A STUDY OF THE FOETAL MEMBRANES AND PLACENTA OF THE AFRICAN CANE RAT (THRYONOMYS SWINDERIANUS) WITH SOME OBSERVATIONS ON THE PLACENTATION IN THE ELEPHANT SHREWS - FAMILY MACROSCELIDIDAE

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A Thesis submitted in fulfilment for the Degree of Doctor of Philosophy of the University of Nairobi.

1979

DECLARATION

I, Dominic Oduor-Okelo, hereby declare that the work contained in this thesis is my original work and has never been submitted for a degree in any other University.

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SUMMARY

This thesis consists of two major studies (sub-theses) both of which are concerned with the female reproductive biology of two unrelated group of mammals which to date have not been extensively studies namely the cane rat, Thryonomys swinderianus Order Rodentia and three species of elephant shrews family Macroscelididae in the order Macroscelidea.

THE CANE RAT - (I. swinderianus) Suborder: Hystricomorpha

Although many studies have been done on the female reproductive biology of the guinea pig and several other species of the suborder Hystricomorpha in South America particularly in relation to the ovaries, foetal membranes and placental morphogenesis similar studies on the African members of this roden suborder are particularly lacking. Two articles by Asibey (1974a, b) dealt with the ecology and reproduction in the cane rat but no detailed macroscopic and microscopic studies were carried out on the reproductive organs.

The guinea pig and the New World hystricomorph rodents have many interesting reproductive characteristics such as the development of many accessory corpora lutea during pregnancy, variations in the relative importance of either the ovaries or placenta in the

production of progesterone for the maintainance of pregnancy; antimesometrial implantation, the absence of the parietal trophoblast and early inversion of the germ layers and the presence of a distinct structure in the placenta - the SUBPLACENTA. Fossil studies (Lavocat 1967) indicate a common ancestry for all the hystricomorph rodents.

This first part of the Thesis is therefore intended to assess the macroscopic and microscopic degree of similarity or dissimilarity in the ovarian, foetal membrane and placental structures between the Old World and the New World hystricomorph rodents. The results may be summarised as follows:
The cane rat resembles the New World hystricomorphs in several ways. During oestrum many follicles develop to Graafian follicle stage but only a few are ovulated and fertilised to develop to full-term foetuses. Secondary corpora lutea develop from many aborted follicles and there is extensive luteinization of the interstitial tissue cells. The chorio-allantoic placenta is haemochorial and is divided into labyrinthine and spongy zones and there is a well developed subplacenta.

The cane rat differs from the other hystrico-morph rodents in the following features: Implantation is mesometrial and secondarily interstitial. The

parietal trophoblast persists throughout pregnancy and in the later stages becomes vascularised by allantoic mesoderm to become a chorio-allantoic membrane (in other words there is no inversion of the germ layers). The chorio-allantois, like the amnion, is studded with many white pustules that do not contain glycogen granules. The umbilical cord contains five blood vessels-two umbilical arteries, one umbilical vein and two vitelline vessels, the latter representing the remnants of the yolk sac (like in man). The decidual reaction is remarkable in possessing numerous multinucleate giant cells that cause a considerable bulge mesometrially. These giant cells are found also in the mesometrium. A similar decidual reaction has been reported in only one other species of the sub-order - The African mole rat, Bathyergus janetta (Mossman and Luckett, 1968).

It is concluded that there is a need to re-examine the foetal membrane morphogenesis in the guinea pig and other hystricomorph rodents with a view to giving a new interpretation to the germ layers. The results of this study show a very close relationship between the Old World and New World hystricomorph rodents. The similarities of the major foetal membrane characters strongly suggest that the hystricomorph rodents evolved from a common ancestor.

THE ELEPHANT SHREWS - Order Macroscelidea

Family Macroscelididae

The literature on the macroscopic and microscopic studies of the female reproduction organs of the elephant shrews is very scanty. Of the 14 living species, only one, Elephantulus myurus has been extensively studied with regards to the morphology of the ovaries, foetal membranes and placenta.

The Macroscelididae is divided into two subfamilies of Rhynchocyoninae and Macroscelidinae on the basis of numerous and distinctive characters (Cobet and Hanks, 1968). These characteristics, however, do not include a comprehensive analysis of the foetal membranes and placenta of the two subfamilies.

The aim of the second part of this thesis is to study and compare the ovarian, the foetal membrane and placental architectures of Rhynchocyon chrysopygus, subfamily Rhynchocyoninae, genus Rhynchocyon and two members of the subfamily Macroscelidinae Petrodromus tetradactylus, genus Petrodromus and Elephantulus rufescens, genus Elephantulus.

The results of this investigation may be summarised as follows: Both Rhynchocyon and Petrodromu are strictly monovulators and give birth to only one offspring per pregnancy while elephantulus releases a maximum of two eggs and carries a maximum of two

foetuses, one in each uterine horn, per pregnancy. Implantation in all the three species is mesometrial and takes place in a uterine crypt, the embryonic chamber. The chorion is richly vascularised by allantois to form a chorio-allantoic membrane which comes into intimate association with the uterine epithelium to establish an epithelio-chorial placental relationship in several places. In all the three species the main placenta is haemochorial and is divisible into columnar, proliferative and spongy zones. There is a large and persistent yolk sac and a prominent allantoic vesicle. The umbilical cord contains only three blood vessels - two umbilical arteries and one umbilical vein.

Rhynchocyon, however, differs from the other genera in its basal placental zone. Here the trophoblastic cells become tall, columnar in appearance and are in contact with a distinct layer of maternal hyaline connective tissue that seems to act as a barrier to any further trophoblastic invasion. The other two genera have a degenerative zone below the spongy zone and their decidua basalis is dominated by the transformed smooth muscle cells in the wall of the coiled uterine artery forming a distinct structure designated as Mesoplacentarium by Starck (1949).

It is concluded that the observations in this study support previous findings in fossil records, osteological analysis, haemoglobin structure and social behaviour patterns which strongly suggest that the Macroscelididae be divided into two subfamilies and that the family deserves an independent order- Macroscelidea with no relationship to the order Insectivora.

ACKNOWLEDGEMENTS

This research was supported by Grant 670 - 092 of the Dean's Committee of the University of Nairobi.

I wish to express my most sincere appreciation to Professors E.C. Amoroso, S. Gombe and R. Tucker who have supervised and directed this research and to Dr. W. Maathai the head of Department of Veterinary Anatomy. I am grateful to Mr. Lawrence Brown and his family (and especially his son Roger) on whose farm the cane rats were trapped. I am also very grateful to Dr. G. Rathbun and Mr. Kashuru Mumba who gave me a large collection of placental material from the elephant shrews.

The technical assistance I received from Messrs. E. Njogu, J. Gichiri, H. Oloo, S. Paye, P. Osula, J. Akin, L. Akwech, F. Mwangi, A. Amukoye and J. Macharia (of the Department of Human Anatomy) is sincerely appreciated.

I extend my sincere gratitude to Mr. Mr. Charanjit S. Bambra who spared his time to help me in photography. My thanks are also due to Mr. D. Aliangana, of the Department of Animal Physiology and Mr. P.E. Siranga, a third year Veterinary student for their assistance.

A word of thanks to Mr. J. Githiomi who, as a senior technician in this department, gave a willing hand in the initial stages of this research. Mr. J. Githiomi has since left the Department.

I would like to express a special word of thanks to Mrs. Alice M. Gacheche for her patience during the typing of this script. Finally I wish to extend my gratitude to my parents, Mr. & Mrs. Okelo for their love and understanding throughout my educational career.

"Advances come more surely from errors which are clearly expressed than from a continuous repetition of the truth". Amoroso, 1976.

"Hypotheses are nets: only he who casts will catch" - Novalis

A. INTRODUCTION

1. EARLY THEORIES OF THE PLACENTA

The mammalian placenta has a most fascinating history dating back to antiquity and yet is one of the most puzzling structures in the eutherian mammalian body whose significance during pregnancy is not yet fully understood. Our present day knowledge of the placenta would not be complete if we do not consider past attitudes towards it. An account of the theories about the mammalian placenta is excellently treated by Needham (1959) and Amoroso (1970).

