metrial triangle, the decidua basalis (D.), and in the metrial gland cells (G.) around the mesometrial blood vessels. PAS-dimedone.



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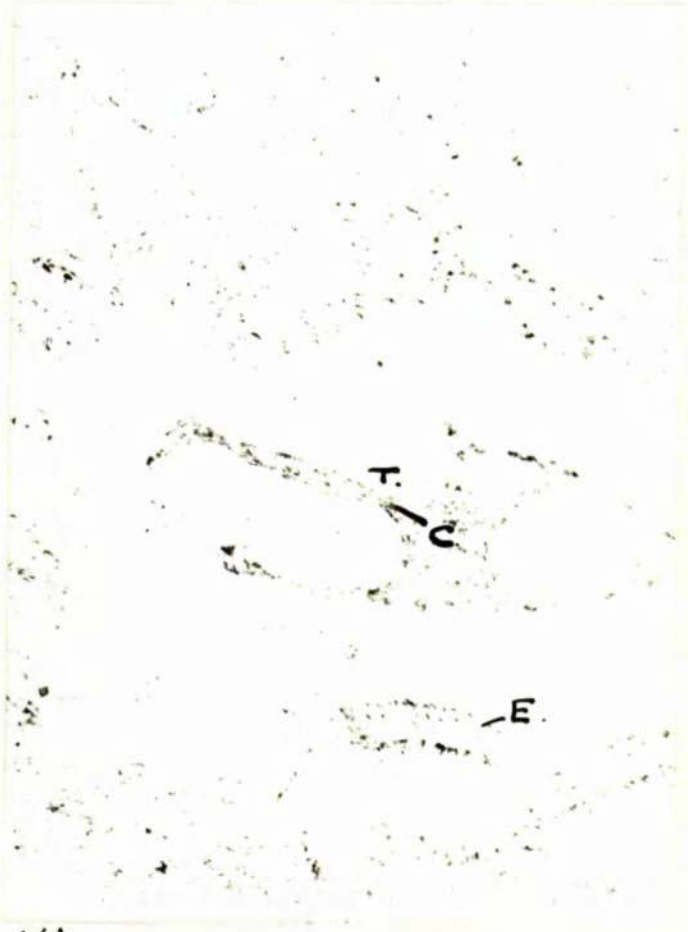

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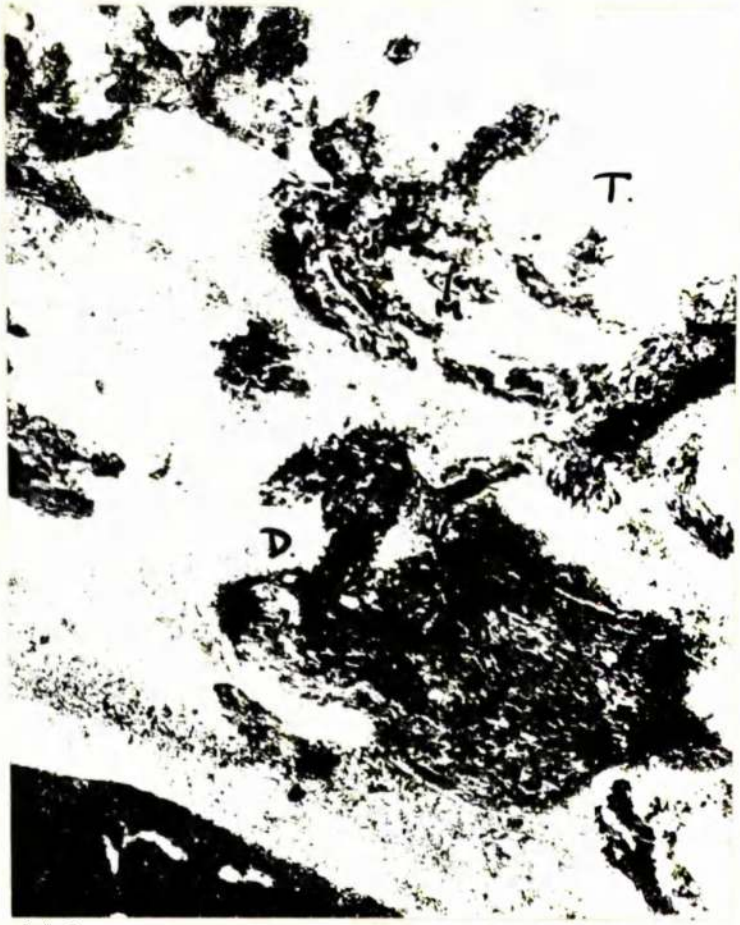
163

Fig. 164. 20 day rabbit embryo placenta. low power, showing large quantities of glycogen in the decidua (D.), and multinucleate decidual cells (M.), but not in the trophoblast (T.). PAS-dimedone.

Fig. 165. 20 day guinea-pig placenta, showing glycogen in the blood vessels deep in the placenta (B.), the placental endoderm (E.), and the junctional zone (Z.). PAS-dimedone.



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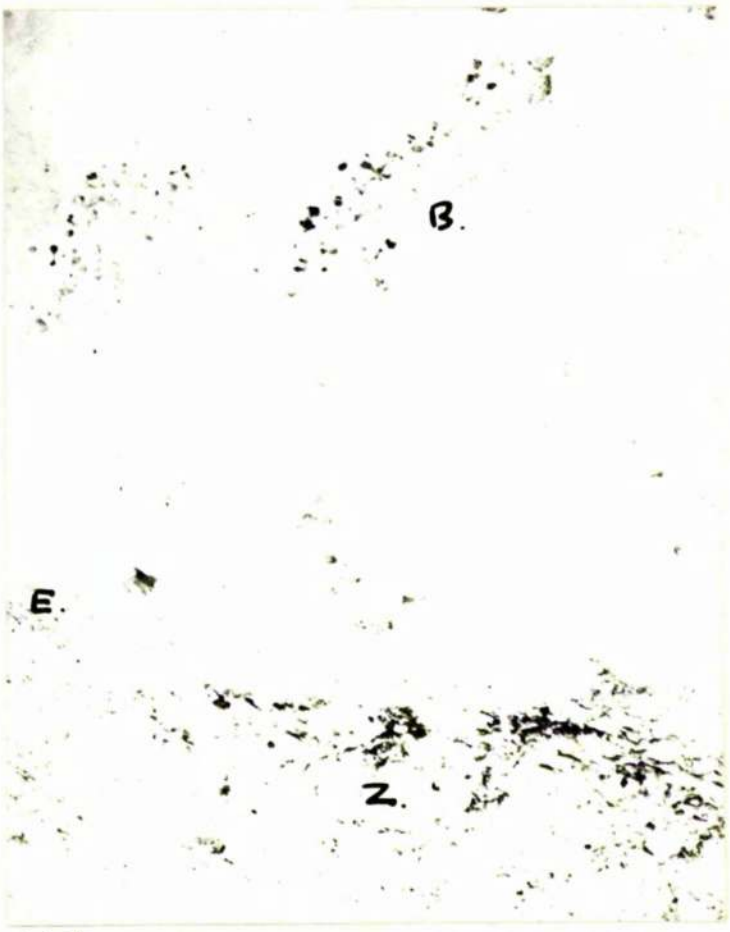
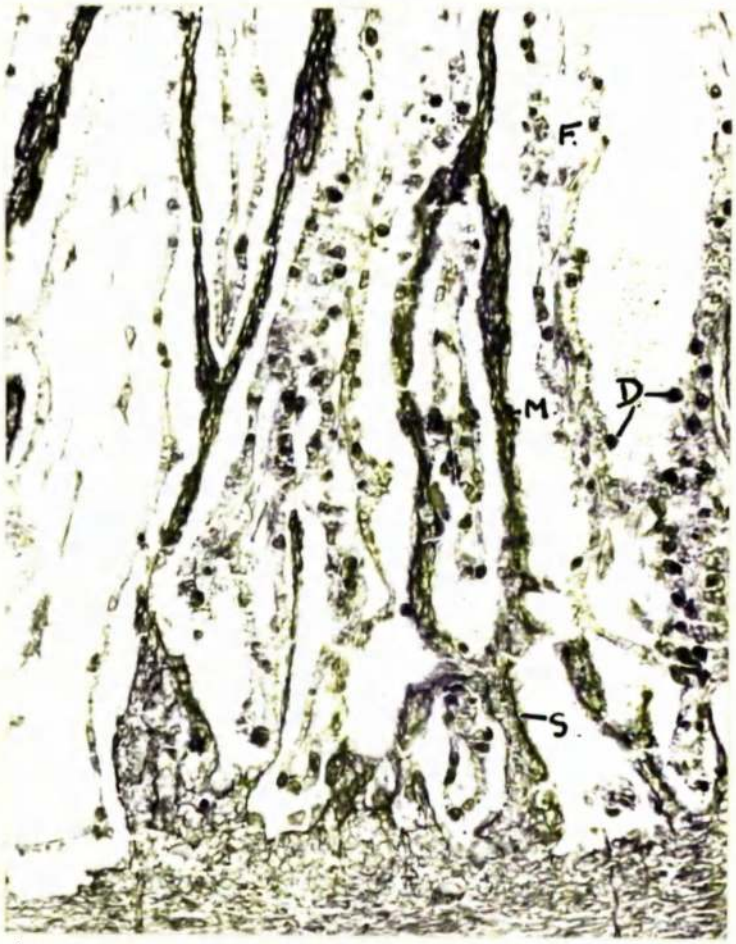


165

Fig. 166. 2.5 cm. sheep placenta, showing an intense reaction in the diplokaryocytes (D.), and maternal stroma (M.), less intense in the foetal stroma (F.), and syncytiotrophoblast (S.). PAS-diastase.

Fig. 167. 25 cm. sheep placenta, showing less reaction than in fig. 166, in the diplokaryocytes (D_1) of the cotyledon, without any change in those of the extra-cotyledonary chorion (D_2). The intense reaction of the uterine secretion (S.), and the epithelial brush border (Ep.) are also visible. PAS-diastase.



166

167

Fig. 168. Placental labyrinth of the ferret, showing the interstitial matrix (arrowed) between the trophoblast (T.), and the thickened maternal endothelium (E.). PAS-diastase.

Fig. 169. Term dog paraplacental, or "green border" chorion showing intra-cellular inclusions (arrowed). PAS-diastase.

Fig. 170. Term cat yolk sac, showing positively staining material in the lumen (L.), and in the endodermal cells (arrowed). PAS-diastase.

168

169

170

Fig. 171. 18½ day rat placenta, showing:-

A) positive inclusions in the visceral endoderm cells, beneath the brush border

B) positive granules in the metrial gland cells.

PAS-diastase.

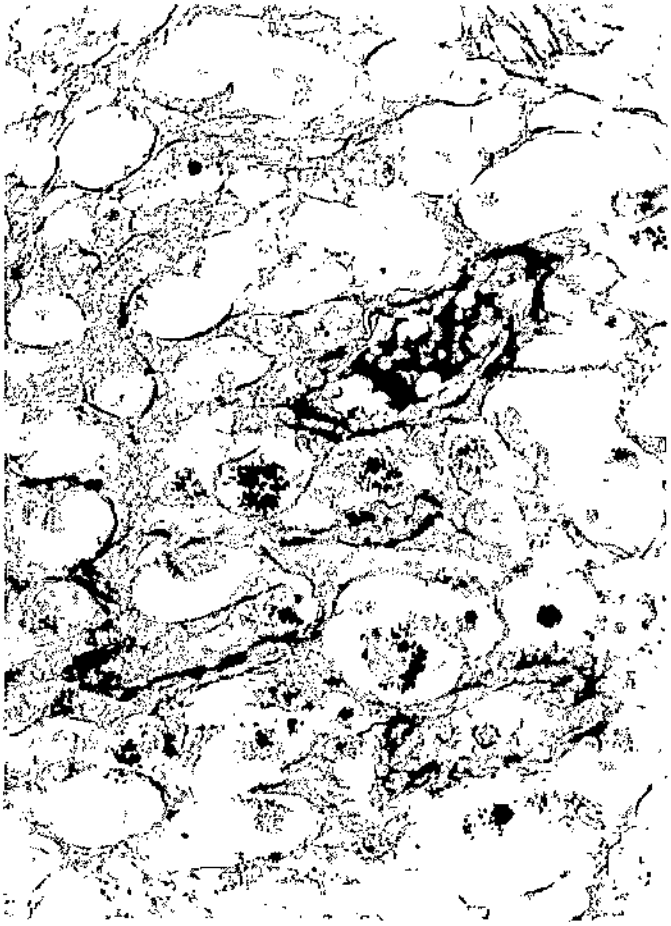
Fig. 172. 20 day rabbit placenta, showing positive lamellae (arrowed) between the trophoblast (T.) and the multinucleate decidual cells (D.).

PAS-diastase.

Fig. 173. 7.5 cm. human placenta, showing an intense reaction in the uterine gland secretion (G.), the fibrin at the base of the placenta (F.), fibrinoid degeneration in the substance of the placenta (D.), and a brush border on the syncytiotrophoblast (S.).

PAS-diastase.

171A



171B

172

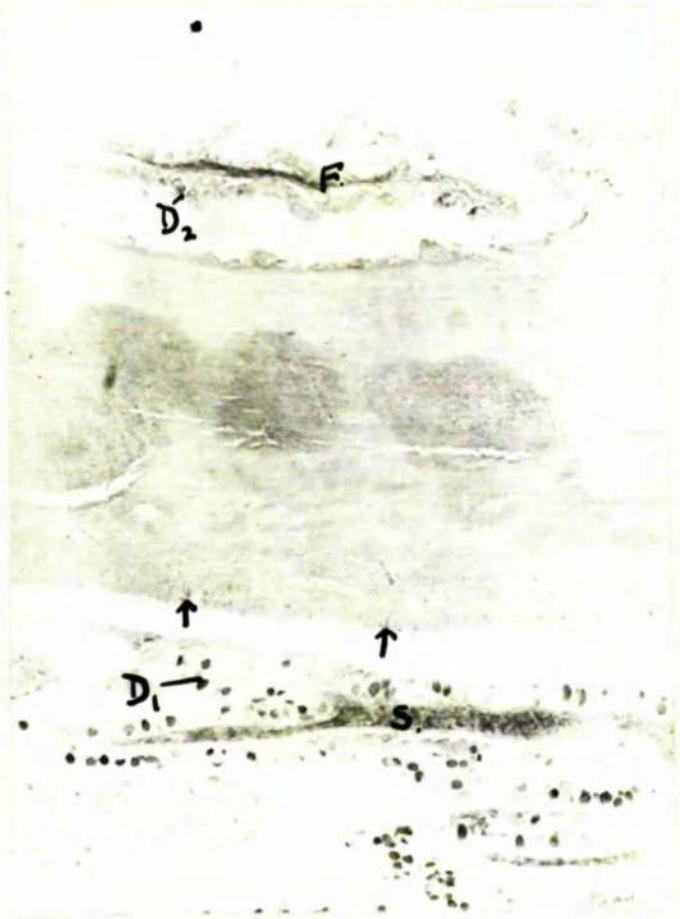
173

Fig. 174. 15 cm. sheep placenta, showing dialyzed iron staining in the chorionic stroma (S.), and diplokaryocytes (D_1), in the foetal stroma (F.), and less in the diplokaryocytes on the surface of the foetal villi (D_2). Cells with a strong reaction (arrowed) are present in the maternal epithelium also.

Fig. 175. Dog placenta, showing dialyzed iron staining in the uterine secretion (S.).

Fig. 176. 18½ day rat placenta, showing dialyzed iron staining in the metrial gland cell granules (G.).

Fig. 177. 13 day rabbit placenta, showing dialyzed iron staining in the uterine secretion.



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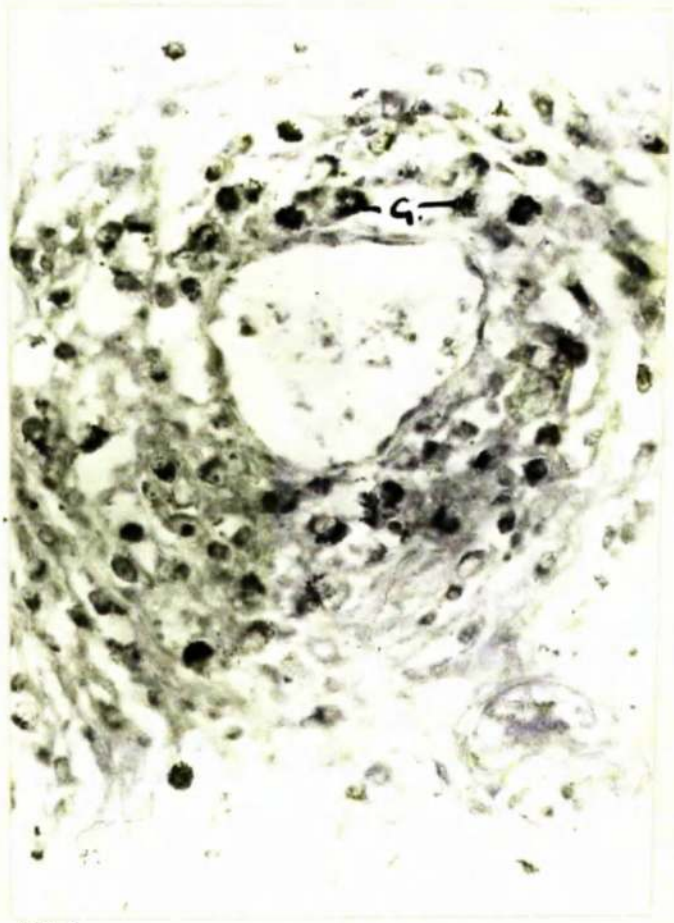


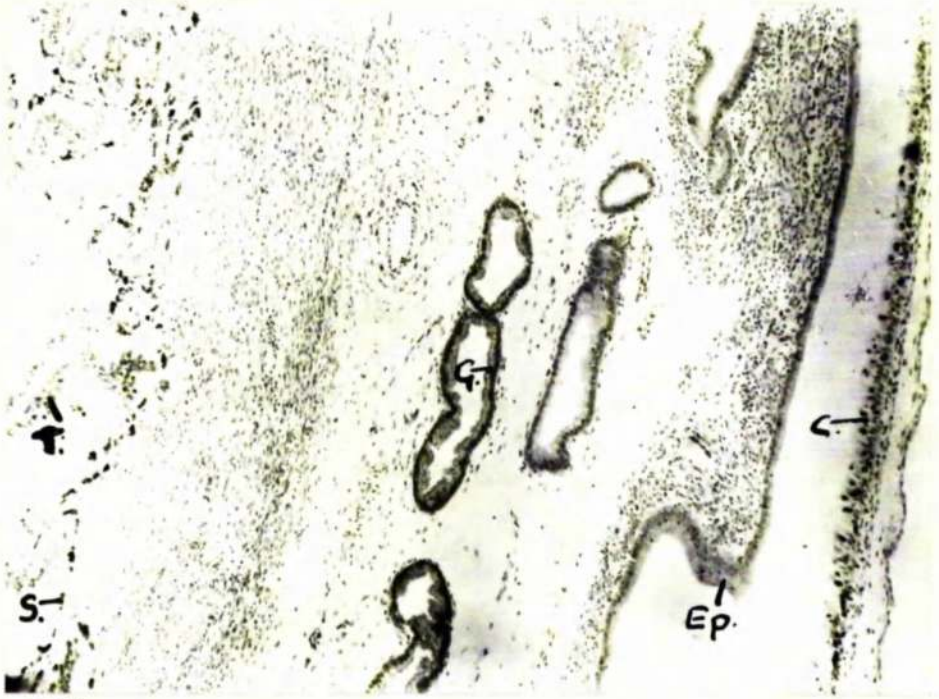
176

177

Fig. 178. 15 cm. sheep placenta, showing RNA in the extra-cotyledonary chorion (C.), the maternal surface (Ep.) and glandular (G.) epithelium, in the cytotrophoblast (T.), and traces in the syncytio-trophoblast (S.). Chrome-alum-gallocyanin.

Fig. 179. 7 cm. cat placenta, showing RNA in the syncytio- (S.) and cytotrophoblast (C.), and in the epithelium of the spongy zone glands (G.). The decidual giant cells (D.) are almost negative.

Fig. 180. Ferret placenta, showing RNA in the maternal endothelium (E.), and in the spongy zone glands (G.). Some is also present in the basal cytotrophoblast (C.), and traces in the syncytiotrophoblast. Chrome-alum-gallocyanin.



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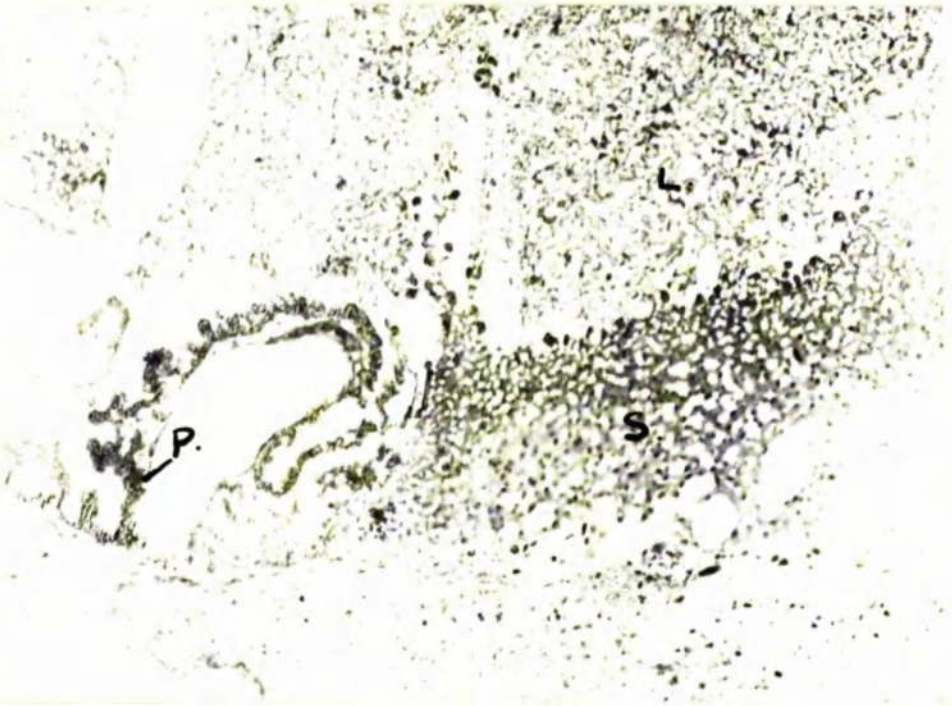
180

Fig. 181. 17½ day rat placenta, showing RNA in the labyrinth (L.), the spongy zone (S.), the giant cells (G.), and in the endovascular plasmodium (P.). Chrome-alum-gallocyanin.

Fig. 182. 20 day guinea-pig placenta, showing RNA in the spongy zone (S.), the labyrinth (L.), and the subplacenta (P.). Chrome-alum-gallocyanin.

181



182

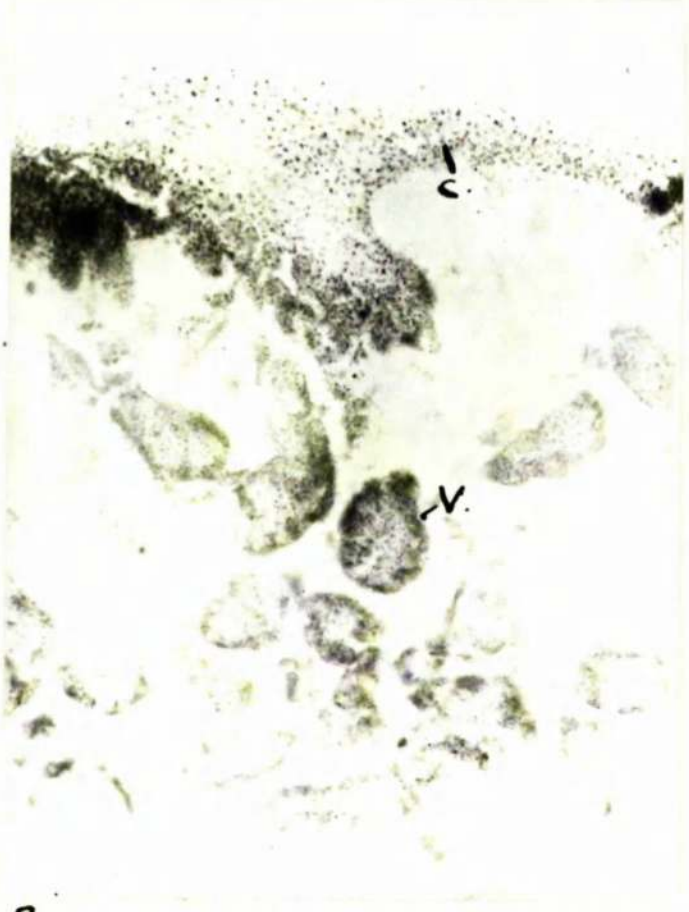
Fig. 183. Term horse placenta, showing enzyme activity in the trophoblast of the villi (V.), and chorionic plate (C.) with:-

A) acid phosphatase

B) non-specific esterase - Naphthol-AS-acetate

C) β-glucuronidase

A



B

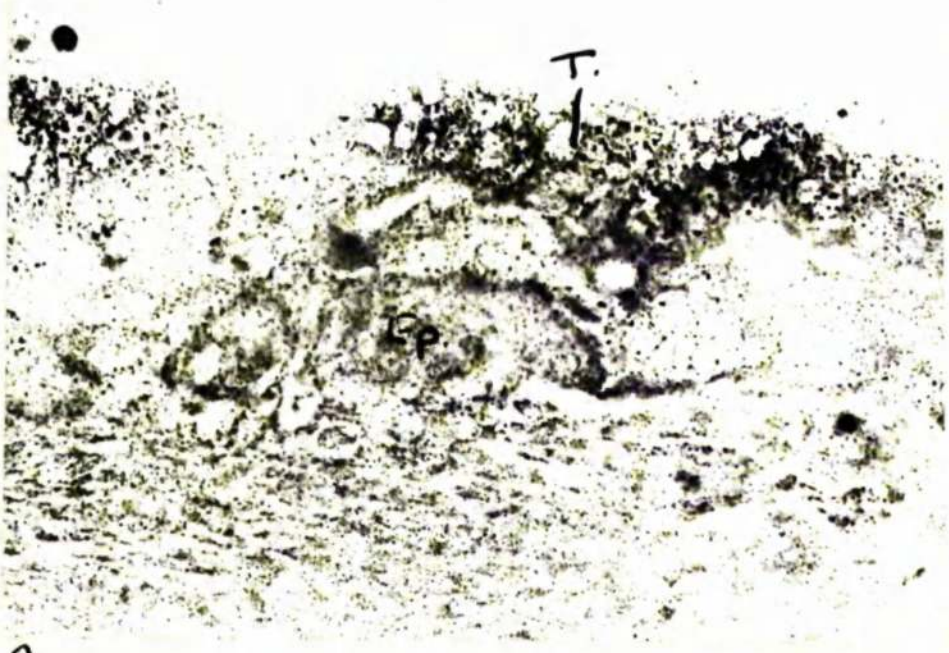
C

Fig. 184. Acid phosphatase in:-

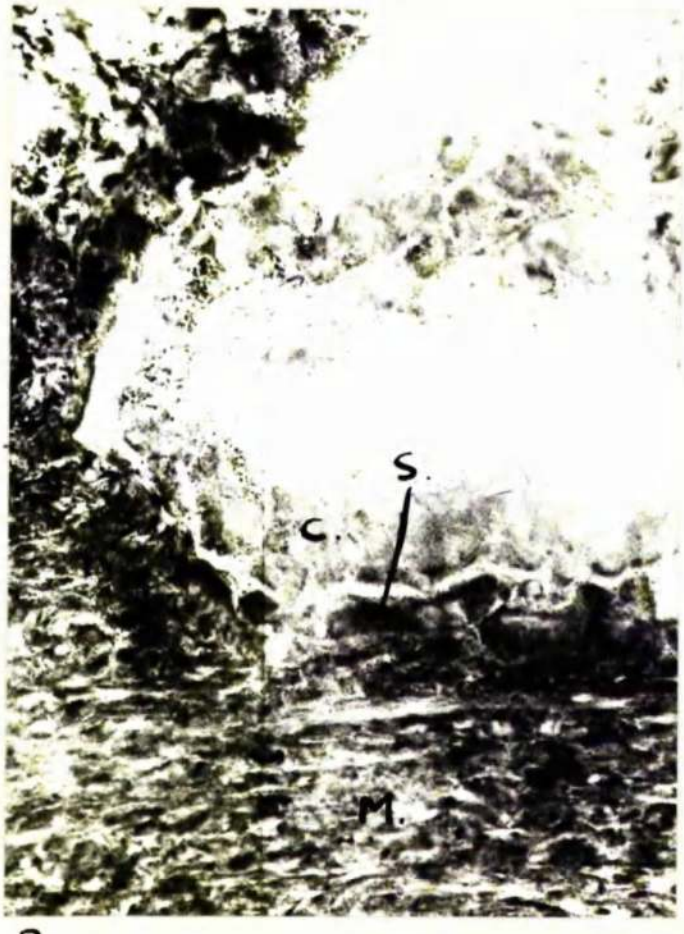
A) the chorionic trophoblast (T.) apposed to the degenerating epithelium (Ep.) on the surface of the sheep cotyledon at 2.5 cm.

B) the cytotrophoblast (C.) and syncytiotrophoblast (S.) and maternal stroma (M.) at 15 cm.

C) in higher concentration in the maternal stroma (M.) of the same specimen as (B) near the bases of the foetal villi



A

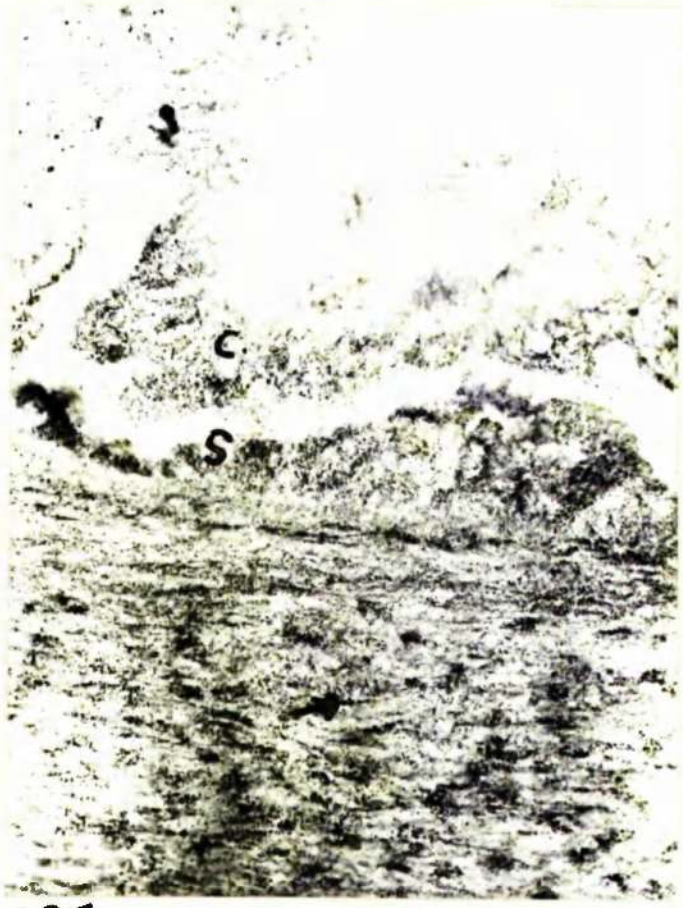


B

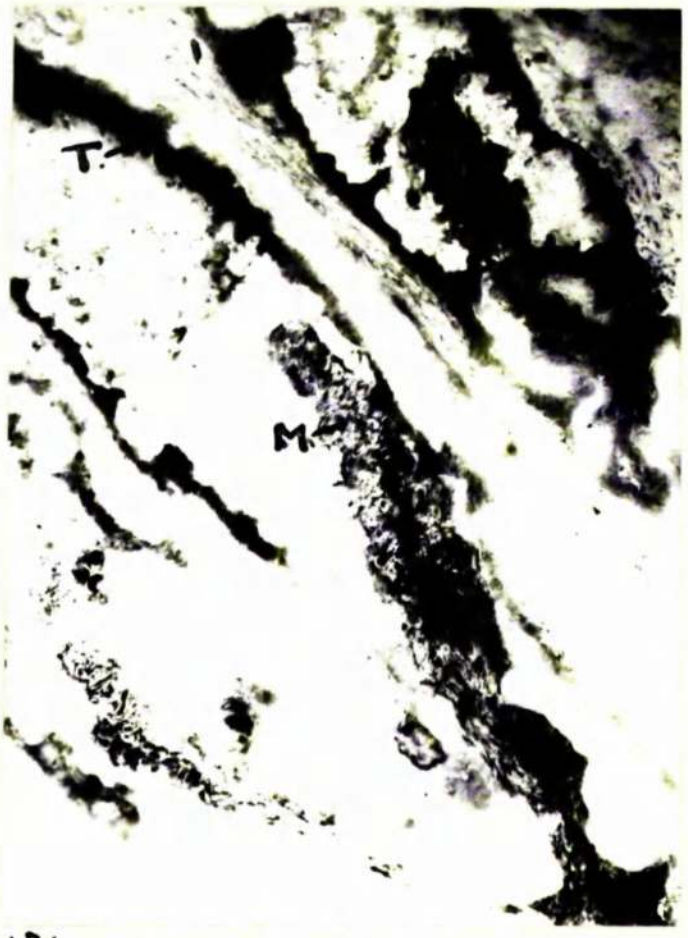
C

Fig. 185. Non-specific esterase in the cyto-trophoblast (C.), and syncytiotrophoblast (S.), and cells of the maternal stroma (arrowed), of a 15 cm. sheep placenta. Naphthol-AS-acetate.

Fig. 186. β-glucuronidase in the maternal stroma (M.), and chorionic trophoblast (T.) of the arcades in the placenta of a 25 cm. sheep foetus.



185



186

Fig. 187. Acid phosphatase in the 7 cm. cat placenta in:-

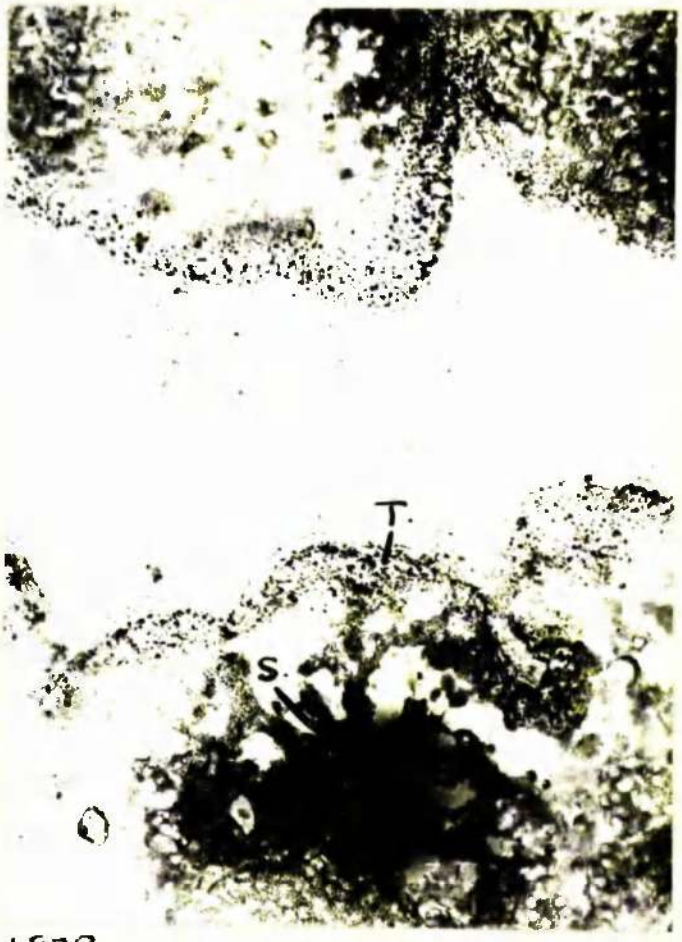
A) the trophoblast of the labyrinth

B) the brown border trophoblast (T.), and a symplasma mass (S.).

Fig. 188. Acid phosphatase in the trophoblast of the term dog placenta.

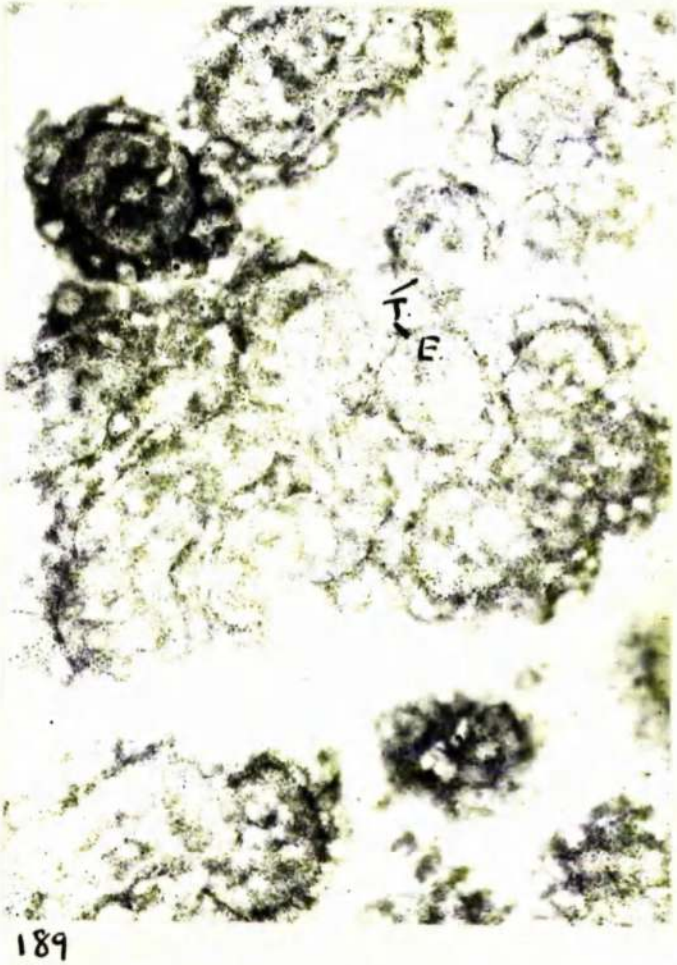
Fig. 189. Acid phosphatase in the labyrinth of the ferret placenta. E - maternal endothelium, T - trophoblast.

187A



187B

188



189

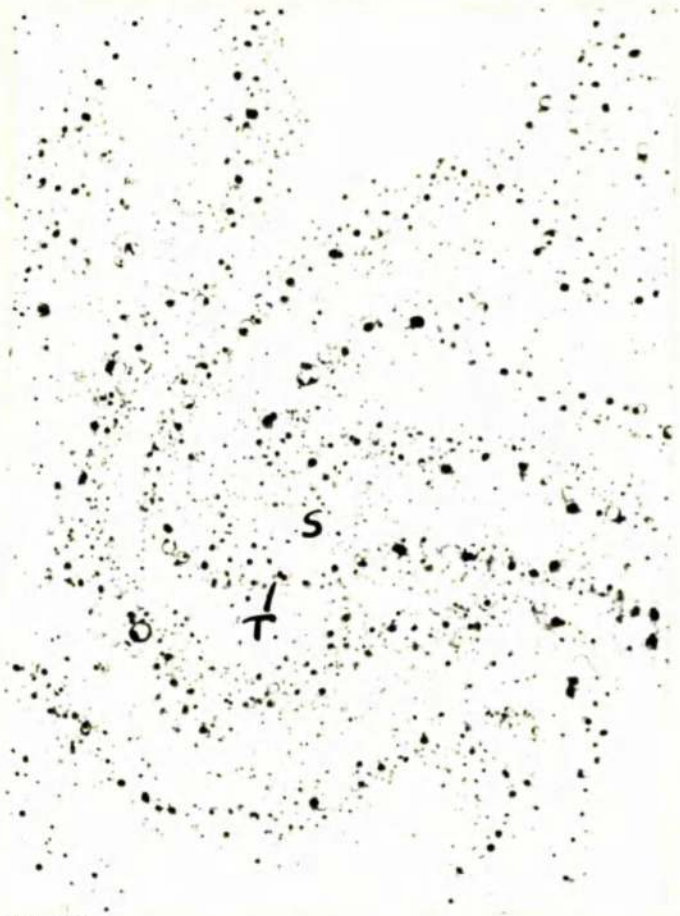
Fig. 190. Naphthol-AS-acetate esterase in the 7 cm. cat placenta, in:-

A) the trophoblast (T.), and maternal stroma (S.)

B) the yolk sac endoderm (E.)

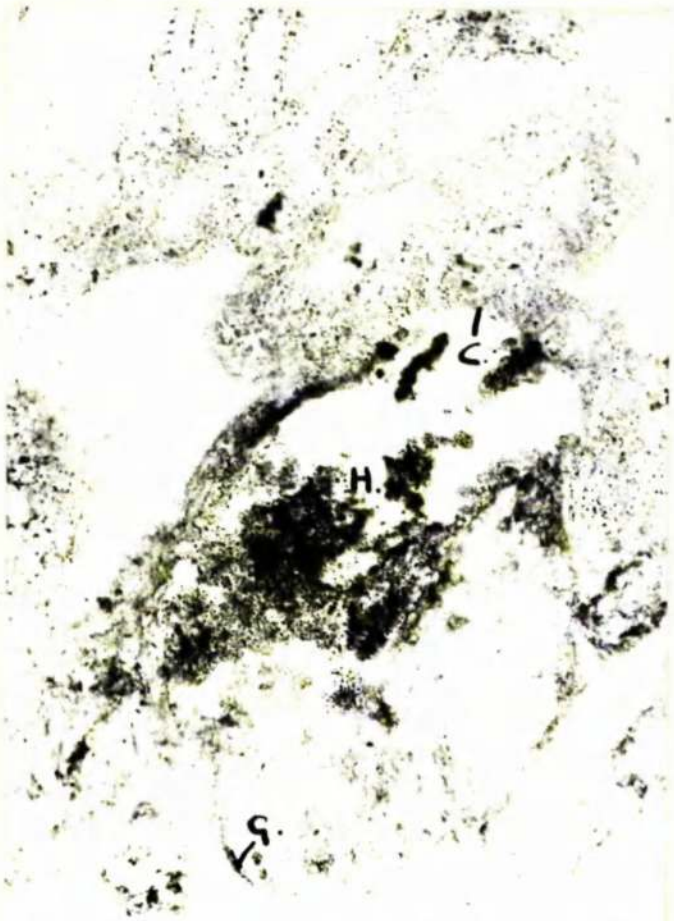
C) the basal cytotrophoblast (C.), the histiotrophe (H.), and the epithelium of the spongy zone glands (G.).