Among the Luos of Kenya the placenta is never buried immediately after birth. It is carried along with the baby to the dwelling house and kept on "tago" (a large piece of broken pottery) until early morning or late afternoon for burial. The burial place is always immediately beneath the eaves of the dwelling house-to the right (as one comes out of the house) in case of a male placenta and to the left in case of a female placenta. Male and female babies are similarly buried to the right and left of the house respectively when they die. A stone is always placed at the place where the placenta is buried (Were,

personal communication). Like many ancient communities, the placenta is regarded by the Luos as the child's nurse. They believe that the future of the new-born child depends to some extent on how the placenta is disposed of (Ogot, personal communication)

It is interesting to note that in ancient Greec the stone "omphalos", which was a cult-object in many temples, was probably intended to mark the buria place of the umbilical cord of the priest-king or Zeus (Needham, 1959). In ancient Egypt the Royal placenta was considered the "Pharaoh's secret Helper" and an effigy of the placenta of the reigning Pharaoh was carried before him during ceremonial processions (Needham, 1959; Amoroso, 1970). A similar custom prevailed among the Baganda of Uganda. Here, the actual placenta of the reigning Kabaka (King) was preserved and was usually carried by a high ranking officer during ceremonial processions (Amoroso 1970).

Fancies and superstitions apart, the role of the placenta in the nutrition of the embryo is not a recent innovation. Aristotle (384 - 322 B.C.) was already familiar with the placenta, the yolk sac and the umbilical cord in a number of animals and he ascribed a nutritional function to the blood vessels "which join to the uterus like the roots of plants and through them the embryo received its

nourishment". Students of the history of Placentology and Embryology will no doubt be familiar with the works of Galen (130 - 200 AD) who described the anatomy of allantois, amnion and placenta in the ruminants. He taught that the embryo excreted its urine into the allantois. He also maintained that the embryo respired through the umbilical cord and that the maternal and foetal blood circulations were continuous. Leonardo da Vinci (1452 - 1519), most of whose work must have been done on the foetal calf, while mistaking the human placenta to be cotyledonary like that of the cow, made the important conclusion that the foetal circulation was not continuous with that of the mother.

Andreas Vesalius (1514 - 1564) that most famous anatomist of all times, described the gross anatomy of the placenta well but thought that the human placenta was Zonary as can be seen from his drawings (Steven, 1970). He, like Galen before him, thought that the foetal and maternal circulation were continuous. Realdus Columbus (1516 - 1559) (who first introduced the term "placenta" into literature) described the human placenta as an affusion of residual matter in the form of a little cake - the placenta. Julius Caesar Arantius (1530 - 1589) introduced two very important concepts about the structure and function of the placenta:

a) he called the placenta "hepar uterinum" (i.e. uterine liver) and

b) he categorically denied the vascular continu between the foetal and maternal circulations especially in the pregnant cow, sow, ewe and bitch, a view which was in contradiction to the teachings of Galen and Vesalius.

Walter Needham (1631 - 1691), Hoboken (1623 - 1678) and John Mayow (1643 - 1679) confirmed the earlier speculations of Aristotle and Galen by establi· shing nutritional and respiratory functions of the placenta. Mayow went further and stated "For it is indeed probable that the spermatic portions of the uterus and its carunculae are naturally adapted for separating aerial particles from arterial blood. These observations premised, we maintain that the blood of the embryo, conveyed by the umbilical arteries to the placenta or uterine carunculae, brings not only nutritions juice, but along with this a portion of nitro-aerial particles to the foetus for its support, so that it seems that the blood of the infant is impregnated with nitro-aerial particles by its circu- lation through the umbilical vessels quite in the same way as in the pulmonary vessels. And therefore I think that the placenta should no longer be called a uterine liver but rather a uterine lung."

William Hunter (1718 - 1783), by a successful injection of the foetal circulation, finally settled the question of the seperate foetal and maternal circulations. His brother, John Hunter, concluded that the decidua was a product of the uterine mucosa and that the chorion was the essential organ of placentation. Baer (1828), with sufficient evidence, confirmed William Hunter's findings and concluded that the two opposing vascular beds were closely associated. He alluded to the possibility of gaseous and nutrient exchange between the two circulations at the placental site.

That the placenta might be concerned in the elaboration of an internal secretion was first suggested by Nattan-Larrier (1900) while Halban (1905) postulated that the internal secretion might be a hormone. Placental hormones have been isolated, purified and finally synthesised. These hormones have since been identified as pregnant mare's serum gonadotrophin (PMSG), Human chorionic gonadotrophin (HCG), progresterone and possibly oestrogens. The presence of a gonadotrophin has also been demonstrated in the urine of the pregnant giraffe (Wilkinson and de Fremery, 1940; Amoroso, 1955). More recently placental "lactogens" have been isolated from a variety of mammals including man, monkey, rats, mice, goats, sheep etc. (Josimovich and Brande, 1964;

McGarry and Beck 1973; Kelly, Robertson and Friesen 1974). Placental lactogens and the HCG are thought to have immunosuppressive qualities such that they may modify the immunologic competence of the maternal lymphocytes and could be important factors in preventing the rejection of the embryo during pregnancy (Contractor and Davies, 1973; Amoroso and Perry, 1975). It has also been suggested by Kelly et al. (1974) that placental lactogens may stimulate progesterone production.

The development of the microtome (which eliminated the vagaries of free hand sectioning) and the introduction of paraffin wax as an embedding medium by Wilhelm His (1874), enabled investigators to study the essentials of placental morphology. The basic pattern of circulation in the intervillous spaces of the human placenta was established and the nutritive role of trophoblast accepted. Although at the turn of this century most of the works on placentation were centered on the human, some noteworthy attempts have been made on the comparative studies on this field. Attempts have been made to classify various types of placentae and to use such classification for phylogenetic grouping of mammals. Earlier workers classified placentae on the basis of external shape. But such a classification proved to be of little phylogenetic significance; among the Carnivora, for instance, placental shapes vary from

zonary to simple discoid (Mossman, 1937). Moreover, the zonary type is found not only among the Carnivora but also among the Hyracoidea and Proboscidea, (species which have no taxonomic relations to the Carnivores). Baer (1828) and Robinson (1904) based their classification on the degree of union between the foetal villi and maternal mucosa. This classification was also of no phylogenetic physiological significance.

The introduction of new histological techniques and the improved resolution of the light microscope in the first half of this century led Grosser (1909, 1927) to formulate the general principles of placental classification based on fine structure. He laid emphasies on the thickness and constitution of the membrane seperating the maternal and foetal blood streams. Grosser thus distinguished four major placental types:

a) the epitheliochorial placenta
b) the syndesmo-chorial placenta
c) the endotheliochorial placenta and
d) the haemochorial placenta

To these four, two more types have since been added:

e) the haemoendothelial placenta found in the latter stages of pregnancy in the rabbit, rat and guinea pig (Mossman 1937) and

f) the endothelio-endothelial placenta found in shrews - Sorex araneus, S. vagrans, S. minutus, S. palustris, S. cinereus, Cryptotis parva and Blarina brevicauda (Mossman and Owers, 1963).

The Grosser classification has been widely used by those workers who have laid down the foundation on comparative mammalian placentation (Wislocki in a series of publications from 1920 to 1941; Mossman 1937; Amoroso 1952, 1955, 1959, 1961). But even the Grosser classification is not phylogenetically foolproof. Comparative studies have shown that within an order there can be all the varieties of placental forms. A few examples will surfice here: it is generally believed that among the primates, the haemochorial type of placenta prevails but the Lemur has an epitheliochorial placenta; the endotheliochorial placenta is widespread among the carnivores yet the hyaena has a haemochorial type (Wynn and Amoroso, 1964); among the rodents, the haemochorial type of placenta is widespread but Mossman (1937) suggested that in the later stages of gestation, the rabbit, rat and guinea pig have the haemo-endothelial type of placenta. It is also known that in some

species two placental may exist simultaneously. Thus in some Chiroptera like Miniopterus schreibersii, marginal endothelial areas supplement the main haemochorial placenta; (Amoroso 1952). Also in Galago demidovii, a primate endothelio-chorial area supplements the epitheliochorial type of placenta (Gerard 1932).