Fig. 191. Naphthol-AS-acetate esterase in the term dog placental trophoblast.



190A

190B



190C

191

Fig. 192. Series of sections, stained by the indoxyl-acetate esterase method, to show the presence of different types of esterase in the brown border of the term cat placenta.

A) uninhibited (A, B, and C esterase)

B) after E600 (A and C esterase)

C) after E600 and p-hydroxy-mercuri-benzoate (C esterase).

A

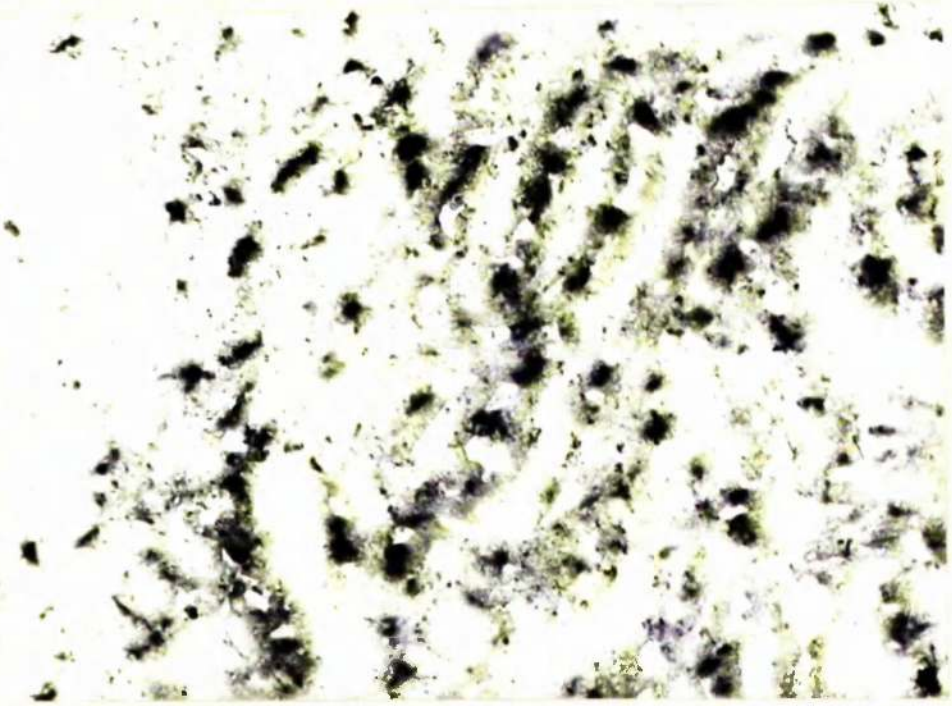
C

Fig. 193. β-glucuronidase in the term cat placenta:-

A) in the maternal stroma of the labyrinth

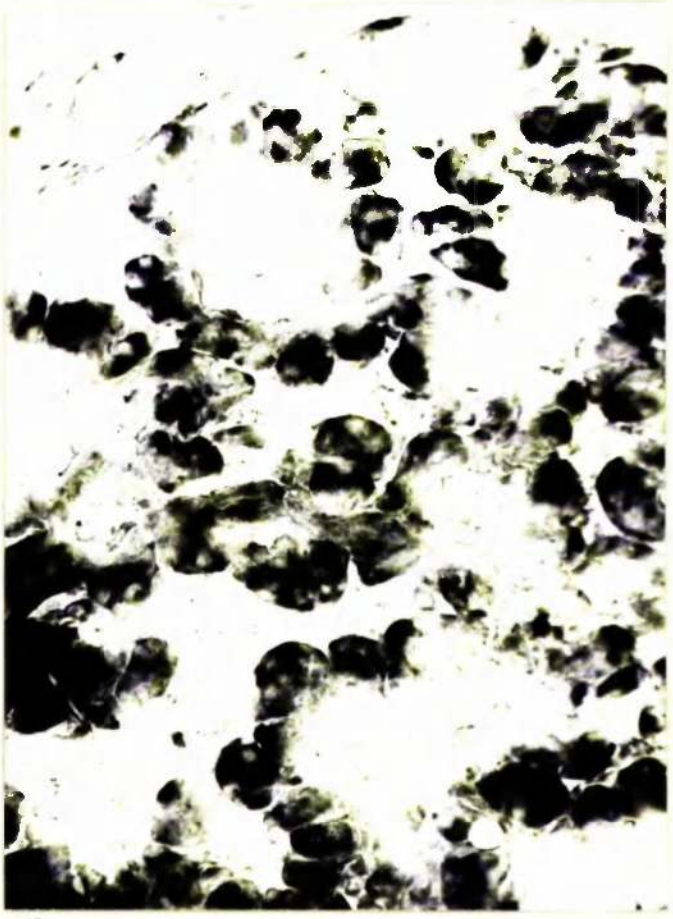
B) in the yolk sac endoderm.

Fig. 194. β-glucuronidase in the trophoblast of the term dog placenta.



193A

193B



194

Fig. 195. 18½ day rat placenta, showing acid phosphatase in:-

A) the yolk sac endoderm (Y.), degenerating parietal endoderm (P.), and maternal epithelium (Ep.)

B) the labyrinth (L.), and spongy zone (S.).

A

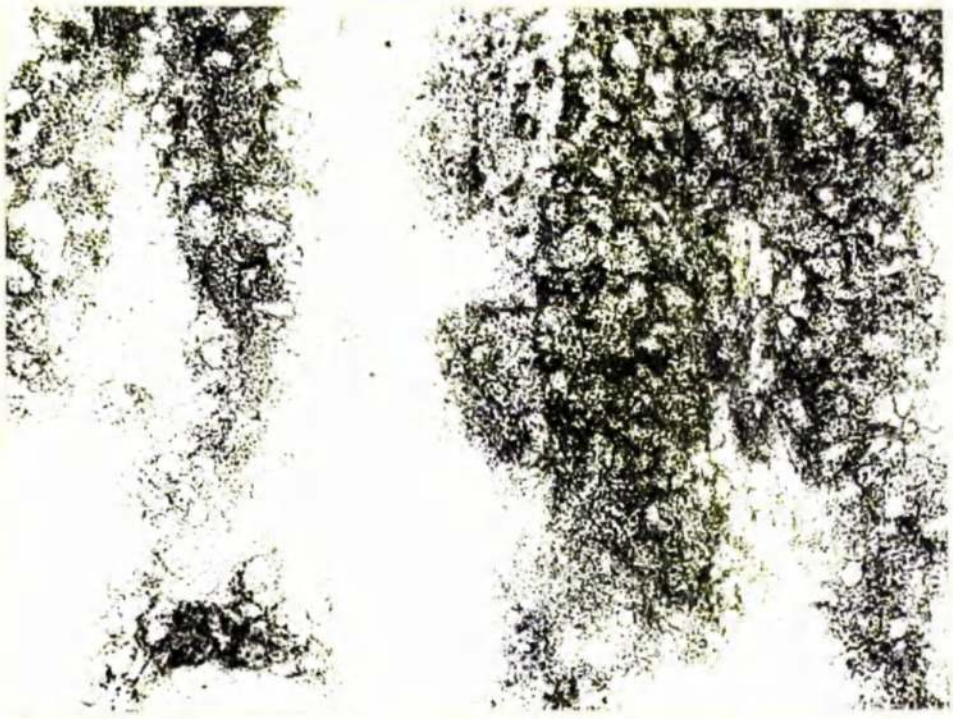
B

Fig. 106. Acid phosphatase in the rabbit placenta:-

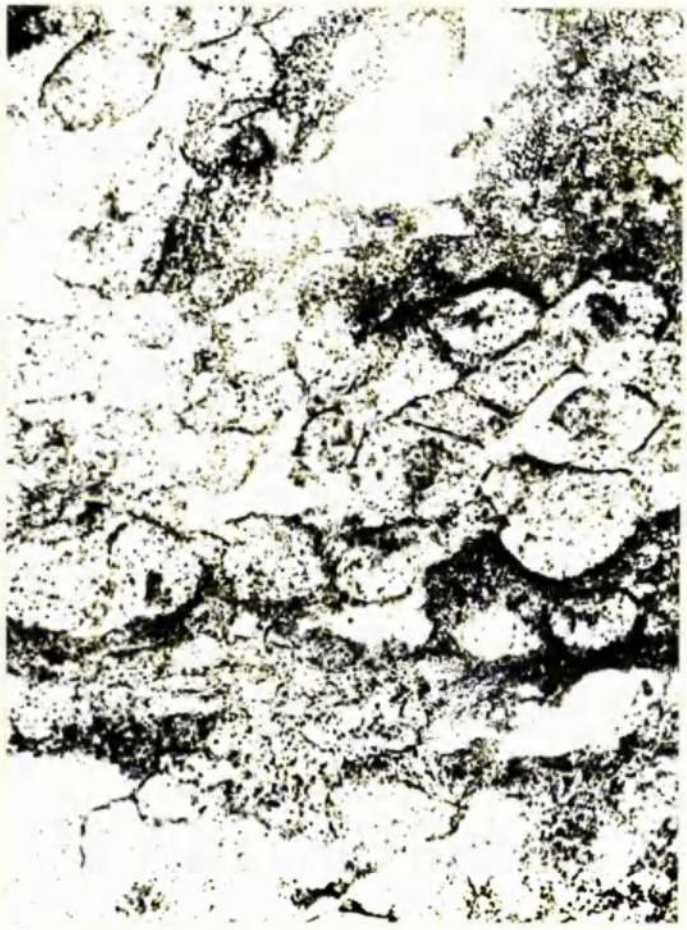
A) in the trophoblast at 13 days

B) in the multinucleate decidual cells at 13 days

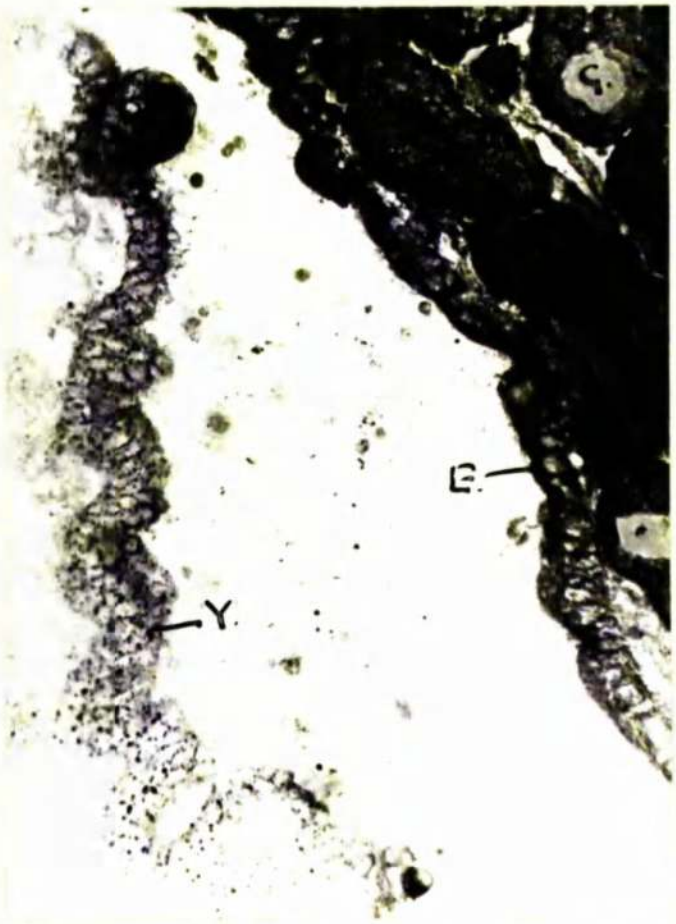
C) in the yolk sac endoderm (Y.), the maternal epithelium (E.), and the obplacental giant cells (G.), at 17 days.



A



B

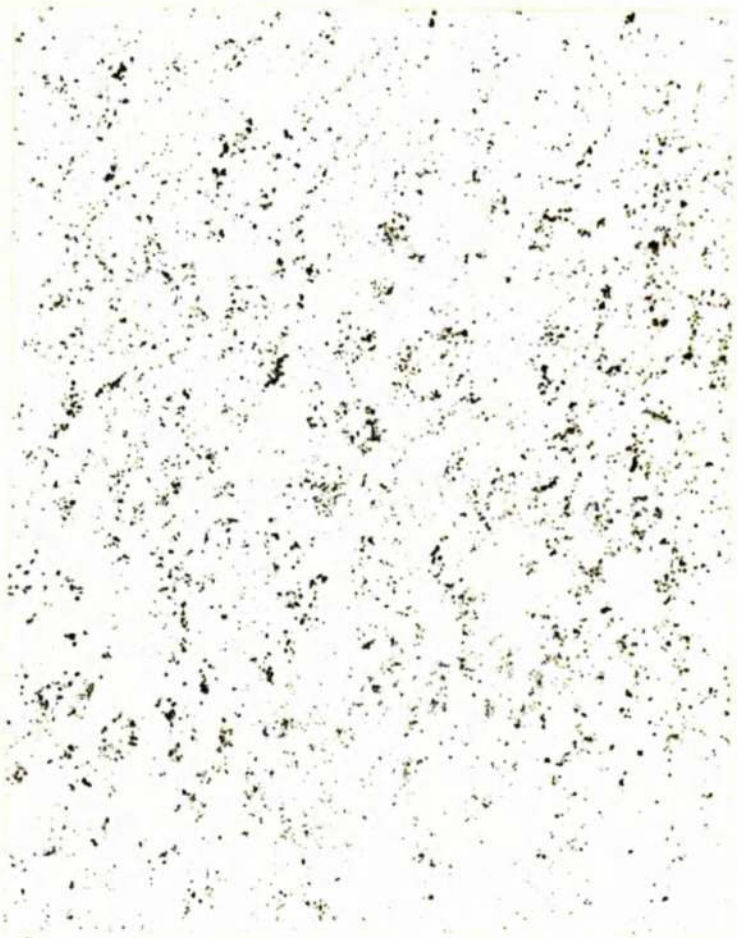


C

Fig. 197. Non-specific esterase in the placenta:-

A) of the rabbit at term in the trophoblast - Naphthol-AS-acetate

B) of the rat at 17½ days in the yolk sac endoderm - Indoxyl acetate



A

B

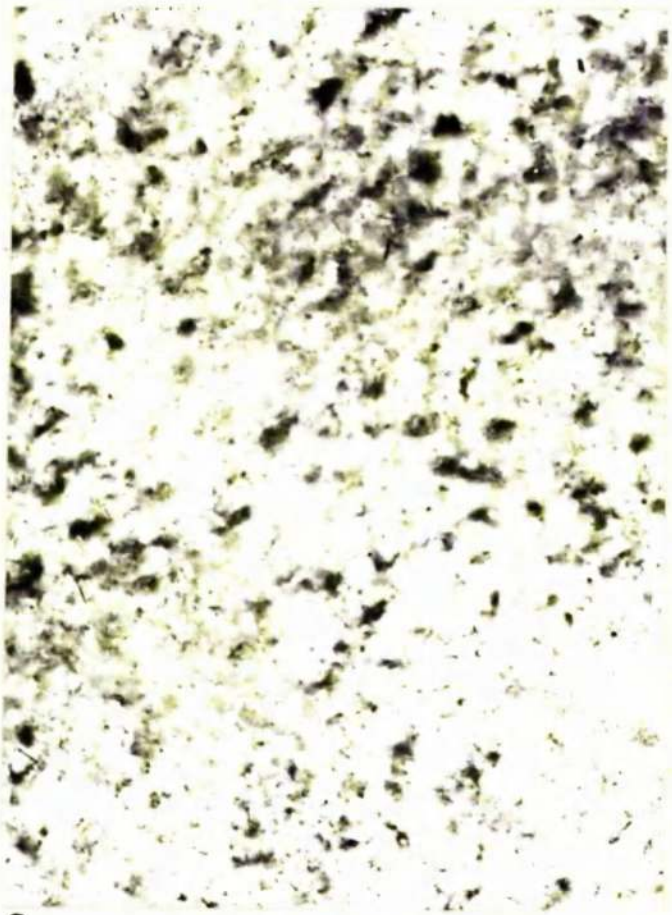
Fig. 198. β-glucuronidase in the placenta of:-

A) the rat at term in the labyrinth

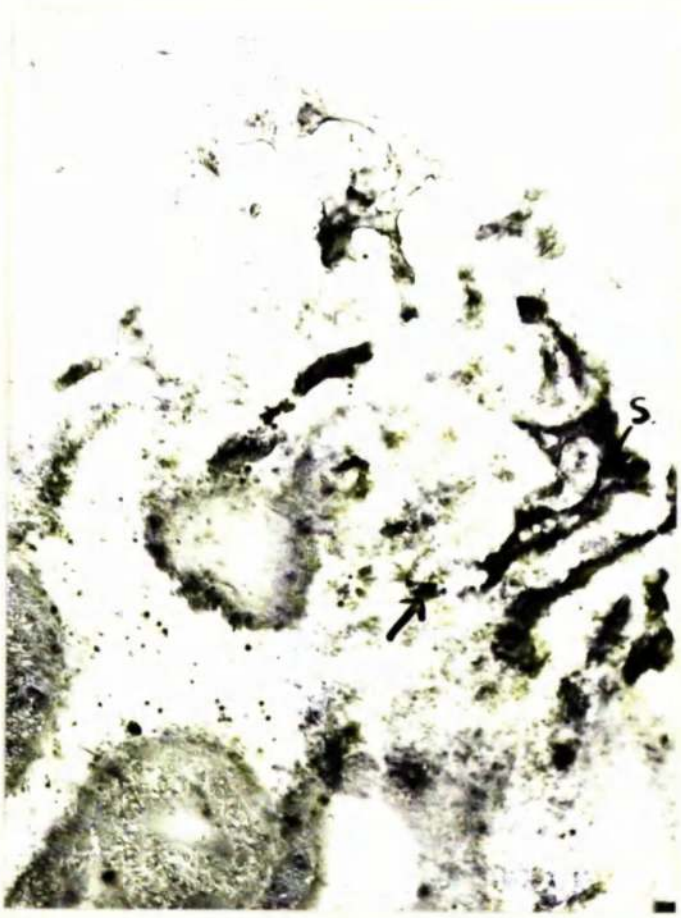
B) the rabbit at 13 days in the degenerating gland symplasma (S.) and related cells (arrowed).

C) the guinea-pig at term in the visceral (V.), and placental (P.) layers of the yolk sac

D) the rat at term, showing activity in the surviving metrial gland cells.