With these few examples it becomes clear that each mammalian species will have to be studied to determine the nature of its placental morphology. This, therefore, constituted one of the reasons for studying the placenta and foetal membranes of the African cane rat and the elephant shrews.

The variations in the types of placentae pose two interesting questions:-

a) Is any one type of placenta functionally more efficient than the others?
b) Is the epitheliochorial placenta phylogenetically more primitive than the haemochorial type?

It is known that the epitheliochorial placenta offers a barrier against large molecules. Hence the newborn of the mare, sow and cow receive their first dose of protective antibodies via colostrum. Amoroso (1952) has described the development of intra-epithelial capillary nets in the chorionic

ridges of the epithelio chorial placenta. He suggested that such an arrangement necessitated much closer contact between the maternal and foetal circulations. Such nets, he further pointed out, constituted the sites of transmission of the more difusable substances.

In those species with the haemochorial placenta, the young are born with full equipment of passive immunity. The route of transmission of the antibodies is either via the yolk sac splanchonopleure or across the chorio-allantoic placenta (Hemmingway and Brambell, 1961). In the carnivores and the elephant (Amoroso, 1952; Perry 1974), a characteristic feature of the placenta is the presence of marginal or central haematomas. Biggers and Creed (1962) and Creed and Biggers (1963) have proposed the term "haemophagous organ" for a similar structure found in the racoon placenta. These authors have subsequently described the haemophagous organ as being equivalent to the haemochorial condition and hence can allow for the passage of more complex substances. (These may include antibodies for passive immunity).

The second question is even more intriguing. Some authorities believe that the epitheliochorial placenta is the most primitive type because, among other things, it is found in the lizards and some marsupials e.g. Didelphys. Also a study of the

development of the haemochorial and endotheliochorial placenta reveals that they pass through stages which can be classified as epitheliochorial and syndesmochorial (Mossman 1937). There are those researchers, however, who think that the haemochorial placenta is the most primitive because:

a) it is found among the Insectivores, a group of mammals considered to be most primitive;

b) many of the most specialised mammals - Artiodactyla, Perisodactyla and Cetacea - have the epitheliochorial placenta. Mossman (1937) expressed the opinion that the modern epitheliochorial placenta of existing mammals was a secondary specialization or reversion. All in all, it is clear that each placental type is efficient for the particular species.

Recent development in histochemical techniques and the advent of the electron microscope in the 1950s have contributed immensely to our knowledge and understanding of the functions and fine structure of the mammalian placenta (Wislocki, Deane and Dempsey, 1946; Wislocki and Dempsey 1945, 1946; Davies, Dempsey and Amoroso 1961 a, b; Enders, 1965; Amoroso and Perry 1975). These workers have not only confirmed the nutritive role of the placental trophoblast, but have also added new information on its

endocrine activities. They have produced suggestive evidence on the protective role of the trophoblast against maternal rejection. We owe a great deal of our knowledge on researches which have been carried out on domesticated species but more so on the laboratory rodents and lagomorphs. The endocrine role of the placenta poses some interesting questions especially its relation with the ovary.

It has been found, for instance, that even within a mammalian family the importance of these two organs during pregnancy varies considerably. In the goat for example, the ovaries produce up to 10 mg. of progesterone daily while the placenta produces none. In the sheep on the other hand, the ovaries produce approximately 2.0 mg. of progesterone daily while the placenta produces up to 14 mg. daily (Linzell and Heap, 1968). Among the laboratory rodents the ovary is necessary for the maintenance of pregnancy throughout most of gestation in the golden hamster, mouse, rat and rabbit while in the guinea pig pregnancy can proceed normally in the absence of the ovary after 30 days post coitus (Amoroso, 1955; Illingworth and Challis 1973). It has recently been shown that among the hystricomorph rodents (a group of rodents which have as one of their most outstanding placental characteristics, the presence of the "subplacenta") the guinea pig and the cuis can

carry live young to term after bilateral ovariectomy. Illingworth and Challis (1973) have shown that the guinea pig placenta produces progesterone. On the other hand the chinchilla, viscacha and casiragua will abort after bilateral ovariectomy (Weir and Rowlands 1974). These three latter species are also characterised by the development, during pregnancy, of numerous accessory corpora lutea in their ovaries. The hystricomorph rodents are widespread in both the New and the Old Worlds. One of them, the guinea pig has been used extensively in the laboratories for numerous experiments many of which are related to the placenta and the ovaries. There has been a total lack of research on the reproductive biology of the Old World hystricomorph.

II. THE SCOPE OF THIS THESIS

It has already been pointed out that the literature contains no description of the foetal membranes or placenta of either:

a) the African cane rat, Thryonomys swinderianus, in the suborder Hystricomorpha; or

b) the elephant shrews namely Rhynchocyon chrysopygus, Petrodromus tetradactylus and Elephantulus rufescens, the three species representing three genera in the family Macroscelididae. The two groups of animals i.e. the cane rat on the one hand

and the elephant shrews on the other, are interesting both from the taxonomic and phylogenetic points of view.

The phylogenetic relationship between the New World hystricomorphs - also referred to as caviomorphs - and the Old World hystricomorphs - also referred to as phiomorphs - has been a subject of considerable debate. In an extensive study of the fossil records of Miocene rodents of Kenya, Lavocat (1967) found that the middle ear of Diamantomys was very similar to that of the present-day African Thryonomys and the South American Lagostomus. Other osteological and dental studies have led him to suggest that the Old and New World hystricomorph had a common ancestral stock living in the Middle and Early Eocene in Africa (Lavocat 1974). Wood (1974), however, contested strongly against Lavocats' theory. He pointed out that the primitive caviomorphs had four crested upper and lower molars with clearly distinguishable cusps and normal replacement of deciduous teeth. The primitive phiomorph on the other hand, had five crested molars and deciduous teeth that were usually not replaced. He further stated that the only known Eocene rodent which could possibly have been ancestral to hystricognathous groups are from U.S.A. and Mexico. He therefore concluded that the two groups had evolved independently in the Old and New Worlds since the late Palaeocene or early Eocene.

Mossman (1937) has stated that the morphogenesis and placental characters of the foetal membranes can be used to throw light on the phylogenetic relationships between various groups since these characters have been least affected by environmental changes. Extensive studies have been carried on the foetal membranes and placenta of the guinea pig and many New World hystricomorph rodents and some characters peculiar to this rodent suborder have been spelt out namely:-

a) An unusually long gestation period ranging from 53 days in the cuis, Galea mustaloides to 283 days in paracana, Dinomys branickii (Weir, 1974);
b) complete interstitial implantation;
c) early and complete inversion of the germ layers;
d) amniogenesis by cavitation;
e) the presence of a subplacenta originating from the chorionic ectoderm;
f) division of the trophoblastic area into labyrinthine and spongy zones (Amoroso, 1952);
g) the decidua basalis which is the maternal component of the placenta.

Among the African species of this suborder no studies have been reported on their foetal membrane and placental morphology except for a brief article by Mossman and Luckett (1968) on the foetal membranes of the African mole rat, Bathyergus janetta, which they described as being similar to those of the New World hystricomorphs. They also reported the unusual and rapid hypertrophy of the basal decidua and the giant cells derived from it.

It is the intention in the first part of this Thesis to study the morphology of the foetal membranes and the placenta of the cane rat, Thryonomys swinderianus and compare the findings with those of the guinea pig and other S. American hystricomorphs and to relate these findings to the possible phylogenetic relationship between these groups of rodents.