A



B

C

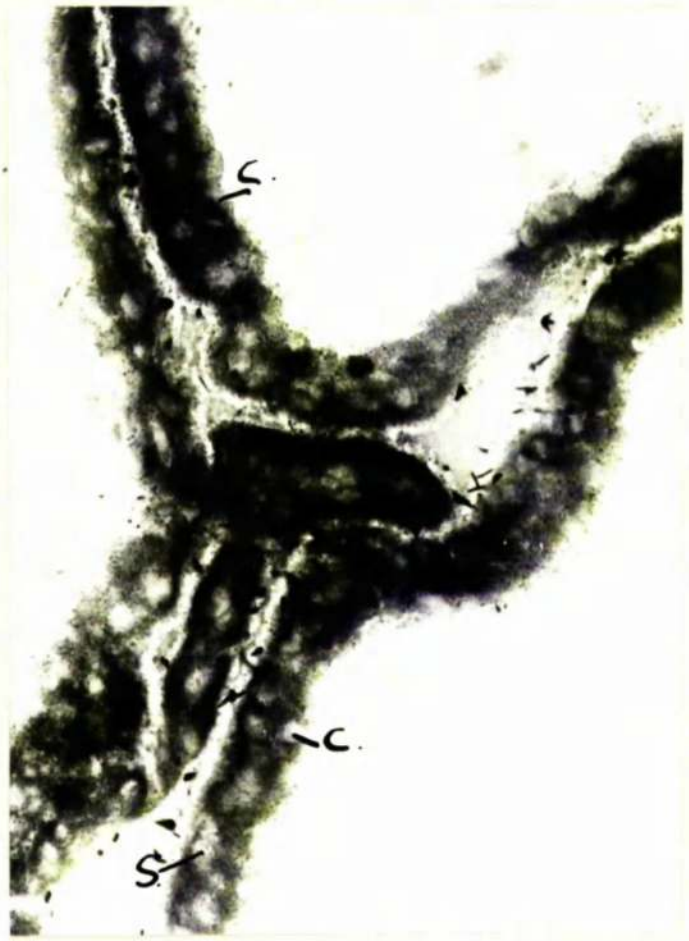
D

Fig. 199. Human placenta, showing acid phosphatase in:-

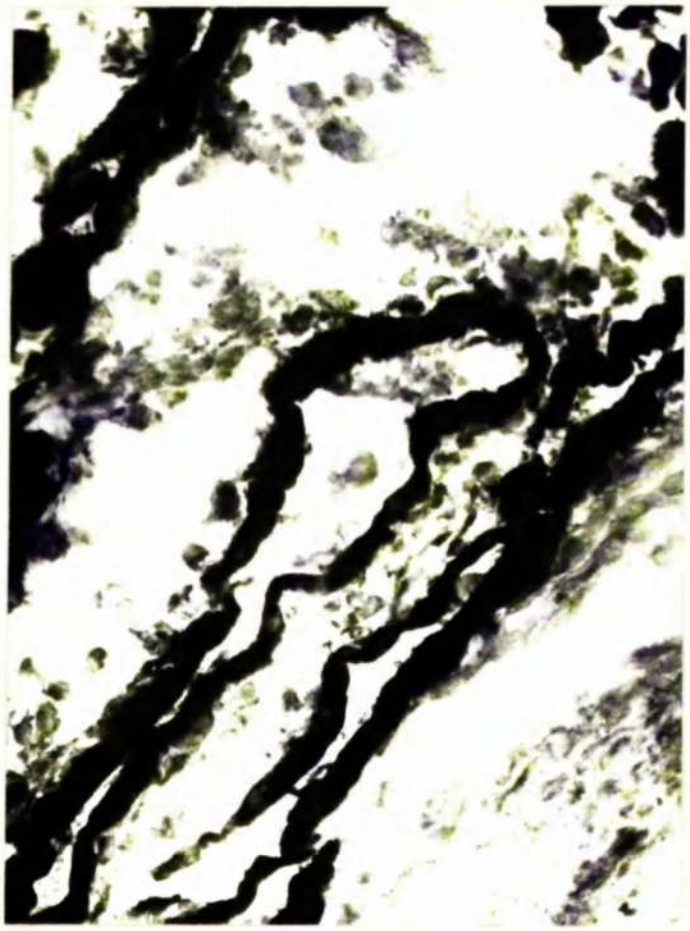
A) the syncytiotrophoblast (S.), and cytotrophoblast (C.), at 1.5 cm.

B) in the epithelium lining the uterine glands at 1.8 cm.

C) the yolk sac endoderm at 1.5 cm.



A



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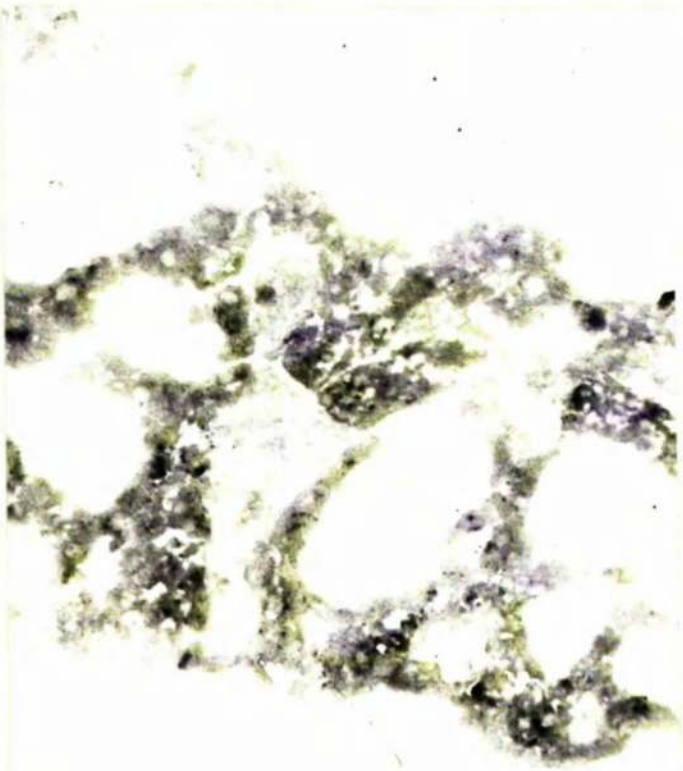


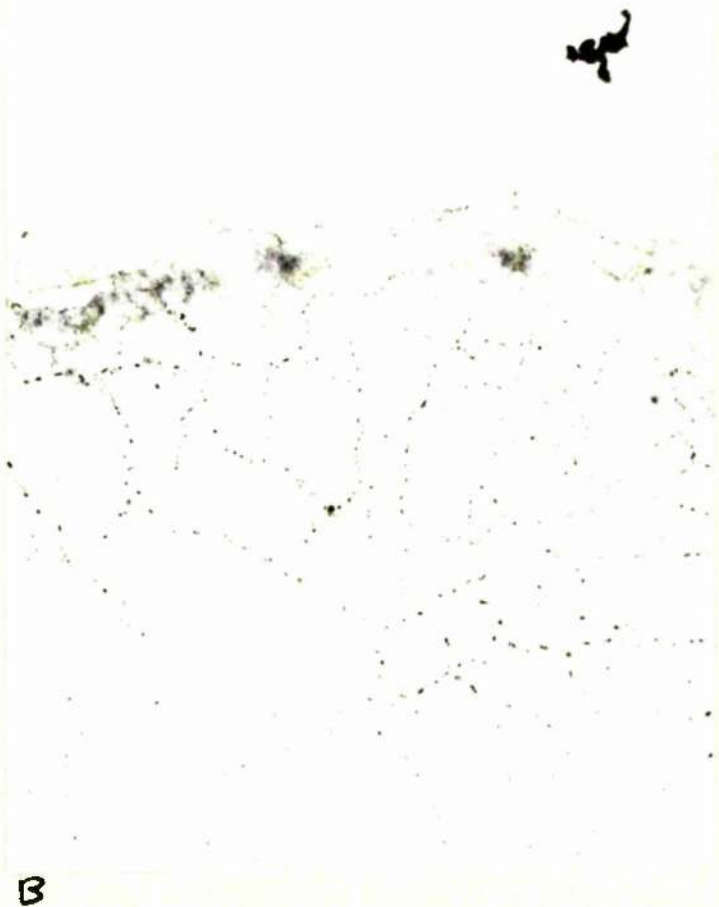
C

Fig. 200. Naphthol-AS-acetate esterase in the yolk sac endoderm of:-

A) the chick at 5 days incubation

B) Limia Maculata in mid pregnancy

A



B

Fig. 201. ATPase in the brush border of the villous trophoblast of the horse placenta.

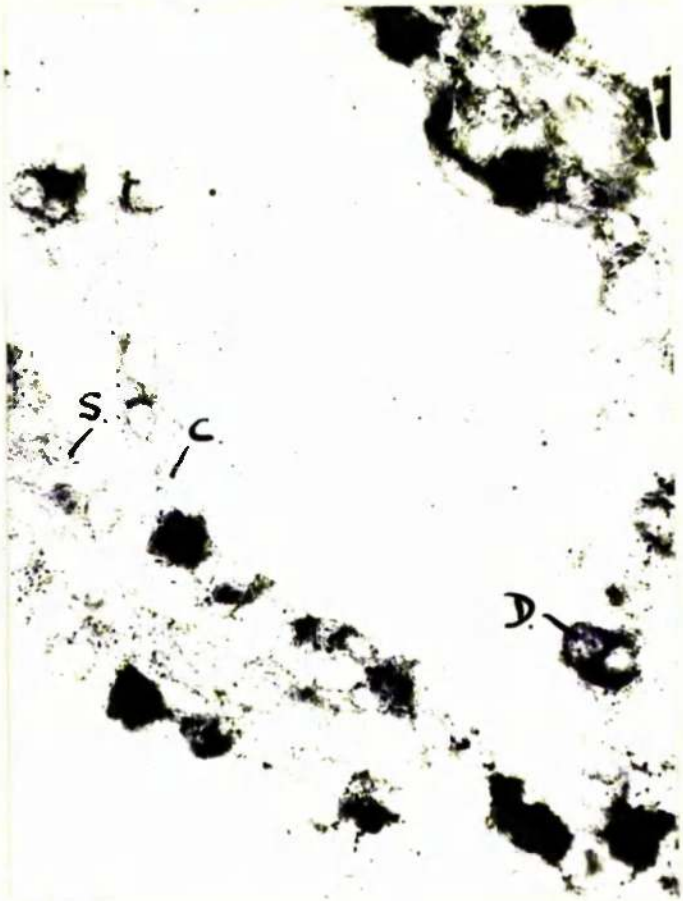
Fig. 202. Non-specific alkaline phosphatase in the sheep placenta:-

A) at 15 cm. in the diplokaryocytes (D.), syncytiotrophoblast (S.), and cellular trophoblast (C.)

B) at term, showing the decrease in staining in the cytotrophoblast(C.), and diplokaryocytes (D.), and increase in that in the syncytiotrophoblast (S.). Uterine glands (G.) are intensely stained.

Fig. 203. AMPase in the cellular (C.) and syncytial (S.) trophoblast, in the 15 cm. sheep placenta. Some secretion appears to be present between the two layers (arrowed). M - maternal stroma.

201



202A

202B

203

204

205

206

Fig. 207. Non-specific alkaline phosphatase in the interstitial matrix of the 7 cm. cat placenta.

Fig. 208. Non-specific alkaline phosphatase in the dog placenta:-

A) at 24 days in the non-placental maternal epithelium

B) at term in the trophoblast.

207

208A

208B

Fig. 209. 7 cm. cat placenta, showing:-

A) AMPase in the trophoblast (T.) of the placental lamellae, and slight activity in the interstitial matrix (arrowed).

B) ATPase in the interstitial matrix (arrowed) and traces in the trophoblast (T.).

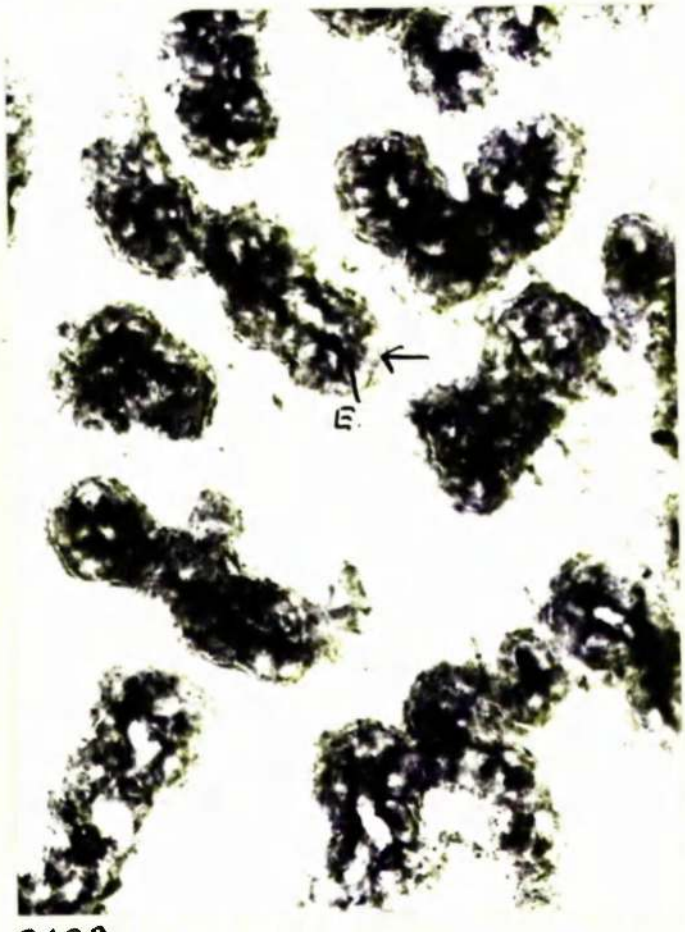
Fig. 210. Ferret placenta, showing:-

A) AMPase in the maternal endothelium (E.). The interstitial matrix (arrowed) is negative.

B) ITPase in the interstitial matrix (arrowed) and trophoblast (T.).

209A

209B



210A

210B

Fig. 211. ATPase in the term cat yolk sac endoderm (E.) and foetal capillaries (C.).

Fig. 212. Non-specific alkaline phosphatase in the 17½ day rat placental labyrinth (L.), visceral endoderm (V.), and giant cells (G.).

Fig. 213. Non-specific alkaline phosphatase in the rabbit at 17 days, in the antimesometrial epithelium (E.), the maternal stroma (S.), and multinucleate bodies in the yolk sac cavity (M.).

Fig. 214. 17½ day rat placenta, showing ATPase in the metrial gland cells (M.), the maternal decidual vessels (V.), the giant cells (G.), and the spongy zone trophoblast (S.).

211

212

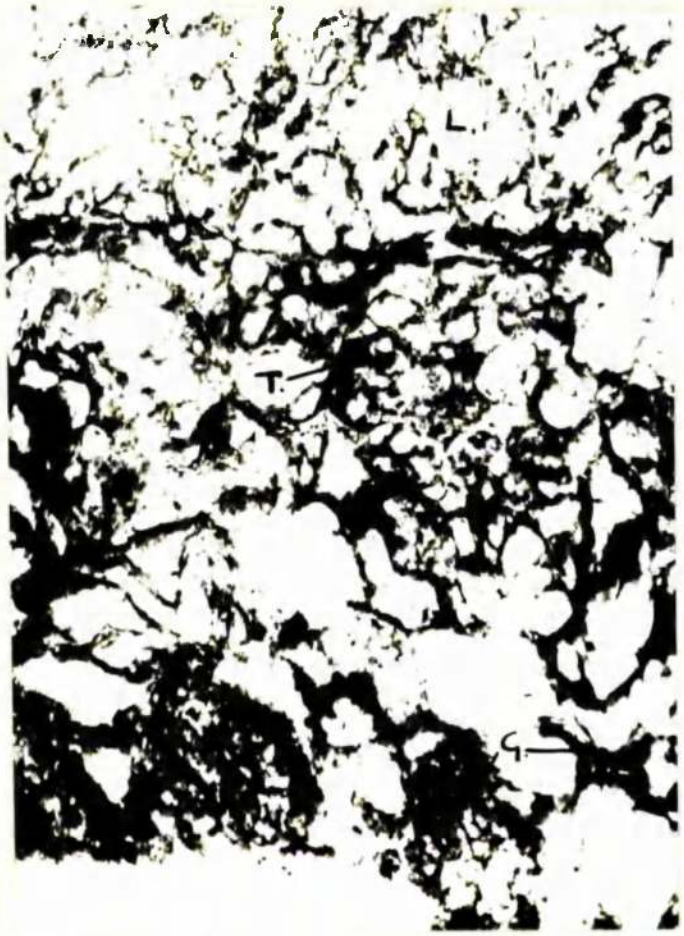
213

214

Fig. 215. Term rat placenta, showing AMPase activity in the labyrinth (L.), the spongy zone trophoblast (T.), and the giant cells (G.).

Fig. 216. 13 day rabbit placental decidua, showing AMPase activity in the lining of the vessels (V.).

Fig. 217. 17 day rabbit placenta, antimesometrial region, showing ATPase in the epithelial (Ep.), and endodermal (E.) brush borders, and in the obplacental giant cells (G.).



215

216

217

Fig. 218. 20 day guinea-pig placenta, showing ATPase in the labyrinth (L.), and spongy zone (S.), the giant cells (G.), and placental endoderm (P.) being negative.

Fig. 219. 1.5 cm. human placenta, showing AMPase in:-

A) the cytotrophoblast (C.) and syncytiotrophoblast (S.), particularly in the brush border. Some activity is also seen in the foetal blood vessels (V.).

B) the yolk sac endodermal brush border (arrowed), and blood vessels (V.).

218

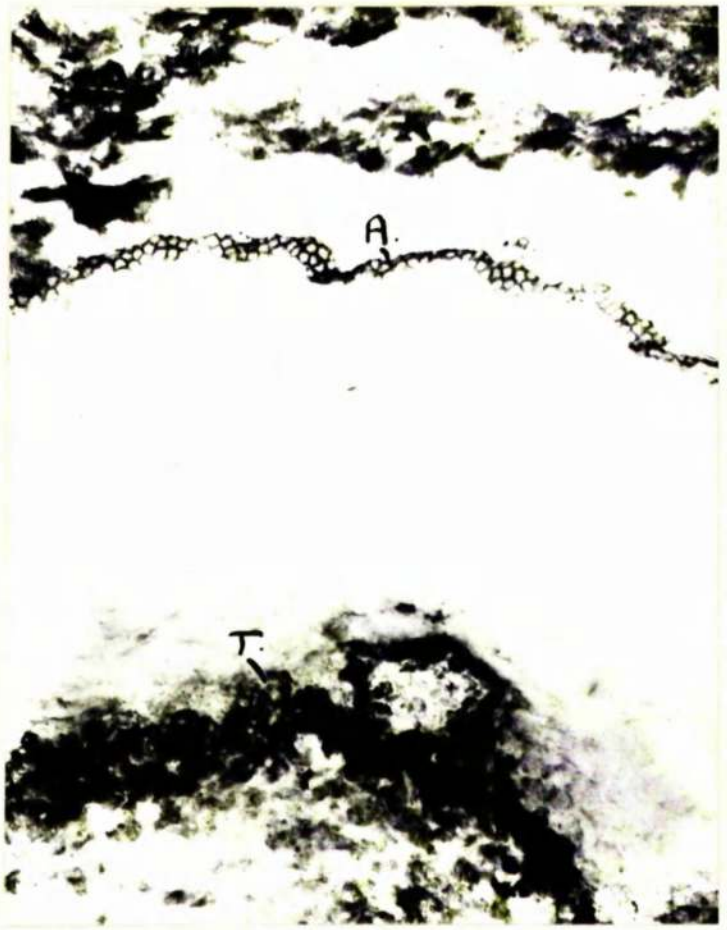
219A

219B

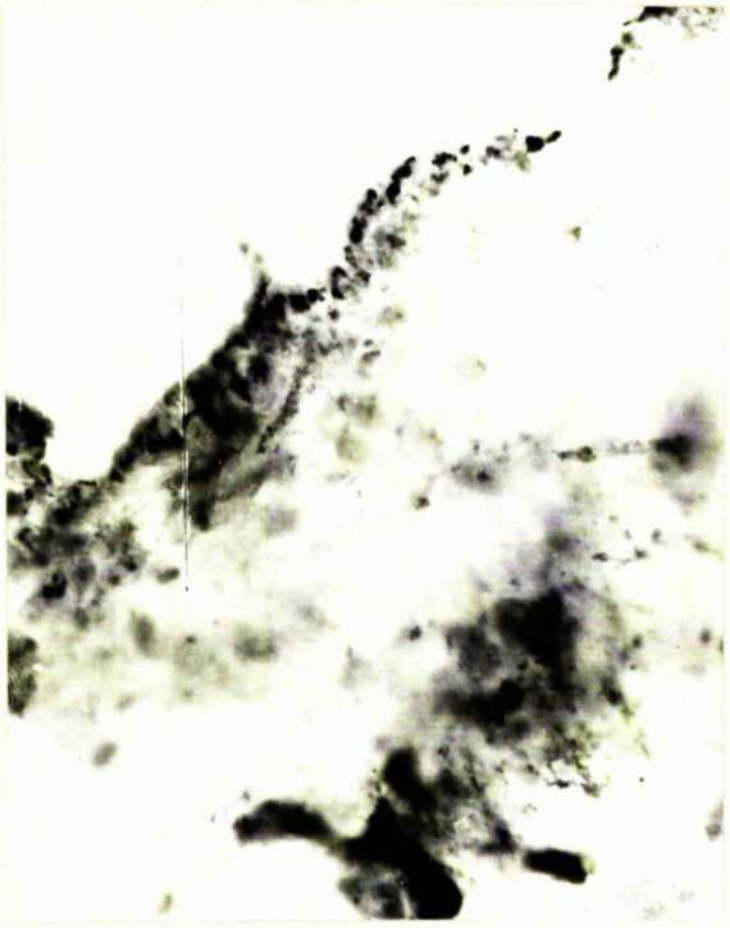
Fig. 220.

A) AMPase in the chorion laeve of the 18 cm. human placenta, in the amnion (A.), and trophoblast (T.).

B) TPPase in the Golgi region of the uterine glands in the pregnant uterus at 1.8 cm.