The elephant shrews, on the other hand pose a different problem. The elephant shrews, family Macroscelididae, are confined to the African continent but their taxonomic position is yet unresolved. They have been classified as insectivores by some researchers and with the primates by others. According to Patterson (1965) fossil records reveal that the Myohyrax oswaldi and Protypotheroides beetzi belonging to the extinct subfamily Myohyracinae and the Mylomygale spiersi of South Africa Pleistocene

period had hypsodont molars very similar to the present-day Macroscelides proboscideus. The same author has also drawn attention to the similarity of the mandible (which lacks the third molar tooth) of Metoldobotes stromeri from the Egyptian Oligocene to those of Petrodromus and Rhynchocyon. He concluded, therefore that on the basis of evidence available the elephant shrew don't seem to have any common ancestry with the Insectivores or with the tree shrews.

The family Macroscelididae has been split into two major subfamilies namely Rhynchocyoninae with one living genus - Rhynchocyon and Macroscelidinae with four living genera. Cobert and Hanks (1968) have listed thirty characters which justify the seperation into the two subfamilies, eight of which are listed below:-

Some diagnostic characters of the two subfamilies of Macroscelididae

(after Cobert and Hanks, 196

Character	Rhynchocyoninae	Macroscelidin
1. Post anal gland	Present	Absent
2. Skeleton of proboscis	partly ossified	wholly carti lagenous
3. Post orbital process	present	absent
4. Upper incisors	absent/rudi-mentary	Present and functional
5. Upper canines	very large	small
6. Ulna	thick through-out	distal half rudimentary
7. Carpal apad	absent	present
8. Uterus	slightly bicornuate	deeply bicornuate

In the second part of this thesis it is the intention to study and compare the foetal membrane and placental morphology of the two subfamilies using Rhynchocyon chrysopygus to represent the subfamily Rhynchocyoninae and Petrodromus tetradactylus (Genus: Petrodromus) and Elephantulus rufescens (Genus: Elephantulus) to represent the subfamily Macroscelidinae. It is also intended to compare the overall findings on Macroscelididae with those which have been described for the tree shrews (Family Tupaidae).

III. LITERATURE REVIEW OF PLACENTA AND FOETAL MEMBRANES IN HYSTRICOMORPH RODENTS

Most of the early literature on the development and structure of the placenta and foetal membranes of the rodent suborder Hystricomorpha relate to the domestic guinea pig, Cavia porcellus (Duval, 1892; Sansom and Hill, 1931; Mossman, 1937, who cited the earlier literature beginning with Bischoff 1842; and Amoroso, 1952). Other hystricomorph rodents whose placental morphology have been studied include the agouti, Dasyprocta aguti, by Strahl (1905) and Becher (1920, 21); the Canadian porcupine, Erethizon dorsatum, by Perrotta (1959); the chinchilla, Chinchilla laniger, by Tibbitts and Hillemann (1959) and by King and Tibbitts (1976); the coypu, Myocastor coypus, by Hillemann and Gaynor (1961); and the plains viscacha, Lagostomus maximus, by Roberts and Weir (1973). More recently Roberts and Perry (1974) have given a comprehensive account of the placenta and foetal membranes in a number of South American hystricomorph rodents, the development of which resembles in several particulars those described for the guinea pig.

1. Implantation in Hystricomorphs

The mode and location of implantation varies considerably with species and especially among the rodents. In those species where multiple conceptions

are common the whole uterus seems to be equally favourable for implantation with the embryos being spaced at equal intervals from each other. It has been suggested that the embryos exhibit spacetaking properties such that after an embryo strikes some sort of a physiological contact with the uterine mucosa, its immediate neighbourhood is rendered refractory to other embryos which must pass on (Mossman 1937; Glasser 1972), reminiscent of changes in the zone pellucida following the ingress of one spermatozoon. Boving (1956) has also suggested that the interaction of forces originated by neighbouring blastocysts may contribute to the equal spacing of the embryos. In mammals with only one conceptus per horn the spacing is with reference to the uterus; for instance in the agouti where only one conceptus is present in each horn, the sites of implantation are symmetrically near the middle of each horn. In the mountain viscacha, Lagidium peruanum, the only hystricomorph rodent that is known to be monovular, the implantation site is localised and is invariably mesometrial, just a short distance above the cornual junction (Wimsatt 1975).

Before implanting itself, the blastocyst of most hystricomorph rodents occupies a rounded bay or a "little nest" (Reichert, 1862) at the antimesometrial extremity of the uterus with the abembryonal trophoblast orientated towards the uterine epithelium. In the guinea pig Blandau (1949 a & b) has described the appearance of pseudopodial processes at the abembryonal pole of the blastocyst. These pseudopodia become more numerous and penetrate the zona pellucida. The zona pellucida gradually disappears. The pseudopodia eventually form an attachment cone which penetrates the uterine epithelium to establish a metabolic relationship with the uterine epithelial cells. In all hystricomorph rodents so far studied, with the exception of the mountain Viscacha, the first attachment of the blastocyst to the uterus is reported to be abembryonic and antimesometrial. The timing of implantation varies from species to species but ranges from 5½ days post coitus in the chinchilla to 18½ days post coitus in the plains viscacha (this appears to be the longest recorded for any rodent, Roberts and Weir, 1973; Roberts and Perry, 1974).

The mode of implantation is well documented for the guinea pig and is of the highly specialised complete interstitial type (Sansom and Hill 1931;

Amoroso 1952; Boyd and Hamilton (1952). Implantation in the cuis, casiragua, degu, chinchilla and plains viscacha have also been described by Roberts (1973) as completely interstitial. In the Canadian porcupine, however, Perrotta (1959) suggested that due to the size of an attached blastocyst, implantation is incompletely interstitial. In the guinea pig, the blastocyst by its own inherent activity embeds itself in the uterine mucosa by penetrating directly through the epithelium; its abembryonic pole constituting the "implantation pole" (Von Spee 1901, Sansom and Hill 1931, Blandau 1949b).

2. Early Post Implantation Development

The early post implantation development of the guinea pig has been described by Duval (1892), Sansom & Hill (1931), and Amoroso (1952). Roberts (1973) has studied the early post-implantation development in the cuis, casiragua, degu, chinchilla, coypu and viscacha. These rodents share some interesting specialisation with the guinea pig. After implantation the blastocyst becomes tubular with its long axis perpendicular to the long axis of the uterus. Due to the growth of the endoderm, the blastocyst elongates into a cylindrical structure (the egg cylinder). The parietal trophoblast disintegrates so that the visceral yolk sac endoderm

becomes directly apposed to the uterine tissue - there is early inversion of the germ layers with no evidence of a parietal yolk sac wall. The amnio-embryonic vesicle is separated from the Trager by the intermniotic or proexocoelomic cavity (Amoroso 1952; Roberts and Perry, 1974) and the new cavity now occupied by the egg-cylinder is called the decidual cavity. Roberts and Perry (1974) reported that abembryonic pole of the blastocyst is composed of ectoplacental trophoblast enclosing an ectoplacental cavity. They further reported that in the chinchilla, coypu and viscacha this cavity is frequently filled with extravasated maternal blood. With the complete inversion of the germ layers, the endoderm comes to form the outer wall of the embryonal formation and is directly bathed by nutritive fluid in the decidual cavity. The endoderm thus plays an important role in the early nutrition of the embryo (Sansom & Hill 1931, Amoroso 1952).

Amoroso (1952) has carefully recorded the postimplantation events that take place in the guinea pig uterus. He says that as the blastodermic vesicle grows the roof of the implantation cavity - the decidua capsularis, comes into contact and fuses with the mesometrial mucosa thus obliterating the uterine lumen on about the 10th day post coitus. A new

lumen later appears antimesometrially on about the 15th day post coitus followed by a rapid thinning and disintegration of the intervening tissue so that the yolk sac eventually comes to lie in the new uterine lumen. The blood vessels of the necrotic zone are opened and blood zoozes freely into the implantation cavity.