220A



B

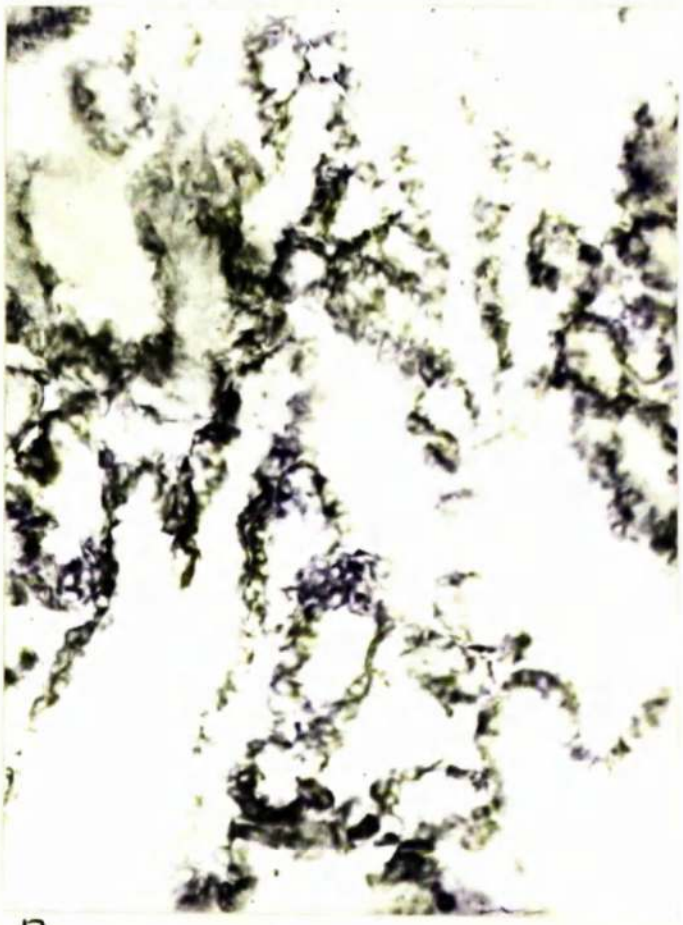
Fig. 221. Term horse placenta, showing:-

A) αGP activity in the allantoic endoderm

B) IDH in the trophoblast

C) G-6-P in the trophoblast - staining is more intense with this enzyme than with the similarly NADP-linked IDH.

A



B

C

Fig. 222. MDH activity in the sheep placenta:-

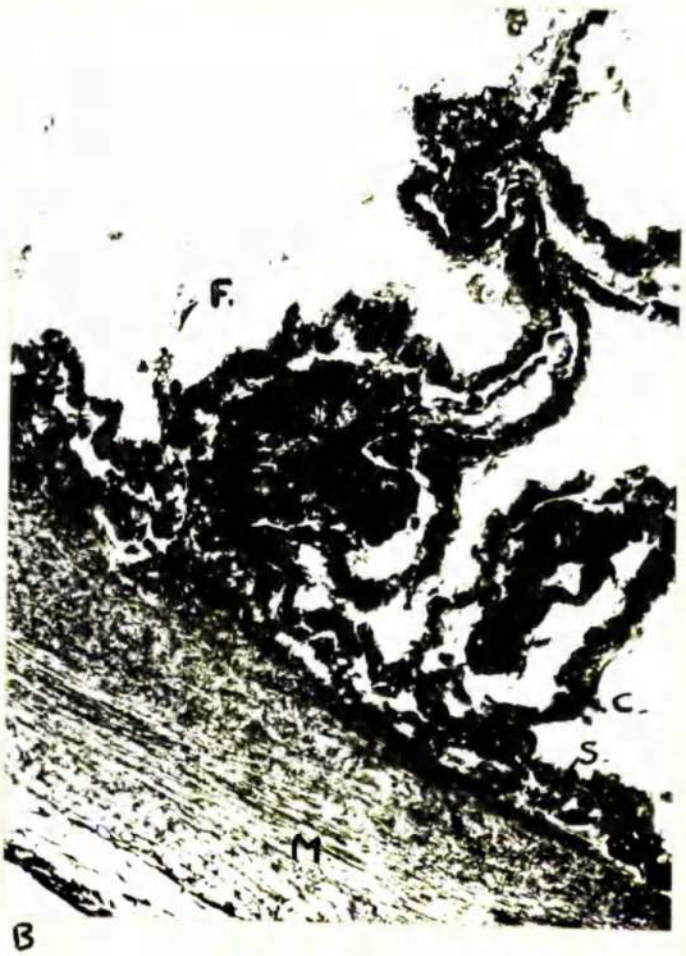
A) at 2.5 cm.

B) at term

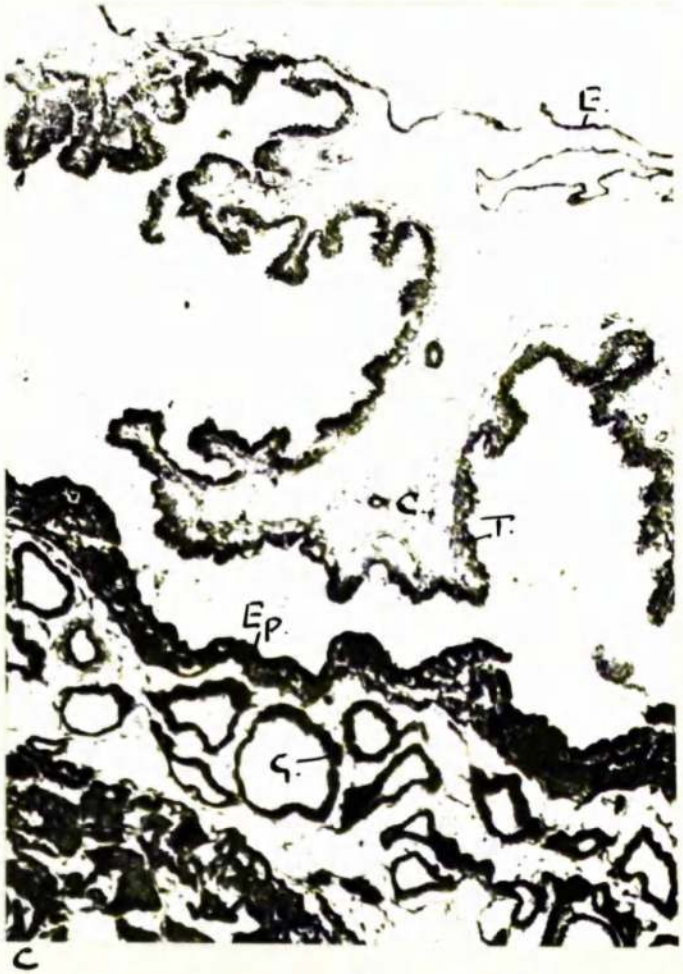
showing staining in the cytotrophoblast (C.), syncytiotrophoblast (S.), maternal stroma (M.), and foetal stroma (F.).

C) in the intercotyledonary area at 40 cm., showing activity in the uterine epithelium (Ep.), and glands (G.), in the chorionic trophoblast (T.), and mesenchyme (C.), and in the allantoic endoderm (E.).

A



B



C

Fig. 223. G-6-P activity in the sheep placenta:-

A) at 2.5 cm.

B) at 15 cm.

showing cytotrophoblastic (C.), minimal syncytiotrophoblastic (S.), and maternal (M.) and foetal (F.) stromal activity.

C) at 40 cm. in the intercotyledonary area, showing activity in the maternal epithelium (Ep.), glands (G.), and stroma (S.), and in the chorionic trophoblast (T.), chorionic (C.) and allantoic (A.) mesenchymes, and allantoic endoderm (E.).

B

A

C

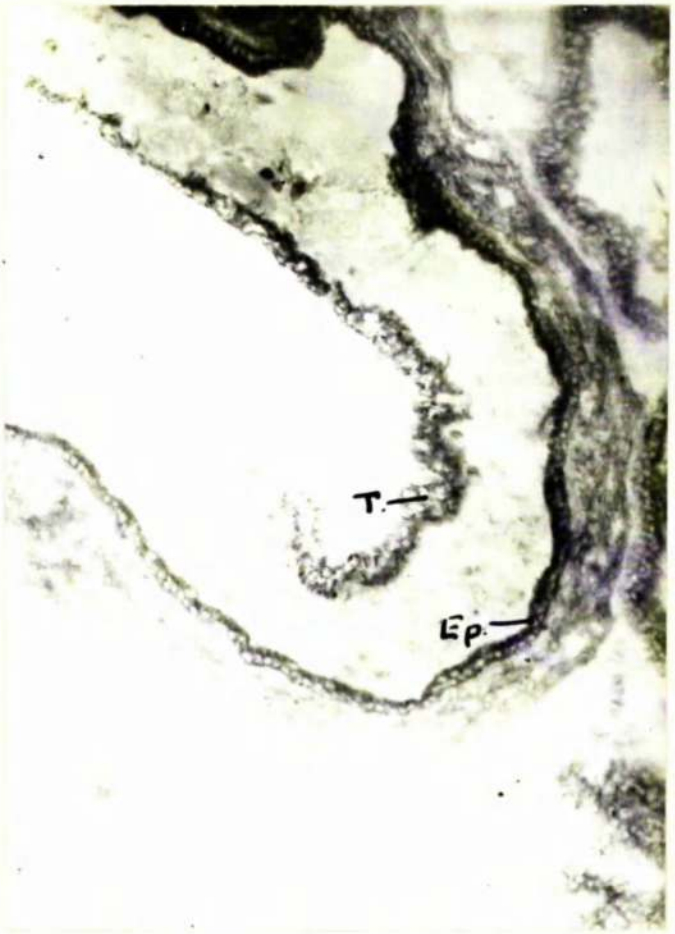
Fig. 224. FDH in the maternal epithelium (Ep.), and chorionic trophoblast (T.) of the 25 cm. sheep placenta.

Fig. 225. GDH in the cytotrophoblast (C.) and maternal stroma (M.) of the sheep placenta at 15 cm.

Fig. 226. αGP activity in the 7 cm. cat placenta:-

A) in the trophoblast (T.) and decidual giant cells (G.).

B) in the histiotrophe (H.).



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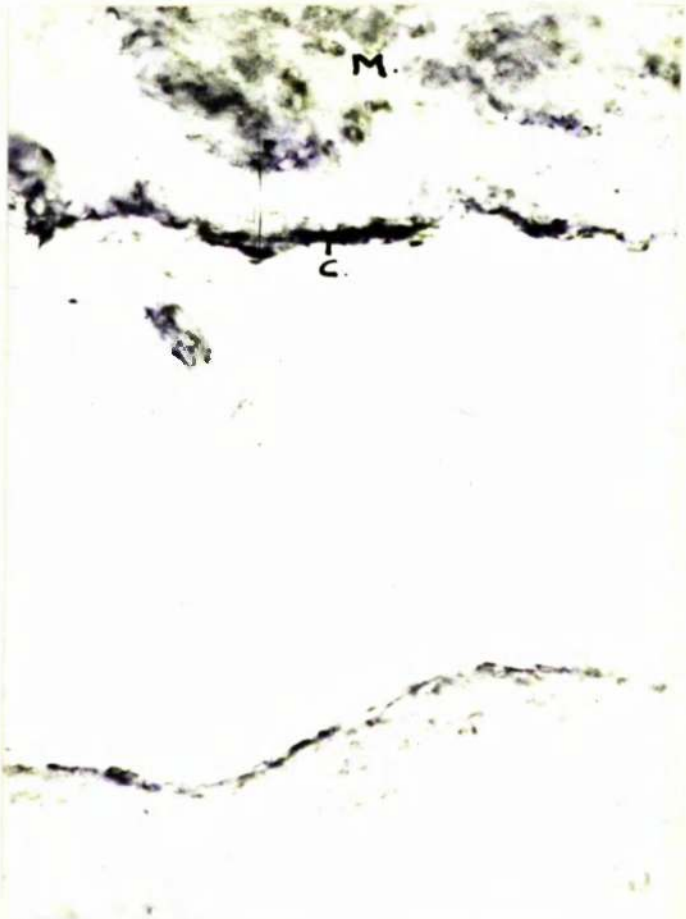

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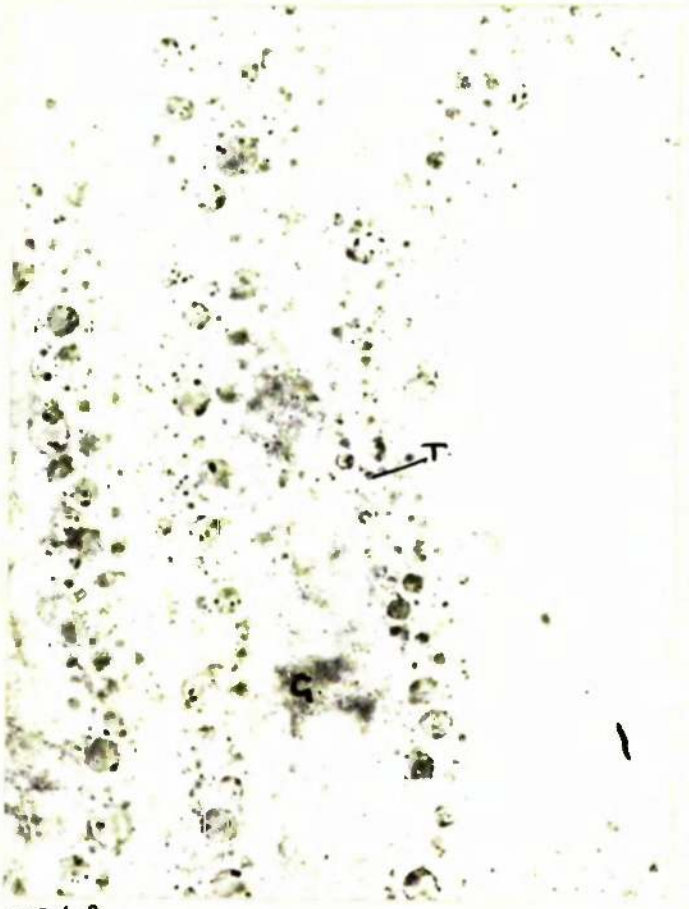


226A

226B

Fig. 227. βOH activity in the brown border chorion (C.) of the term cat placenta.

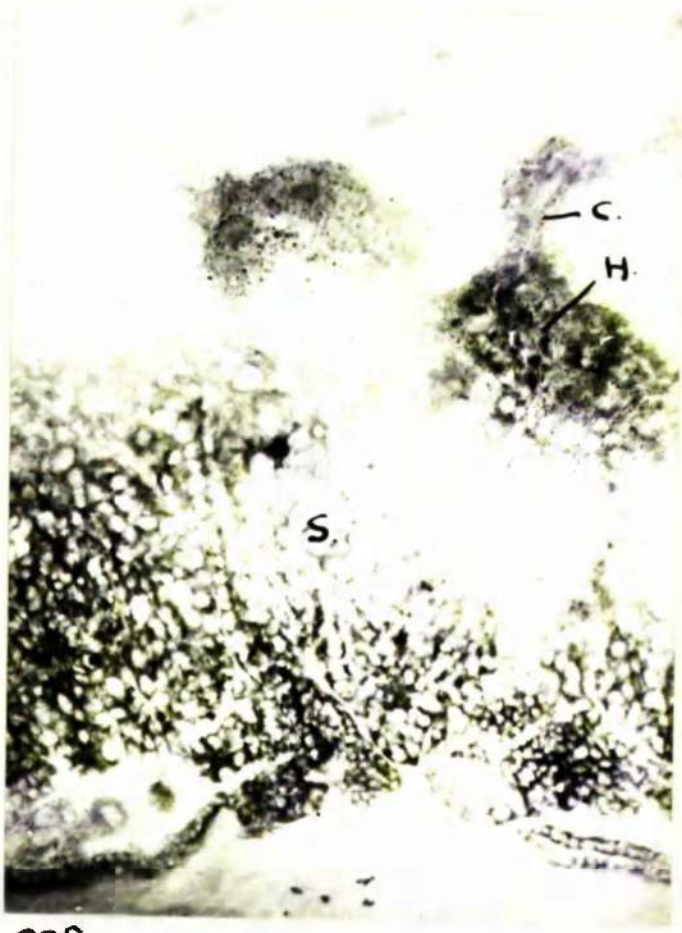
Fig. 228. αGP activity in the ferret placenta, in the spongy zone glands (S.), histiotrophe (H.), and basal cytotrophoblast (C.).

Fig. 229. 24 day dog placenta, showing:-

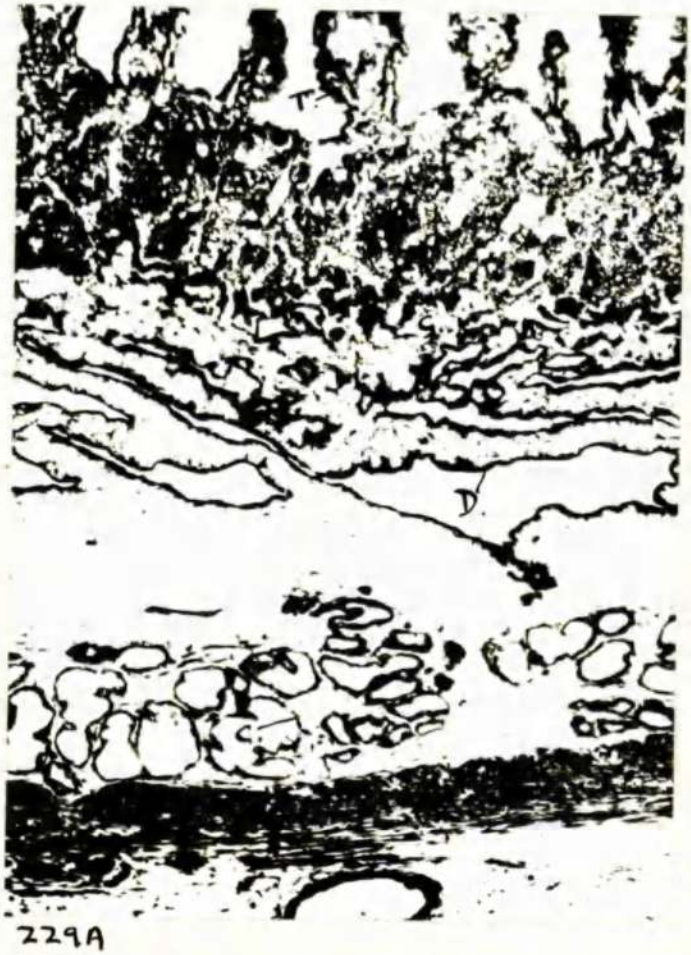
A) LDH in the trophoblast (T.), uterine glands (G.), and spongy zone glands - contracted (C.), and dilated (D.).

B) MDH in the non-placental trophoblast (T.), and uterine glands (G.).

227



228



229A

229B

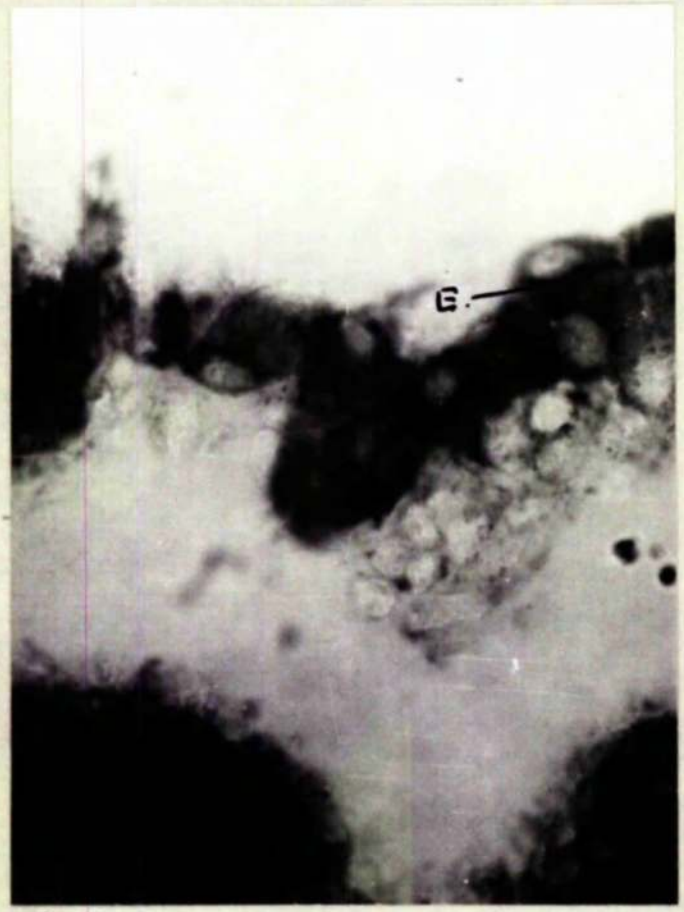
Fig. 230. 32 day dog placenta, showing:-

A) LDH in the labyrinthine (L.), green border (G.), and non-placental (N.), trophoblast, and in the uterine glands (U.).