3. Development of the Placenta

The chorio-allantoic placenta in the guinea pig and all the other hystricomorph rodents develops mesometrially. It is formed by the growth of a solid mass of richly vascularised allantoic mesoderm with no endodermal cavity (compare mouse). This solid extraembryonic stalk of mesoderm projects into the exocoelomic cavity and eventually becomes applied to the mesoderm underlying the thickened part of the placental trophoblast. The vascular allantoic mesenchyme spreads over the chorionic surface which thus becomes vascularised to constitute the chorio-allantoic placenta. The trophoblast continues to advance deeply into the decidua while at the same time it is being penetrated on the embryonic side by outgrowths of mesoderm containing branches of allantoic vessels. The extensive branching of allantoic vessels gives rise to the lobate appearance of the definitive placenta (Harman and Prickett, 1932; Amoroso 1952; Roberts and Perry, 1974).

In this way a complex labyrinth is formed consistint of two sets of closely paralleled tubes namely:

a) trophoblastic tubules composed of fine-meshed syncytial trophoblast enclosing maternal blood and,
b) the allantoic capillaries ensheathed in delicate and discontinuous foetal mesenchyme (Amoroso 1952).

Roberts and Perry (1974), without giving an adequate bibliographic reference, have quoted a report by Contreras (1965) on the presence of two chorio-allantoic placental discs for each embryo in the tuco tuco. This interesting finding, although it has been confirmed by Roberts (1973), has not been recorded for any other hystricomorph rodent.

4. The Subplacenta

The subplacenta is a structure of considerable size and in the guinea pig lies at the bottom of the central excavation. It has been studied extensively in the guinea pig by Amoroso (1952), Davies, Dempsey & Amoroso, (1961 a & b) and in the chinchilla by Tibbitts & Hillemann (1959) and King & Tibbitts (1976). Its presence has been recorded in all the hystricomorph rodents that have been studied. Unlike the spongy zone which it adjoins on its

foetal side, its cells are closely packed in ovoid groups with few blood sinuses. The presence of PAS-positive material within the subplacenta has been recorded in the guinea pig by Davies et al. (1961 a), and in the cuis, casiragua, chinchilla and Viscacha by Roberts (1973). It is a structure which appears to be peculiar to the hystricomorph rodents.

5. The decidua

The Order Rodentia exhibits some of the most interesting variations and specializations in the morphology, amount and position of the decidual tissue. In the rabbit there is a relatively large amount of decidua consisting of three types of cells namely unicellular, large vesicular multinucleate cells and "myometrial gland" cells. Most rodents have, however, relatively little decidual tissue in the full term placenta. As already mentioned, very little information is available on the decidual reaction in the African hystricomorph rodents except for a brief report by Mossman and Luckett (1968) on the African mole rat, Bathyergus janetta.

6, The Junctional zone

The junctional zone forming the foeta-maternal contact region has been described in the

guinea pig (Amoroso 1952) and the South American hystricomorphs (Roberts and Perry, 1974) as being composed largely of necrotic material derived from the decidual tissue.

7. The Foetal Membranes

a) The Amnion: The amnion in all hystricomorphs so far studied is formed by cavitation and throughout gestation it appears to be avascular (Roberts and Perry, 1974) although Tibbitts and Hillemann (1959) have reported the presence of primordial blood cells in the somatic mesenchyme of the chinchilla amnion. The presence of white rounded pustules on the surface of the amnion has been reported only in one species - the plains viscacha, L. maximus (Roberts and Perry 1974). These authors stated that histologically the pustules consisted of a series of coiled layers of loose fibrous tissue with small, scattered, deeply staining nuclei. No vascular elements were present within them.

b) The Yolk Sac Placenta

Mossman (1937) stated that in the guinea pig and possibly in man, the first intimate apposition between the blastocyst and uterine mucosa is made at the unilaminar stage thus

forming a definite non-vascular yolk sac placenta. Later when the area vasculosa spreads and vascularises the bilaminar omphalopleure the result is a chorio-vitelline placenta.

It has been alleged by a number of researchers that a peculiarity of the guinea pig yolk sac is that its lower wall never develops and in the area of the placental pole its cavity becomes lined with the ectoplacental trophoblast instead of the endoderm and the sinus terminalis is especially very prominent. After the dissintegration of decidua capsularis the visceral yolk sac. endoderm comes into contact with the uterine epithelium. It soon becomes vascularised by the splanchnic mesoderm and thus plays an important role in the nutrition of the embryo (Amoroso 1952; Roberts and Perry 1974).

IV. LITERATURE REVIEW OF PLACENTATION IN THE ELEPHANT SHREWS, MACROSCELIDIDAE

Unlike the hystricomorph rodents reviewed above, the elephant shrews are unique to the African continent (Kingdon, 1974). Van der Horst & Gillman (1942) reported that E. myurus is a polyovulator, releasing up to 120 eggs per ovulation. Yet only two eggs can develop beyond the four cell-stage and be carried to term. They further stated that in E. myurus the four cell stage egg implants at a restricted area in the caudal part of each horn, the embryo chamber, thus bypassing the morula stage. Tripp (1970), while confirming the polyovular characteristic of E. myurus, did not agree with Van der Horst (1950) that the fertilized eggs lose their zona pellucida as they enter the oviduct. Similar disagreement was also expressed by Amoroso (personal communication). Amniogenesis is by cavitation and the yolk sac is in intimate contact with the actively secreting bed of uterine glands during the early stages of development before the establishment of chorio-allantoic placenta (Van der Horst, 1950). Later, the yolk sac is completely separated from the chorion by the appearance and extension of the exocoelom. Van der Horst also noted that while the main placenta, which is haemochorial, is the dominant organ of nutrition for the embryo, it is

supplemented by obplacental areas in which the chorion is vascularised by allantoic mesoderm thus establishing a "rudimentary secondary chorionic placenta". Van der Horst (1950) has described the definitive placenta of E. myurus in great depth and recognised three distinct zones:

a) a columnar zone consisting of trophoblastic syncytium separating maternal blood from the allantois vessels;

b) a spongy zone comprising the greater part of the placenta and made up of a maze of relatively broad cords of syncytial trophoblast enclosing maternal blood spaces but lacking allantoic vessels;

c) An anchoring or attachment zone restricted to a narrow area related to the placental arteries and veins.

In the early stages of placenta formation, he also described a well defined zone of darkly staining aggregates of cells lying between the columnar and spongy zones. He termed this "zone of proliferation". This zone disappears in the later stages of pregnancy. Starck (1949) made similar observations on the placental architecture of M. proboscideus. But he also described the presence of a "mesoplacentarium" in the subplacental region of

M. proboscideus, in which there is considerable hypertrophy of the cells in the wall of the coiled artery and the whole gland-like structure is surrounded by "mesoplacentarial epithelium."

The literature contains no adequate description of the placenta and foetal membranes of the golden-rumped elephant shrew, Rhynchocyon chrysopygus (known as Njule in Kiswahili); the four-toed elephant shrew, Petrodromus tetradactylus (Isanje in Kiswahili) and the short nosed elephant shrew, Elephantulus rufescens (Sengi in Kiswahili).

Fig. 1a

Adult female cane rat, Thryonomys swinderianus.

Fig. 1b

Adult female elephant shrew, Rhynchocyon chrysopygus.

1a

1b

V. MATERIALS AND METHODS

i) ANIMALS:

A total of 13 female cane rats and 15 female elephant shrews were used in this study. The cane rats were trapped in Mr. Lawrence Brown's farm at Athi river, 30 Km. Southeast of Nairobi, between December 1975 and April 1977 (see Table 1). The cane rats move in pairs, usually male and female and occasionally both male anf female were trapped together. Many times the animals avoided the traps and this made it difficult to catch them in large numbers. There was also a communications problem and often a trapped animal would be one or two days dead before it was brought to the laboratory.