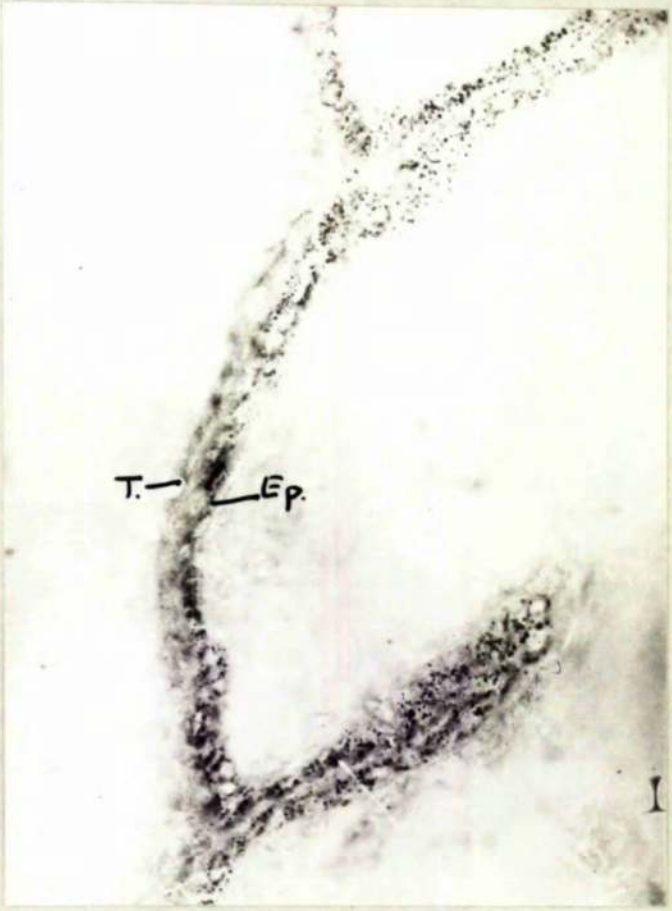
B) MDH in the endoderm (E.) of the chorio-vitelline placenta. C - trophoblast of the chorio-allantoic placenta.

C) IDH in the non-placental trophoblast (T.), and uterine epithelium (Ep.).

A



B



C

Fig. 231. LDH in the trophoblast (T.), and maternal (M.) and foetal (F.) endothelia in the term dog placenta.

231

Fig. 232. 7 cm. cat placenta, showing:-

A) MDH in the trophoblast (T.), and decidual giant cells (G.).

B) IDH mainly in the decidual giant cells (G.), with some in the trophoblast (T.) also.

C) SDH in the non-placental uterine glands

D) LDH in the yolk sac endoderm (E.), and mesoderm (M.).

A

B

C

D

Fig. 233. Ferret placenta showing:-

A) LDH in the spongy zone (S.), basal cytotrophoblast (C.), labyrinthine syncytiotrophoblast (T.), and thickened maternal endothelium (E.).

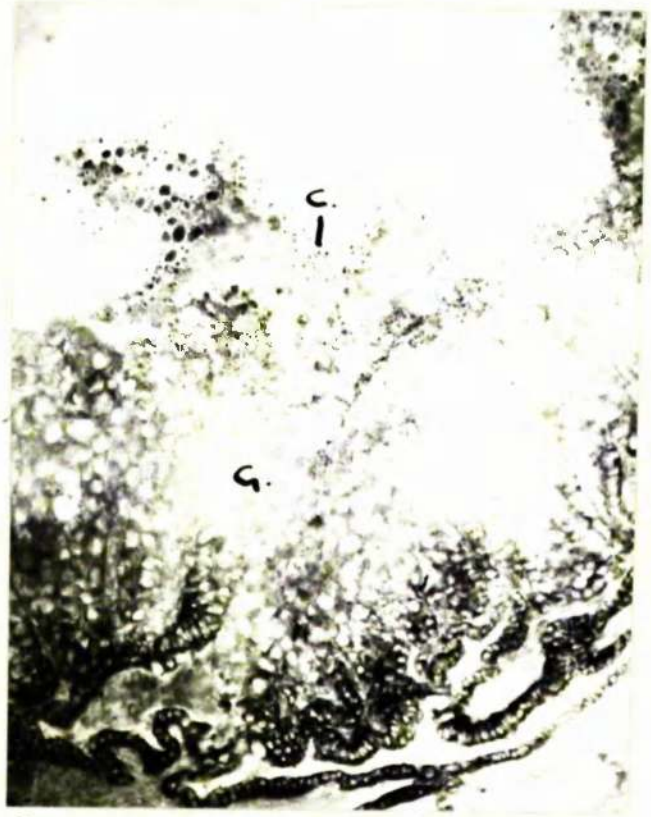
B) MDH in the labyrinthine trophoblast (T.), and maternal endothelium (E.).

C) SDH in the spongy zone glands (G.), and basal cytotrophoblast (C.).

D) MDH in the non-placental uterine glands (G.), and chorionic trophoblast (T.).

233A

B



C

D

Fig. 234. G-6-P in the placenta and related structures of the dog, at:-

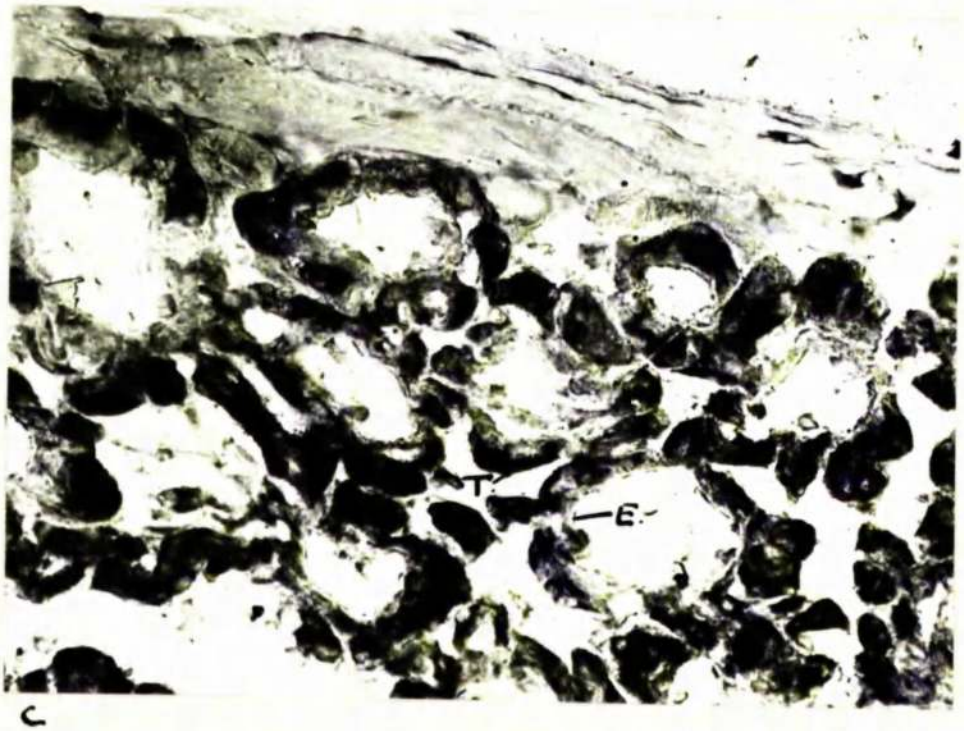
A) 24 days, showing activity in the green border (G.), and non-placental (T), trophoblast, and in the maternal glands (M.).

B) 45 days, showing little activity in the contracted part (C.) of the spongy zone glands, with considerable staining in the dilated part (D.), the basal cytotrophoblast (T.), and the areas of decidualization (arrowed).

C) term, in the trophoblast (T.), and maternal endothelium (E.).

A

B



C

Fig. 235. G-6-P in the 7 cm. cat placenta, in:-

A) the decidual giant cells (G.), and trophoblast (T.). Upper part - superficial labyrinth, lower part - deep labyrinth.

B) the spongy zone epithelium (Ep.), areas of decidualization (D.), and basal cytotrophoblast (T.).

C) the brown border trophoblast (T.), and maternal epithelium (Ep.).

D) the yolk sac endoderm (E.), and mesoderm (M.).

A

B

C

D

Fig. 236. Ferret placenta showing, G-6-P in the spongy zone glands (G.), and basal cytotrophoblast (C.).

Fig. 237. 7 cm. cat placenta, showing:-

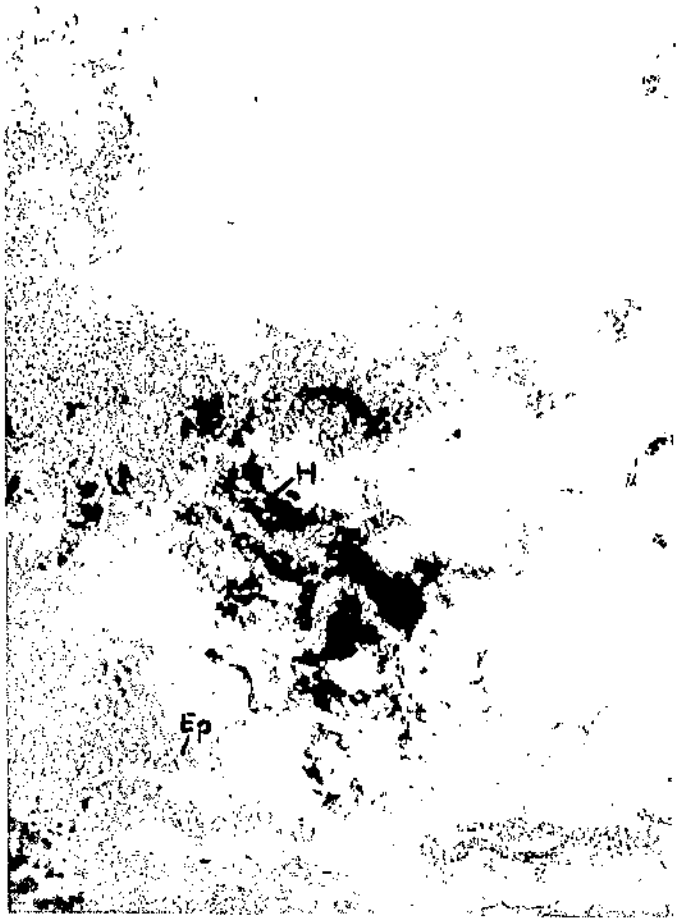
A) FDH in the basal cytotrophoblast (C.), and histiotrophe (H.).

B) GDH in the histiotrophe (H.), and spongy zone epithelium (Ep.).

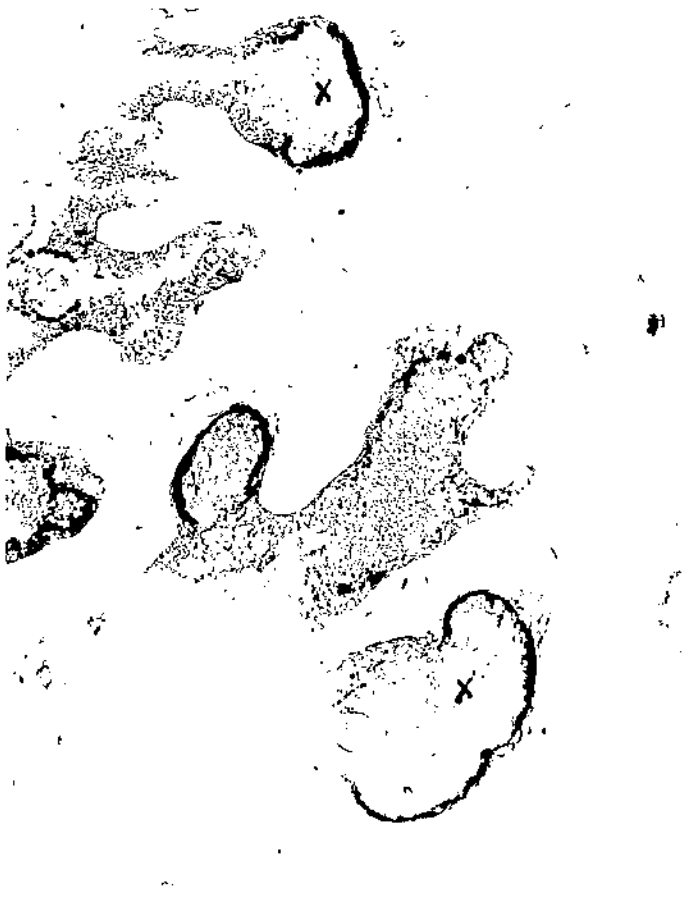
Fig. 238. Term cat placenta, showing GDH in areas of brown border associated with absorption of degenerating material (X.).

236

237A



237B



238

Fig. 239. Ferret placenta, showing SorbDH in the spongy zone glands (G.), and basal cytotrophoblast (T.).

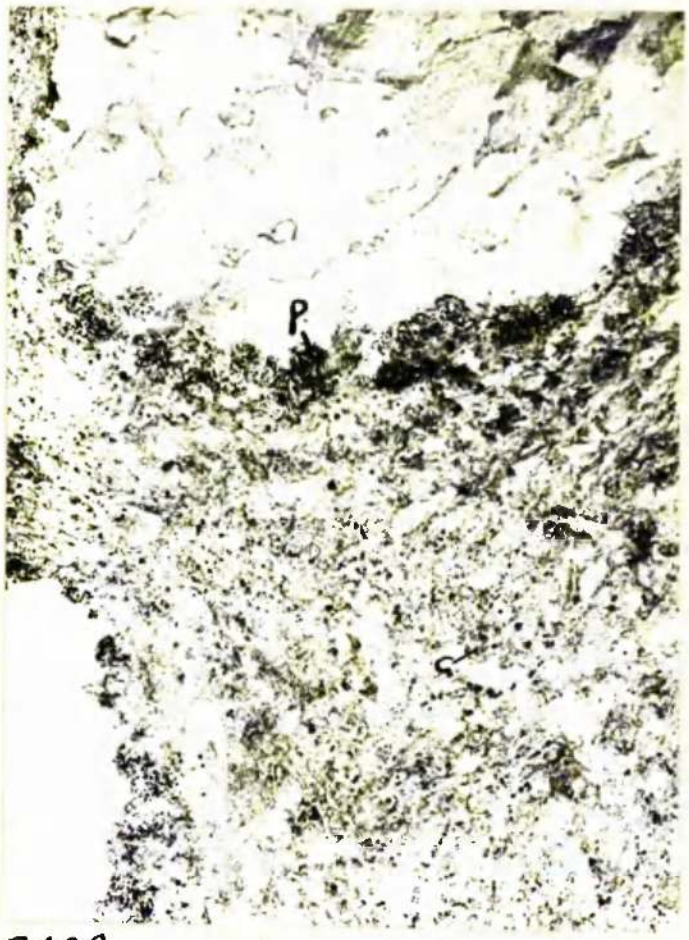
Fig. 240. αGP activity in the rat placenta at:-

A) 17½ days in the endovascular plasmodium (P.), and metrial gland cells (G.).

B) term in the yolk sac endoderm (V.), labyrinth (L.), and endodermal sinus (S.).

Fig. 241. αGP in the junctional zone of the guinea-pig placenta.

239



240A

240B

241

Fig. 242. αGP in the amnion (A.), and amniotic mesenchyme (M.), of the human placenta at term.

Fig. 243. Rat placenta, at:-

A) $14\frac{1}{2}$ days, showing MDH in the labyrinth (L.), giant cells (G.), and decidua basalis (D.), but not in the spongy zone (S.).

B) $17\frac{1}{2}$ days, showing MDH in the same areas as (A) and in the visceral endoderm (V.), and endodermal sinus (E.). Increase in activity as compared to (A) is visible.

C) term, showing LDH in the labyrinth (L.), and endodermal sinus (E.). The parietal endoderm of the sinus (P.) is also active.

242

243A

243B

243C

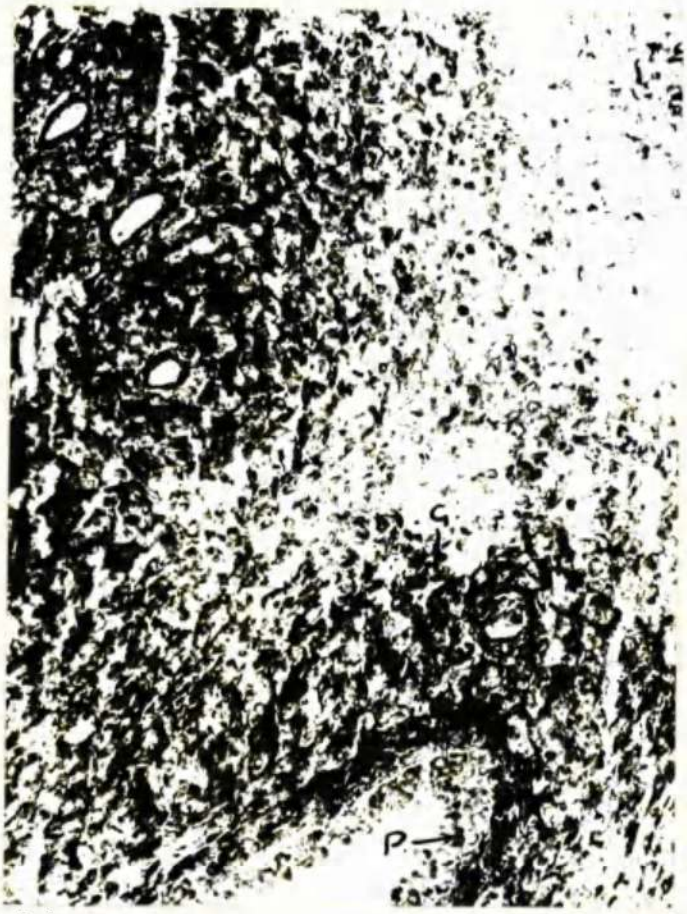
Fig. 244. LDH in the endovascular plasmodium (P.), and metrial gland cells (G.) at 18½ days in the rat.

Fig. 245. LDH in the guinea-pig placenta, at:-

A) 20 days in the labyrinth (L.), spongy zone (S.), and visceral endoderm (E.).

B) term, lettering as in (A). Activity is also seen in the giant cells (G.), and placental endoderm (P.).

Fig. 246. MDH in the subplacenta (S.), of the guinea-pig at 20 days.



244

245A

245B

246

Fig. 247. SDH in the visceral endoderm (V.), and antimesometrial epithelium (Ep.) of the term guinea-pig placenta.

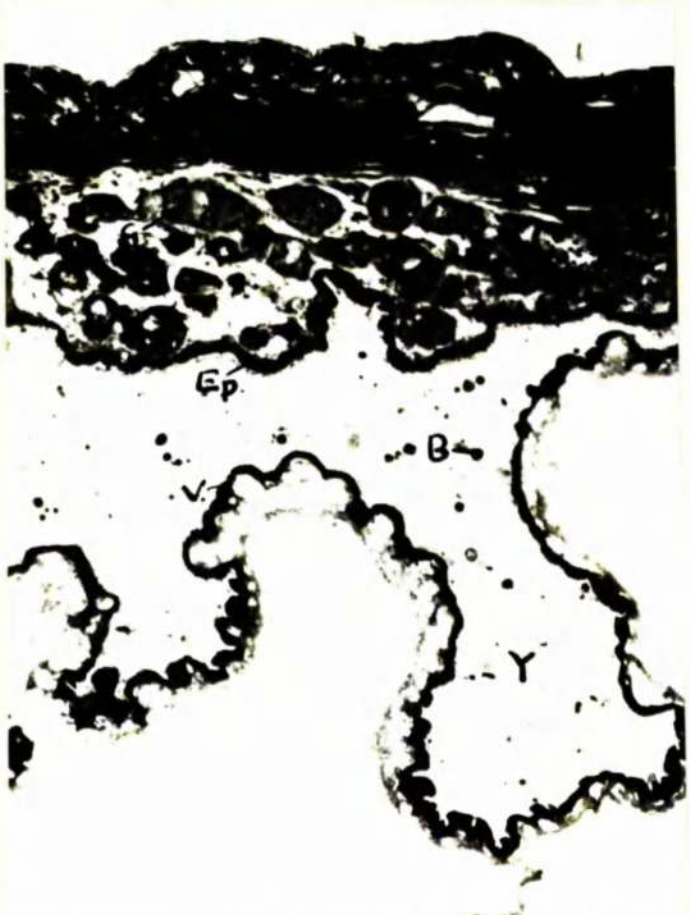
Fig. 248. LDH in the trophoblast (T.), multi-nucleate decidual cells (M.), and uni-nucleate decidual cells (D.) of the 13 day rabbit placenta.

Fig. 249. MDH in the antimesometrial (obplacental) giant cells (G.), and epithelium (Ep.) of the 17 day rabbit placenta. The visceral endoderm (V.) and degenerating multinucleate bodies (B.) in the yolk sac lumen (Y.) are also positive.

Fig. 250. IDH in the term human placental syncytio-trophoblast (S.), and decidua (D.).

247

248



249

250

Fig. 251. G-6-P in the term rat placenta:-

A) in the labyrinth (L.), spongy zone (S.), and giant cells (G.).

B) in the visceral endoderm (V.), and labyrinth (L.).

C) in the endovascular plasmodium (E.), and surviving metrial gland cells (G.).

A

B

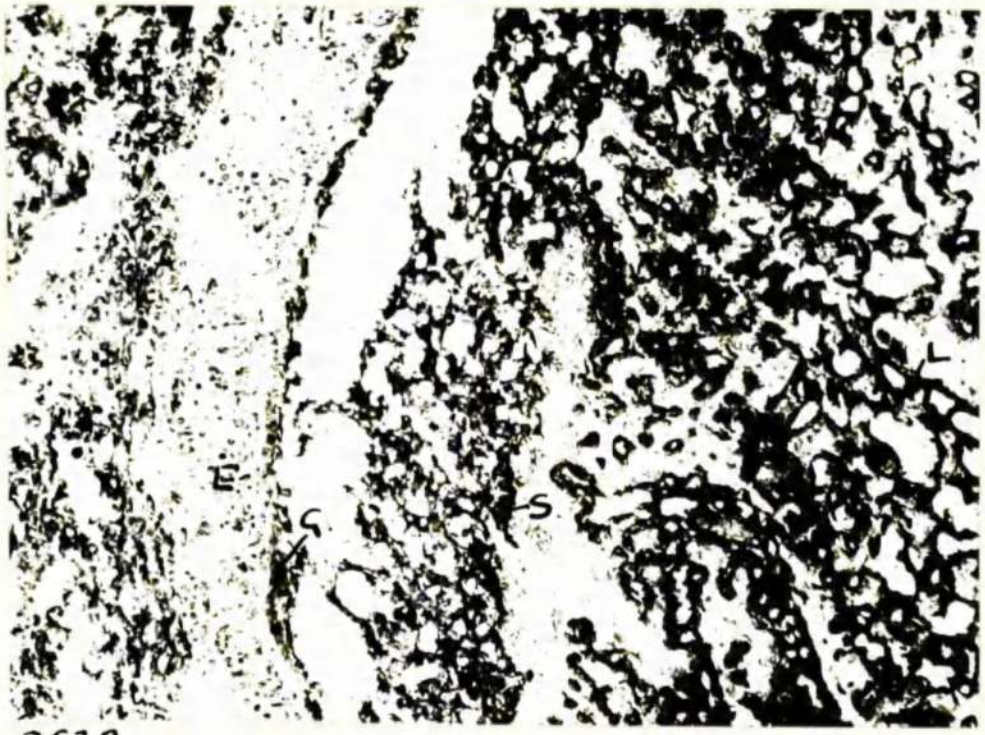
C

Fig. 252. G-6-P in the 20 day guinea-pig placenta, in:-

A) the labyrinth (L.), spongy zone (S.), giant cells (G.), and placental endoderm (E.).

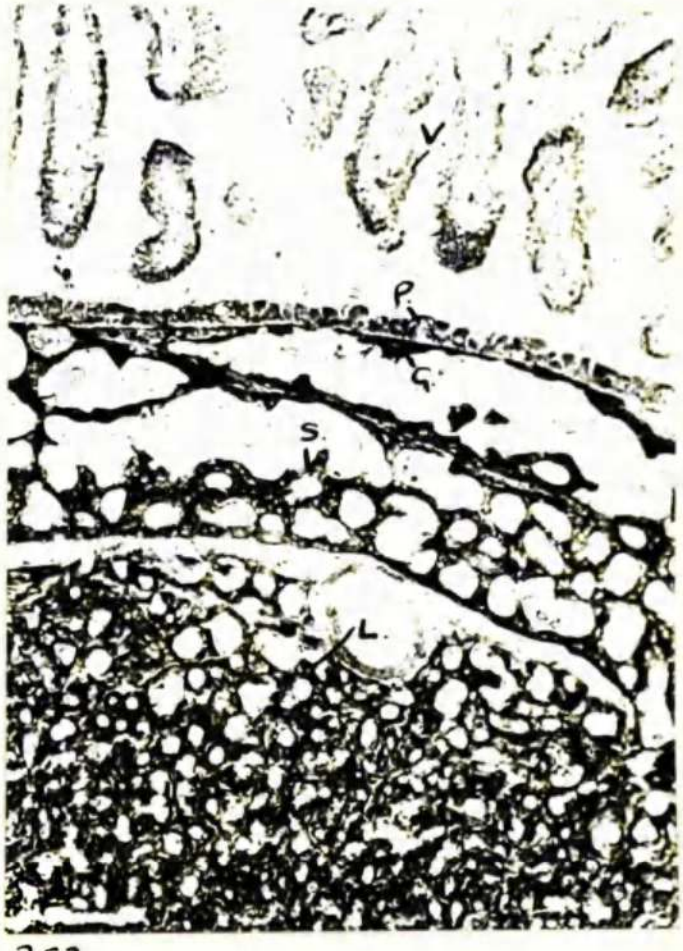
B) the subplacenta (B.).

Fig. 253. G-6-P in the term guinea-pig placenta, in the labyrinth (L.), spongy zone (S.), giant cells (G.), placental (P.) and visceral (V.) layers of endoderm.



252A

252B



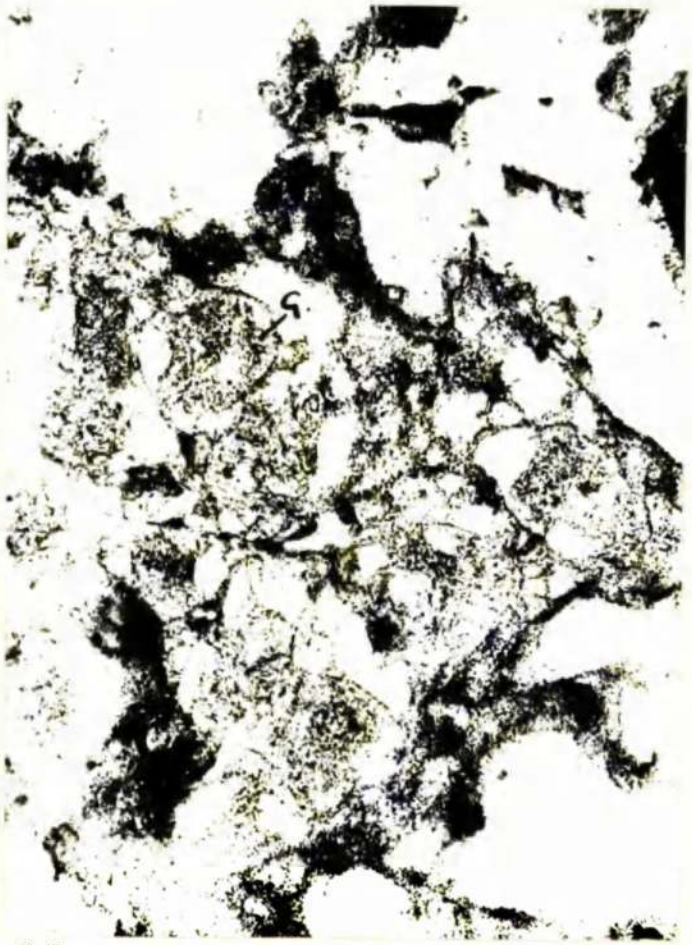
253

Fig. 254. G-6-P in the 13 day rabbit placenta, in:-

A) the multinucleate decidual cells (G.)

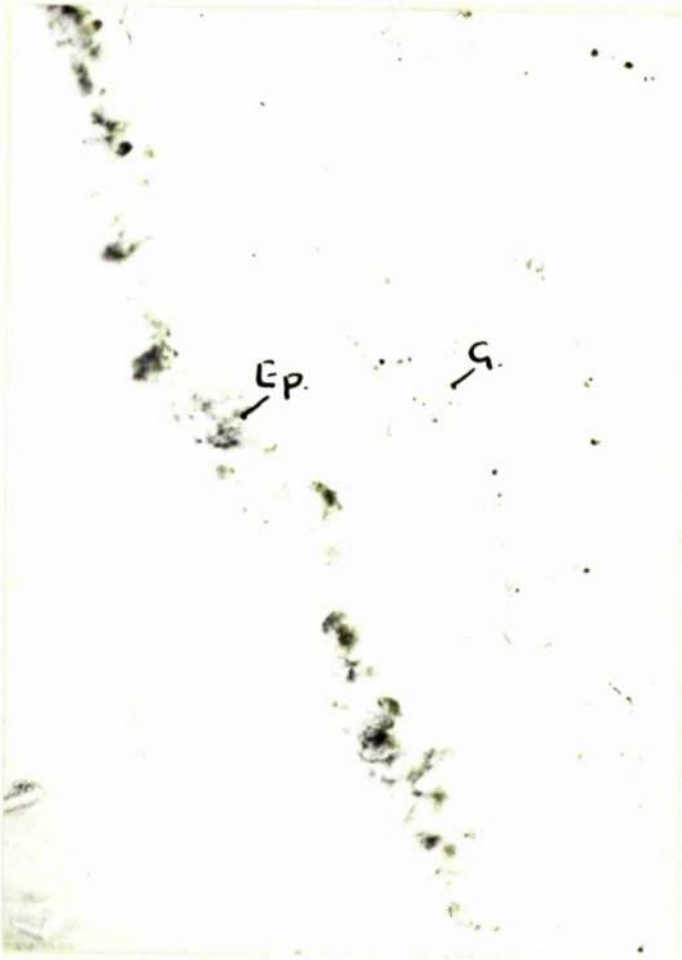
B) the decidual blood vessels (V.).

Fig. 255. GDH in the regenerating uterine epithelium related to the term rat placenta (Ep.). Some activity is also seen in the giant cells (G.).



254-A

254B



255

Fig. 256. Term guinea-pig placenta, showing:-

A) GDH in the antimesometrial epithelium.

B) SorbDH in the visceral endoderm

Fig. 257. Rabbit placenta, showing:-

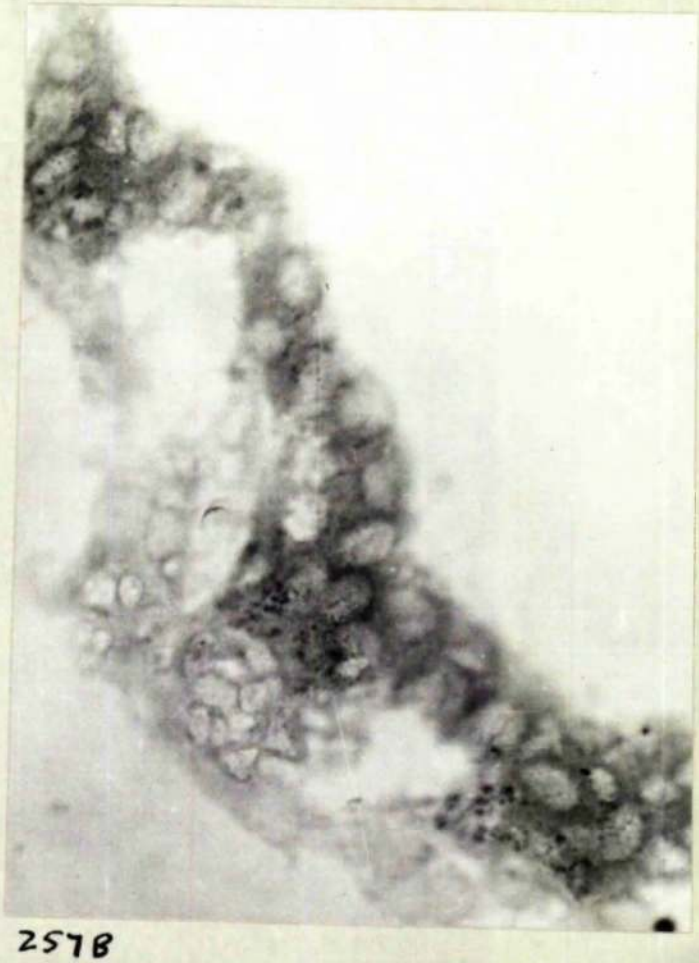
A) GDH in the cells of the separation zone, at term

B) FDH in the visceral endoderm at 13 days.

256A

256B

257A



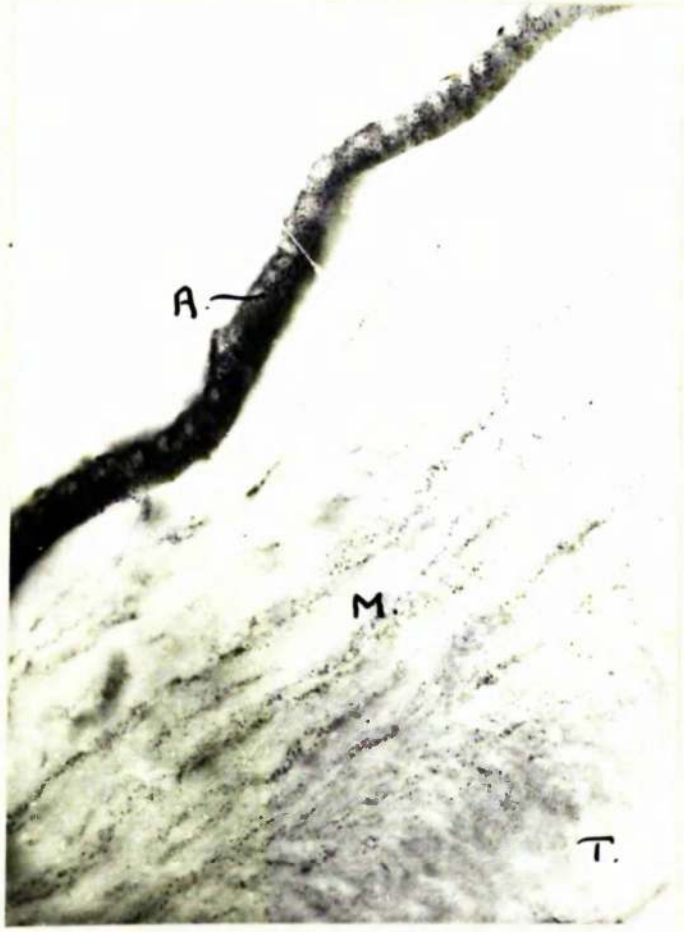
257B

Fig. 258. FDH in the amnion (A.), amniotic and chorionic mesenchymes (M.), and traces in the trophoblast (T.), of the human chorion laeve at term.

Fig. 259. 10 day incubation chick yolk sac, showing endodermal activity, with:-

A) FDH

B) βOH



258

259A

259B

Fig. 260. Yolk sac endoderm (E.) of Limia maculata, with:-

A) MDH

B) G-6-P

C) FDH, which also stains intensely the developing skeletal muscle of the embryo (S.).

Fig. 261. 1.5 cm. human yolk sac showing G-6-P in the endoderm.

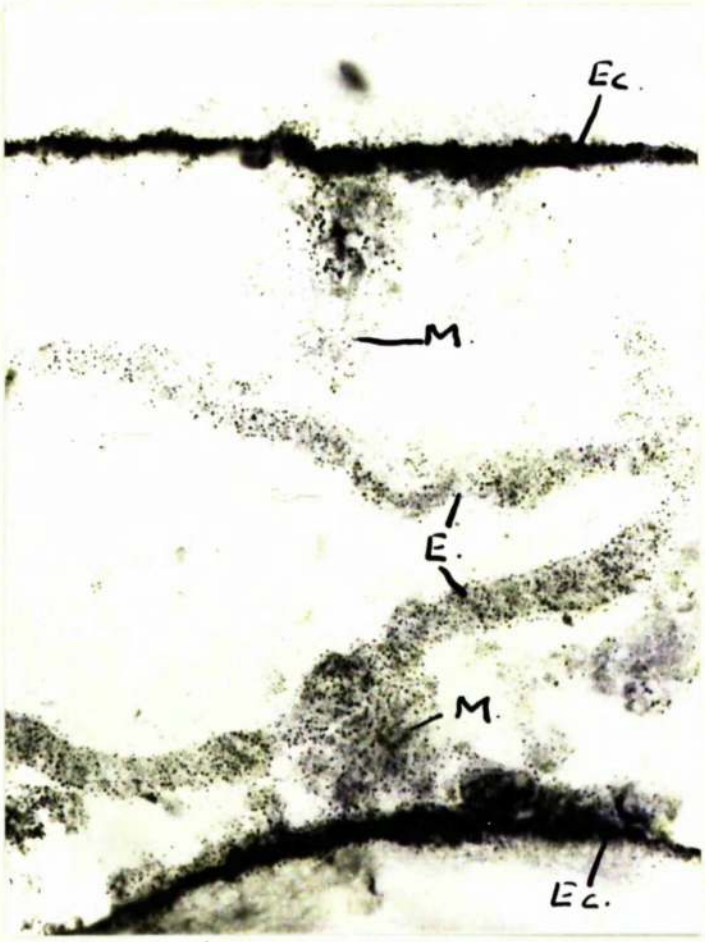
260A

260B

260C

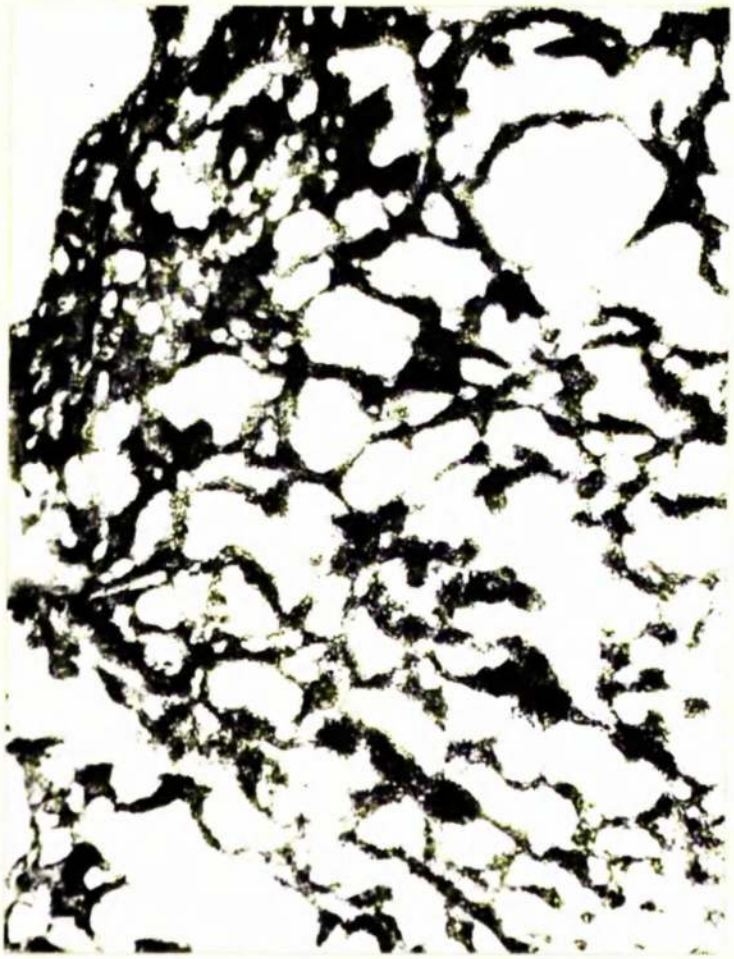
261

Fig. 262. Chorio-allantoic membrane of the chick, showing NADPH diaphorase in the ectoderm (Ec.), mesoderm (M.), and endoderm (E.).



262

Fig. 263. 20β-HSD in the trophoblast of the term horse placenta.



263

Fig. 264. Sheep placenta, showing HSD activity, with:-

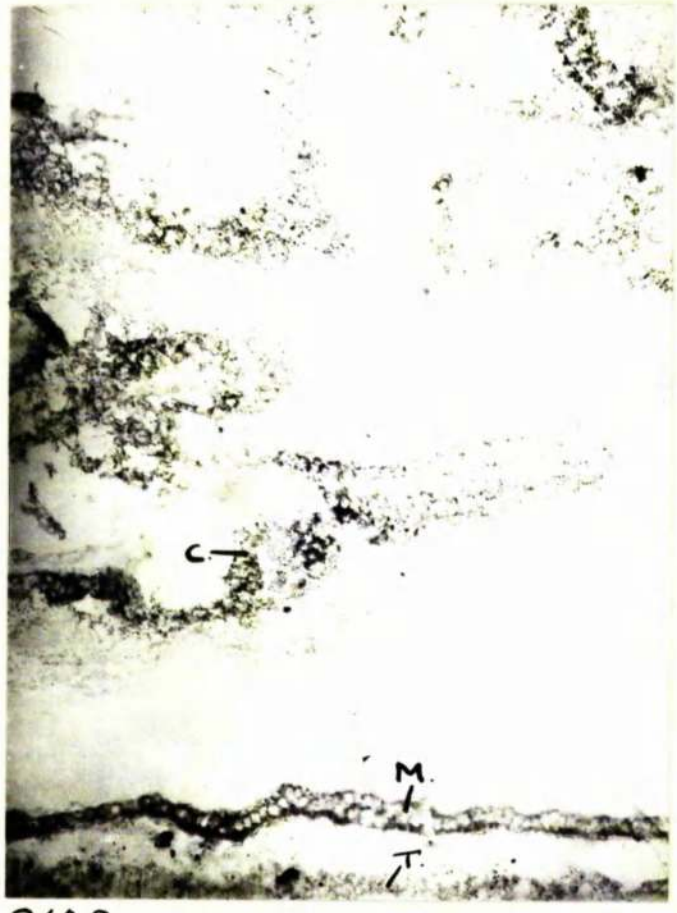
A) $\beta 3\beta$ at 15 cm. in the cytotrophoblast (C.), maternal epithelium (M.), and extra-cotyledonary trophoblast (T.).

B) 16β(androgen) at term, in the cytotrophoblast (C.), and syncytiotrophoblast (S.).

Fig. 265. Sheep intercotyledonary membranes, showing HSD activity, with:-

A) 16β(androgen) at 15 cm. in the trophoblast (T.), the maternal epithelium (M.) being negative.