The elephant shrews were obtained from two different locations by Dr. G. Rathbun between April 1971 and January 1976. The golden rump elephant shrew, R. chrysopygus and the four toed shrew P. tetradactylus were trapped in the Gedi Ruins, a 44 - hectare National Historic Monument 20 Km. South of Malindi on the coast of Kenya; while the short nosed elephant shrews E. rufescens were trapped at Bushwahacker's, a holiday camp 20 Km. northeast of Kibwezi on the west bank of the Athi River within the Tsavo National Park. For details of vegetation and rainfall patterns and the distribution of these animals in both areas, Rathbun

(1976) should be consulted.

ii) PROCESSING OF MATERIAL FOR MICROSCOPY

The placental tissues were fixed in either Bouin's or 4% formalin solutions, dehydrated through ascending grades of alcohol and embedded in paraplast. Five to seven micron thick sections were stained in haematoxylin and eosin (H & E), PAS, alcian blue and Mason's Trichone or Goldener stains. When fresh materials were available, small pieces of placental tissues were fixed in formaldehyde - gluteraldehyde-trinitro cresol solution (FGC), post fixed in osmium tetroxide (Ito and Karnovosky, 1968), dehydrated through ascending grades of alcohol and embedded in Epon-Araldite. For light microscopy, sections of 2 to 3 microns were cut with glass knives on a Sorvall Porter-Blum ultramicrotome MTI. and stained with toluidine blue. Thinner sections were stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and examined with Zeiss EM 9 A electron microscope.

The date of capture, the number of conceptuses per uterine horn and the number of corpora lutea per ovary were all recorded and tabulated (Table I & II).

The sizes of the cells were measured using a calibrated eyepiece graticule at the magnification of X 10 and the resultant readings multiplied by a constant figure of 0.08 to convert to microns according to a method described by Neaves (personal communication).

SOME REPRODUCTIVE FEATURES OF THE CANE RAT

Animal No. & Sex	Date and weight in Kg.	Reprod. state	No. Rt ovary	Cl. Lft. ovary	No. of foetuses Rt. uterine Horn	Lft. uterine Horn	Wt. of foetus in gr.	C-R length of foetus in cm.
CR 1(F)	20.12.75 (2.9)	Pregnant (late)	5	6	0	3	60	12.4
CR 2(F)	20.12.75 (2.5)	Preg. (V. early)	2	2	2	0	blastocyst	
CR 3(F)	6.1.76 (2.7)	Pregnant	6	5	3	1	2.7 gr	27
CR 4(M)	6.1.76	Mature	-	-	-	-	-	-
CR 5(F)	22.4.76 (2.6)	Pregnant	3	5	2	1	1.8	2.4
CR 6(F)	27.4.76 (2.6)	Pregnant	3	2	1	1	Fixed whole	-
CR 7(F)	12.8.76 (0.7)	Not pregnant	-	-	-	-	-	-
CR 8(M)	12.8.76 (3.0)	Mature	-	-	-	-	-	-
CR 9(M)	13.8.76 (0.7)	Young	-	-	-	-	-	-
CR 10(F)	13.8.76 (0.6)	Not pregnant	-	-	-	-	-	-

Animal No. & Sex	Date and weight in Kg.	Reprod. state	No. Rt. Ovary
CR 11(M)	5.10.76 (3.4)	Adult	-
CR 12(F)	5.10.76 (2.3)	Pregnant	7
CR 13(F)	5.1.77 (2.4)	Pregnant	6
CR 14(F)	15.1.77 (3.0)	Pregnant	3
CR 15(F)	19.1.77 (1.5)	Not pregnant	-
CR 16(F)	25.1.77 (2.2)	Not pregnant	-
CR 17(M)	6.5.77 (3.3)	Adult	-
		Mean	1.37
		SEM	±0.64
Mean	2.62		
SEM	±0.08		

Cl. Lft. ovary	No. of foetuses Rt. uterine Horn	Lft. uterine Horn	Wt. of foetus in. gr.	C-R length of foetus in cm.
-	-	-	-	-
2	2	1	Blastocyst	-
3	3	0	Early preg. (fixed whole)	-
5	1	2	41.2	9.3
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
3.75 ±0.58	2.00 ±0.30	1.50 ±0.33		

CR	=	cane rat
F	=	female
M	=	male
Rt.	=	right
Lft.	=	left
C-R	=	crown-rump
CL	=	corpus luteum

TABLE 2

SOME REPRODUCTIVE FEATURES OF ELEPHANT SHREWS

A. Rhynchocyon

Date & No.	No. Corpora lutea		No. conceptuses	
	Right ovary	Left ovary	Right uterine Horn	Left uterine Horn
31.7.71 342	1	0	1	0
3.8.71 365	1	0	1	0
12.2.72 414	0	1	0	1
5.8.72 483	1	0	1	0
5.8.72 484	0	1	0	1
7.10.76 506	0	1	0	1
26.7.76 552	1	0	1	0
26.7.76 553	1	0	1	0
Mean ±SE	1.00 ±0.00	1.00 ±0.00	1.00 ± 0.00	1.00 ± 0.00

B. Petrodromus

Date & No.		No. corpora lutea		No. Conceptuses	
		Right ovary	Left ovary	Right uterine horn	Left uterine horn
3.8.71	361	1	0	1	0
3.8.71	362	1	0	1	0
4.8.71	367	0	1	0	1
4.8.71	368	1	0	1	0
5.8.71	381	0	1	1	0
5.8.71	383	0	1	0	1
23.4.72	434	1	0	1	0
23.4.72	480	1	0	1	0
Mean ± SEM		1.0 ± 0	1.0 ± 0	1.0 ± 0	1.0 ± 0

C. Elephantulus

Date & No.	No. corpora lutea		No. Conceptuses	
	Right ovary	Left ovary	Right uterine horn	Left uterine horn
20.7.74 543	1	1	11	1
1.8.74 550	2	0	1	1
10.12.74 593	1	1	1	1
12.12.74 596	1	0	1	0
15.12.74 598	0	1	0	1
10.4.76 603	1	1	1	1
13.7.76 607	1	0	1	0
Mean ± SEM	1.16 ± 0.14	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00

"Advances come more surely from errors which are clearly expressed than from a continuous repetition of the truth". Amoroso, 1976.

VI. RESULTS

The results are described in two parts: Part one deals with the marsh cane rat, T. swinderianus. and Part two deals with the elephant shrews.

PART I. THE MARSH CANE RAT (T. swinderianus)

1. HABITS AND HABITAT

The cane rats live in reed beds and grassy areas in valleys and along river banks and lake shores They feed mainly on roots and shoots of such plants as elephant grass (which are plentiful along the banks of Athi River especially during the rainy season). When, during the dry periods, the river dries up and grass becomes scarce, the animals migrate to places where water is still found. The adult male cane rat weighed an average of 3.5 Kg. while the adult female weighed between 1.5 and 2.5 Kg.

Although pregnant females were not obtained every month of the year, the variations in the ages of conceptuses, even those caught within the same month, were such that breeding throughout the year was indicated (Table 1).

2. THE EXTERNAL GENITALIA

In both the non-pregnant and the pregnant female the external genitalia was not easily visible, although the urinary papilla and the anal openings were distinct (Fig. 2). The vagina opened through a transverse slit between the urinary papilla and the anus (Fig. 2) and it was closed by a thin membrane during pregnancy and in immature females. This membrane disintegrates at parturation and for a few days at oestrus.

3. THE OVARIES

The ovaries, which were only partially enclosed by the ovarian bursa, were ovoid to pear-shaped and lay dorso-lateral to the kidneys. In non pregnant females, both ovaries were about equal in size. They measured between 1.2 to 1.5 cm in length and about 0.5 cm across their greatest width. The ovaries weighed about 0.5 g. Mature follicles that were about to rupture were appreciably visible on the surface. The corpora lutea did not protrude above the ovarian surface.

HISTOLOGY OF THE OVARY

The ovary at oestrus:

In the Cane rat both ovaries are functional (Table I). Cane rat 15 was a non-pregnant mature female. The vaginal closure membrane was absent and histologically both ovaries had many large and medium sized follicles (Fig. 3a). This animal was, therefore, presumed to be in oestrus. Numerous primordial follicles were embedded in the outer region of the zona parenchymatosa beneath the tunica albuginea (Fig. 3b). The secondary and tertiary follicles lay deeper in the ovary (Fig. 3a) and were scattered throughout the substance of the ovary.

Both secondary and ripe follicles were surrounded by a relatively thick, highly vascularised theca interna, whose cells were as yet undifferentiated (Fig. 3c). The interstitial gland tissue was abundant and was subdivided into masses of varying sizes by thin septal of stromal tissue. Many of these masses contained remnants of zona pellucidae thus indicating that these interstitial gland cells are derived from relatively undifferentiated theca interna cells of atretic follicles. These glandular masses were well vascularised (Fig. 3d).

Fig. 2

Gross specimen of a female cane rat to show the external genitalia.

a = anus; p = urinary papilla.

The vaginal opening is indicated by the arrow.

Fig. 3a

A low power photomicrograph of a cane rat ovary at oestrus showing numerous follicles scattered in the substance of the ovary.

H & E Stain. X 10.

2

3a

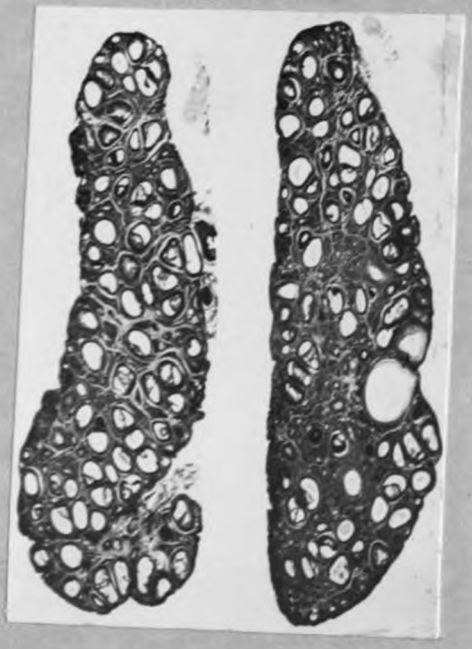
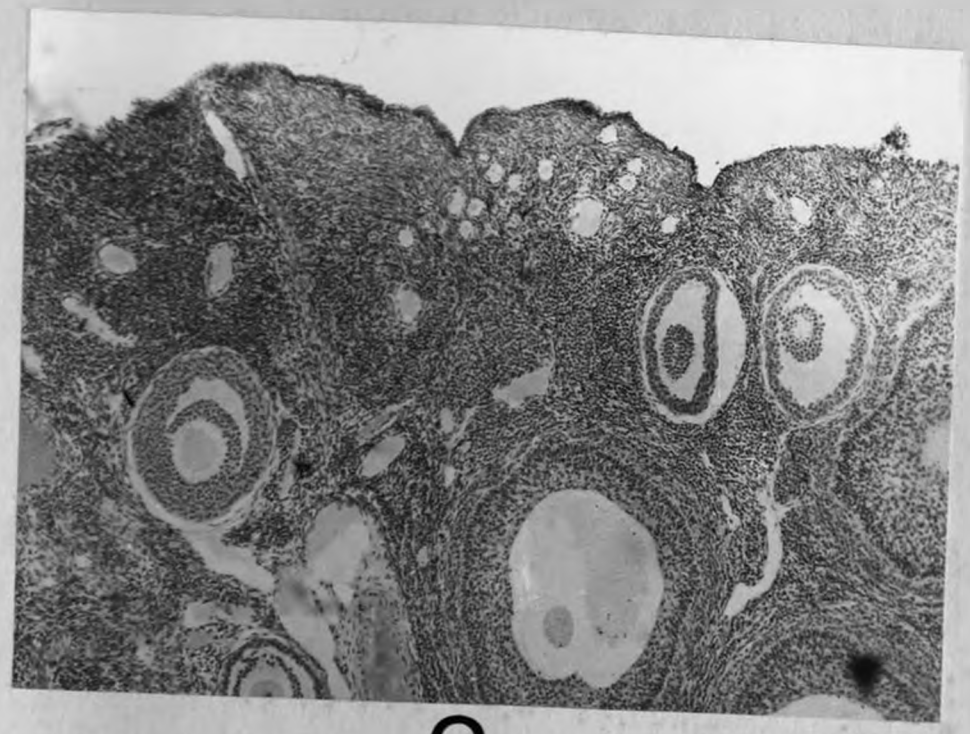


Fig. 3b

A high power section of the cane rat ovary at oestrus showing many primary germ cells, secondary and tertiary follicles.
H & E. stain X 75.

Fig. 3c

A section of the cane rat ovary at oestrus. Note the undifferentiated but well vascularised theca interna.
H & E. stain. X 200.



3b

3c

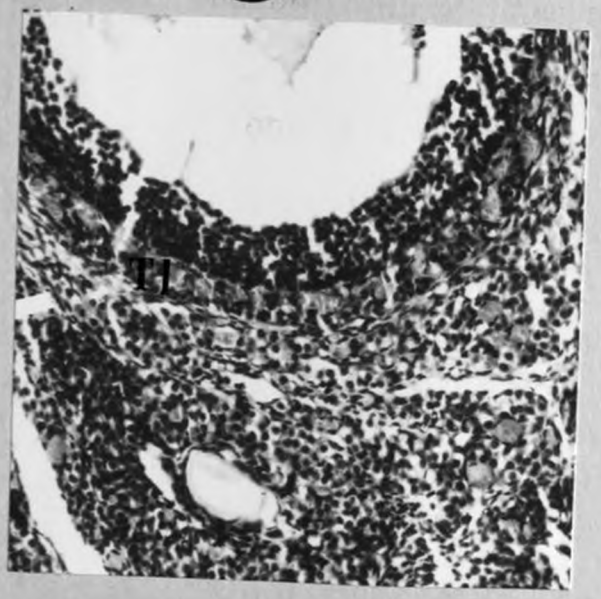
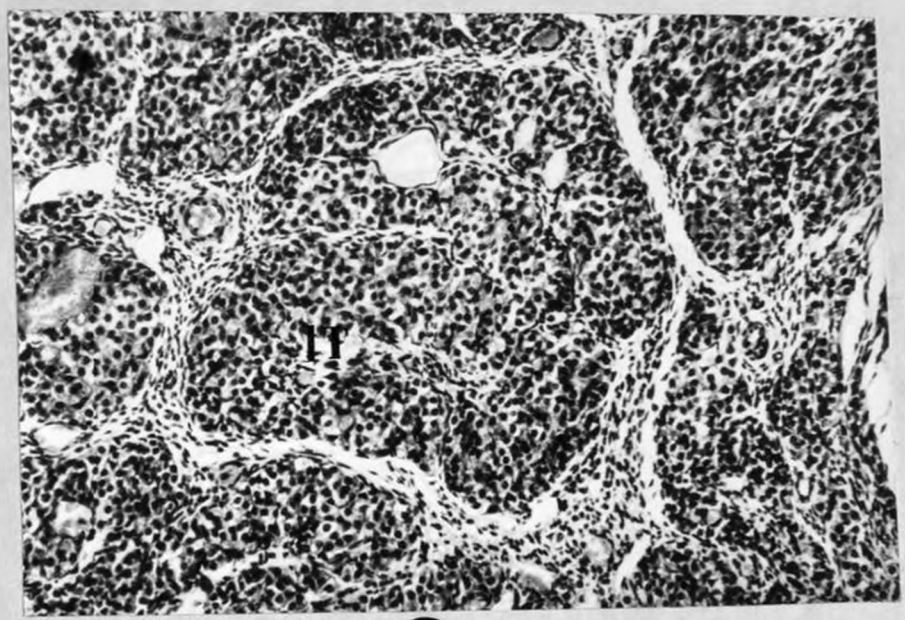


Fig. 3 d

A section of the cane rat ovary at oestrus showing the interstitial tissue (I.T.) arrangement.

H & E. X 200.