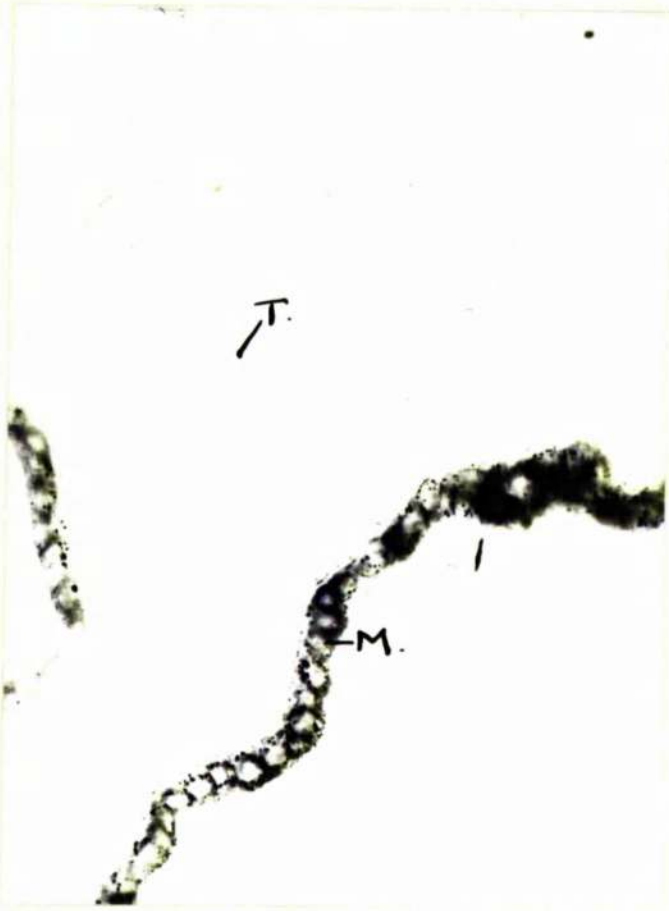
B) C-ol at term in the maternal epithelium (M.). The trophoblast (T.) is negative.



264A

264B

265A



265B

Fig. 266. C-ol activity in the thickened maternal epithelium (M.) of the ferret placenta.

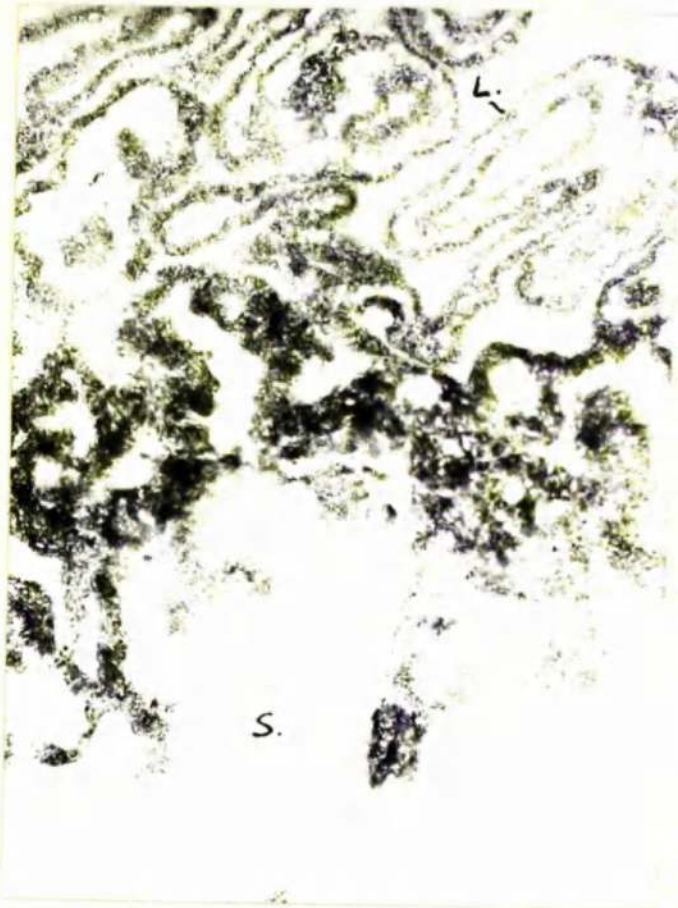
Fig. 267. 7 cm. cat, showing 16β(androgen)HSD activity in the labyrinth (L.) increasing near the base. The spongy zone (S.) is negative.

Fig. 268. 12.5 cm. cat, showing HSD activity in the yolk sac (Y.), and labyrinth (L.), with:-

A) testosterone

B) 16β(androgen)

266



267

268A

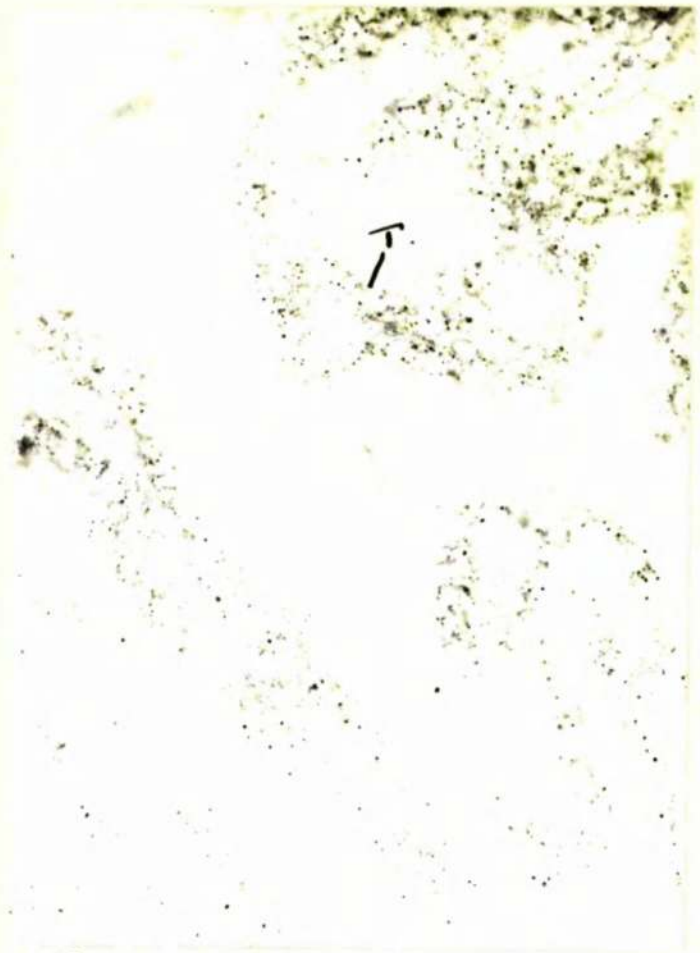
268B

Fig. 269. 12.5 cm. cat, showing C-ol activity in the labyrinthine trophoblast (T.).

Fig. 270. 12.5 cm. cat, showing testosterone HSD activity in the spongy zone (S.), and brown border trophoblast (T.).

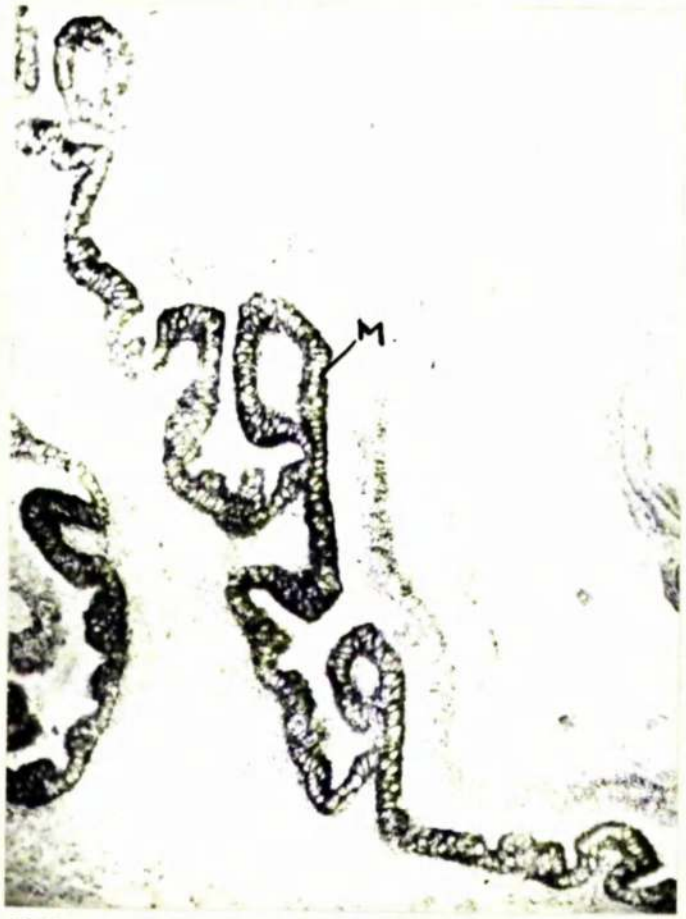
Fig. 271. 12.5 cm. cat, showing C-ol activity in the maternal epithelium (M.) of the brown border.

Fig. 272. 12.5 cm. cat, showing $\beta 3\beta$ activity in the brown border trophoblast (T.), and symplasma (S.).

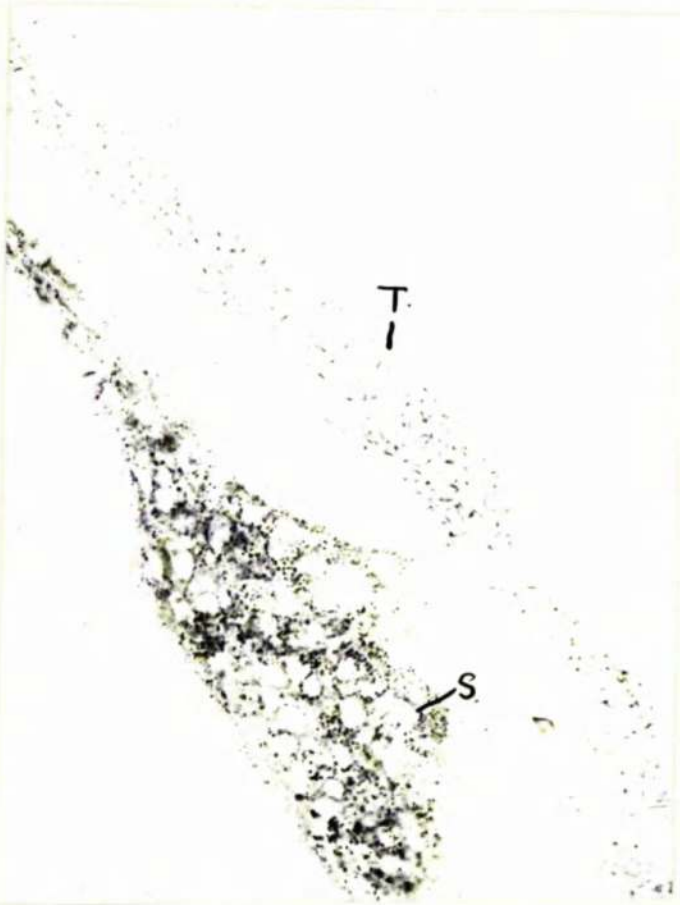


269

270



271



272

Fig. 273. HSD activity in the spongy zone of the dog placenta, with:-

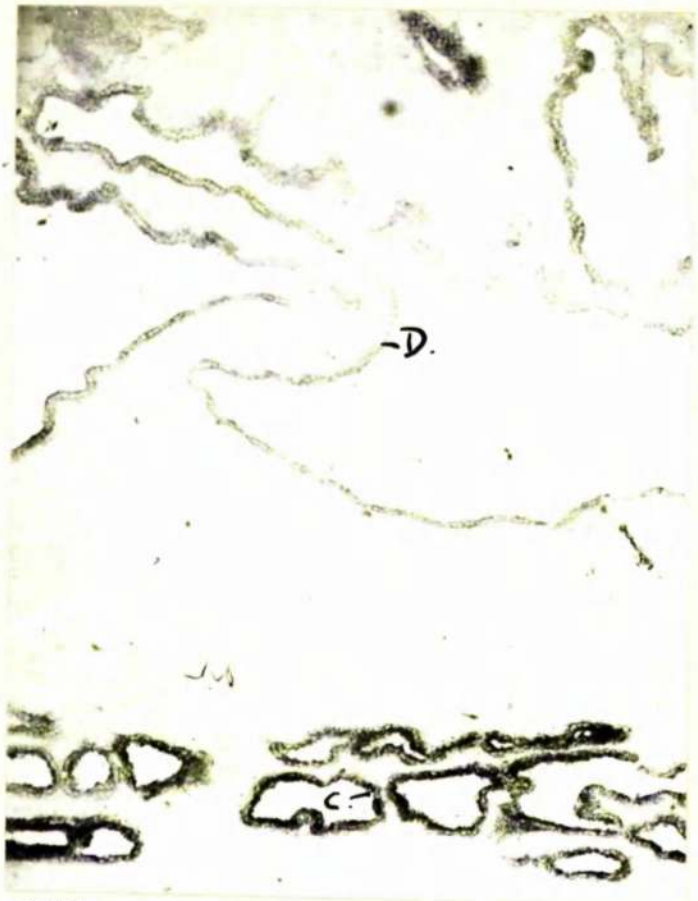
A) 16β(oestrogen) at 24 days. C - contracted part, and D - dilated part of the glands.

B) 16β(androgen) at 45 days, in the contracted part of the spongy zone glands.

Fig. 274. Ectoplacental cone and lateral giant cells of the 10½ day rat embryo.

A) 16β(oestrogen) activity in the cone (C.), and giant cells (G.)

B) 3β-HSD activity in the giant cells only (G.).



273 A

273 B

274 A

274 B

Fig. 275. 3β-HSD activity in the lateral (L.), and abembryonic (G.) giant cells of a similar specimen to fig. 274.

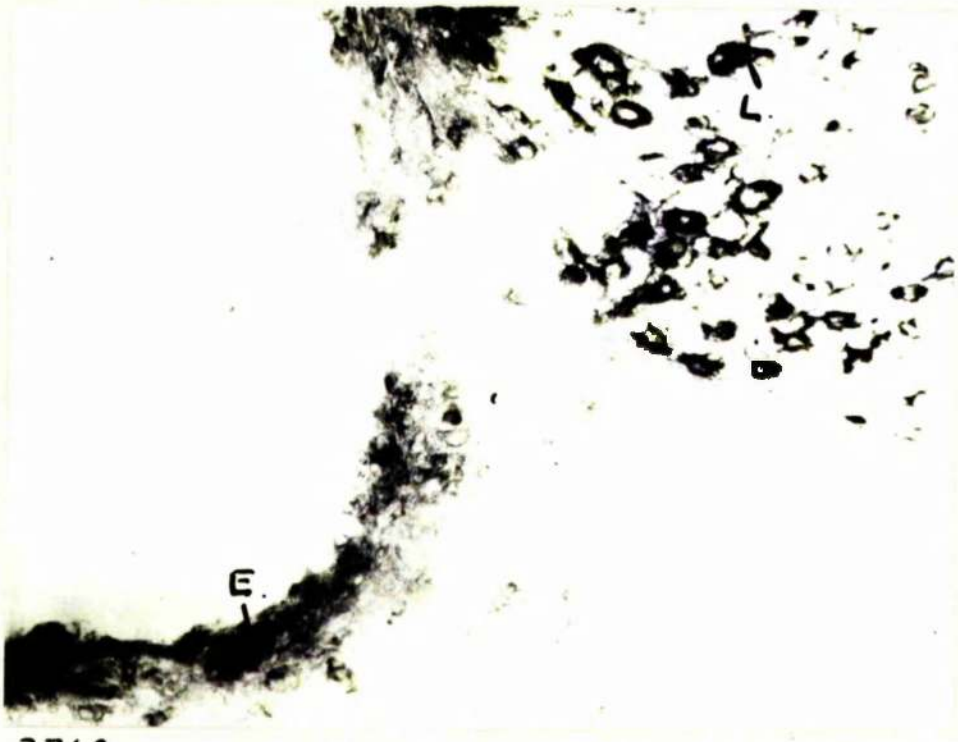
Fig. 276. Term rat placenta showing HSD activity, with:-

A) oestradiol, in the labyrinth (L.), and endovascular plasmodium (E.).

B) C-ol in the region of the fibrinoid capsule.

275

276B



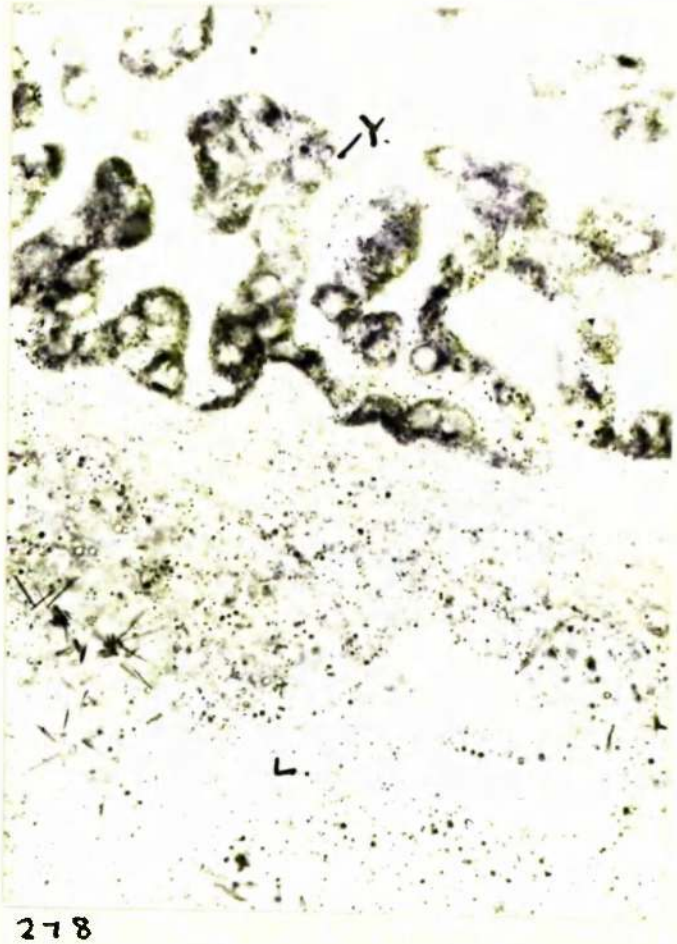
276A

Fig. 277. 20 day guinea-pig placenta, showing oestradiol HSD activity in the yolk sac (Y.), and cells in the decidua capsularis (D.).

Fig. 278. Term rabbit placenta, showing 16β(androgen)HSD activity in the yolk sac (Y.), and labyrinth (L.).

Fig. 279. 20 day rabbit placenta, showing 16β(androgen)HSD activity in the yolk sac (Y.), and multinucleate bodies (M.).

277



278

279

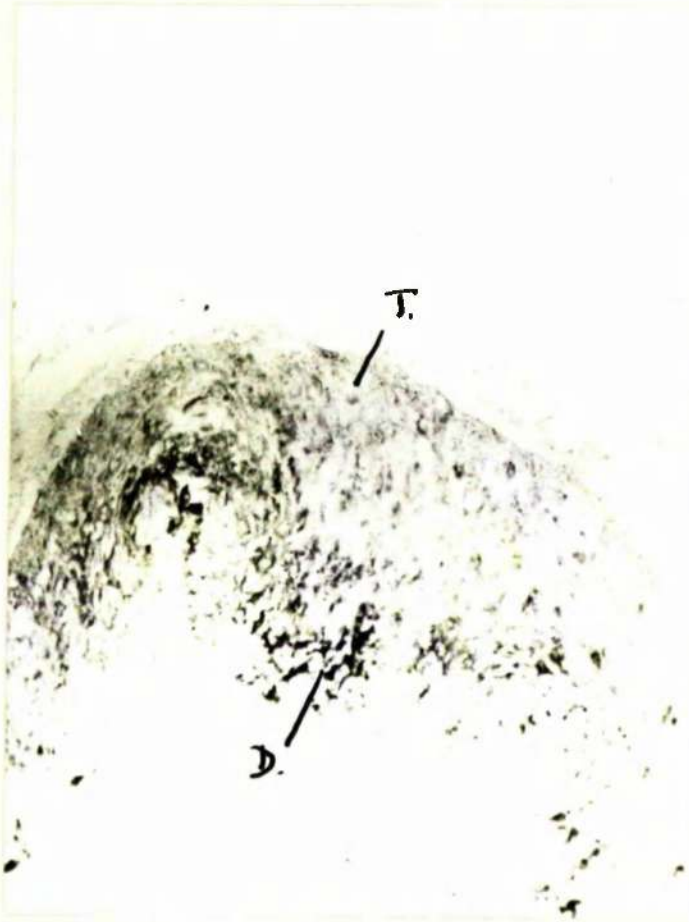
Fig. 280. Human term membranes, showing HSD activity, with:-

A) 3β in the trophoblast (T.).

B) 16β(androgen) in the trophoblast (T.), and decidua (D.).

C) oestradiol in the amnion (A.), and amniotic (M.), and chorionic (C.) mesenchymes.

A



B

C