3d

The ovary during pregnancy:

A conspicuous feature of the Cane rat ovary during pregnancy was the presence of many atretic follicles. These atretic follicles were distinguished by the presence of remnants of the zona pellucida (Fig. 4). In some of these the granulosa cells were partially luteinised to give rise to many secondary corpora lutea (Fig. 4 & 5).

In all the pregnant Cane rats studied there were always more large primary corpora lutea in both ovaries than the number of conceptuses present in the uterine horns. The distribution of these corpora lutea in each ovary was not often consistent with the number of embryos in the ipsilateral uterine horn. Their distribution is illustrated in Fig. 6a and Table I. During pregnancy the theca interna was several layers thick; the cells had undergone considerable hypertrophy and were fully differentiated (Fig. 6b). The theca interna cells were distinctly more eosinophilic than the corpora luteal cells.

In the very late stages of pregnancy as represented by Cane rat 1 (foetus was fully furred, weighed 60 gms. with a crown-rump length of 12.4 cm) the luteinisation included also the stromal cells and in some instances it was difficult to define the

boundary between one corpus luteum and another (Fig. 6c). The theca interna in this late stage of pregnancy appeared to grow larger while the actual corpus luteum diminished in size (Fig. 6d). The thickness of the theca interna increased from an average width of 75 u in Cane rat 12 which had 3 embryos in the blastocyst stage to an average of 258 u in Cane rat I.

Postpartum ovary:

The postpartum ovary was found in Cane rat 7. This animal had recently given birth as evidenced by the presence of milk in the mammary glands. The vaginal closure membrane was present. Histologically the ovaries had many primary follicles but a few large secondary and tertiary follicles.

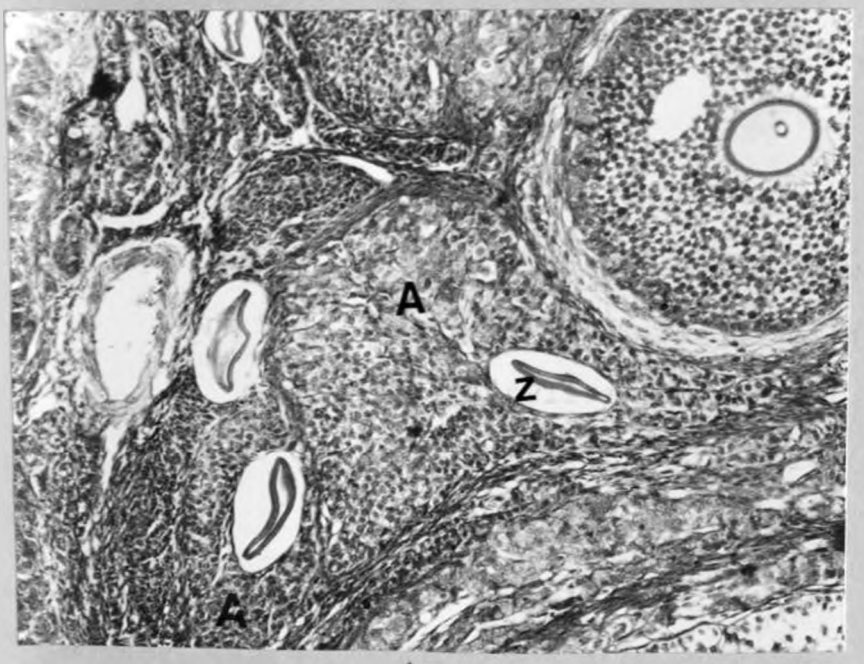
The most striking feature of these ovaries was the extensive amount of interstitial gland tissue. In these ovaries whatever was not a follicle was interstitial gland tissue. Compared to the interstitial gland tissue of pregnant animals, those of postpartum appeared to have undergone dedifferentiation and were very similar both in size and appearance to the theca interna cells (Fig. 7a, & b).

Fig. 4

A section of the cane rat ovary during pregnancy showing atretic follicles (A). Note the luteinization of these follicles and the remnants of the zonae pellucidae (Z).
H & E. X 200.

Fig. 5

A section of the ovary during pregnancy. Note the extensive luteinization of the interstitial tissue. It = intersititial tissue
cl = corpus luteum.



4

5

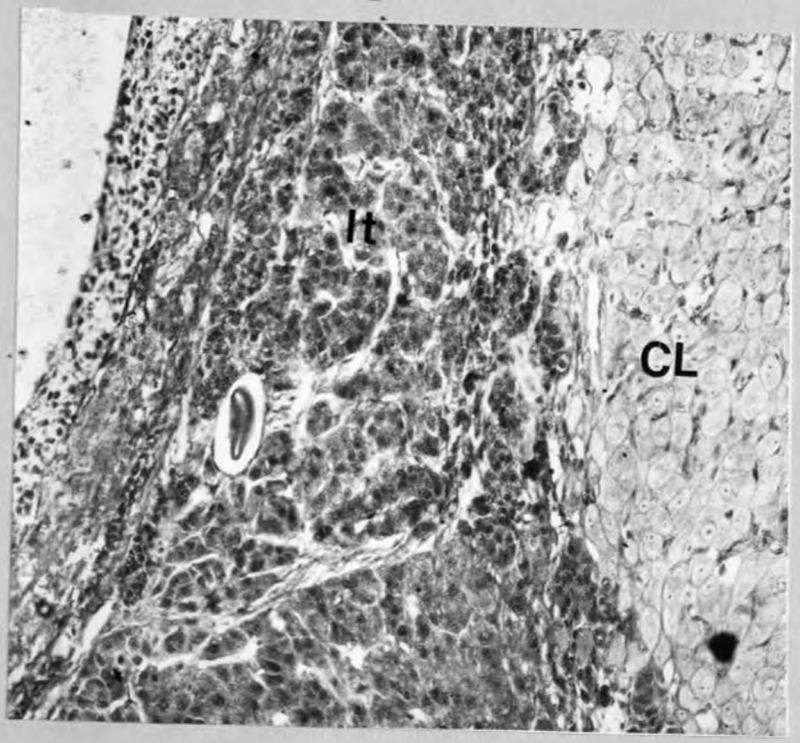
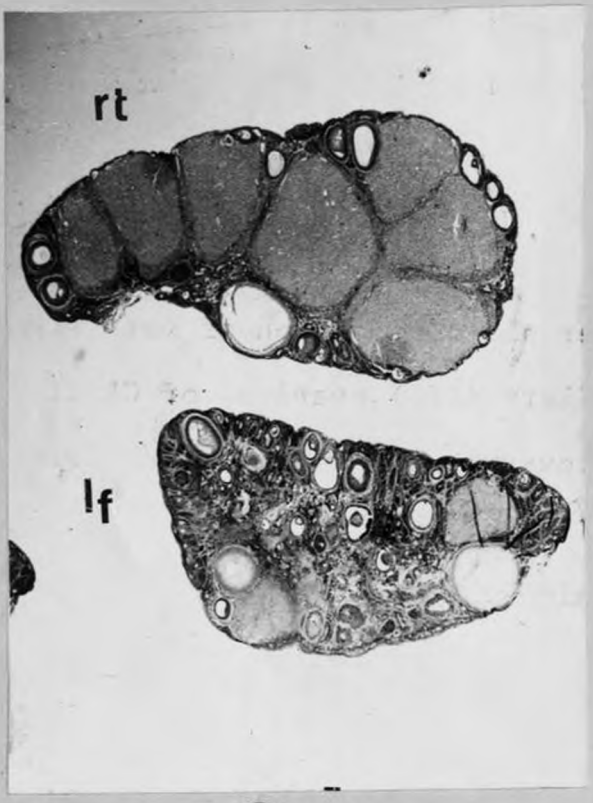


Fig. 6a

A low power photomicrograph of both right (rt.) and left (lf.) ovaries of CR 12. The right ovary has 7 cl while the left ovary has 2 cl (see arrows).
H & E. stain X 10.

Fig. 6b

A section of the cane rat ovary during pregnancy showing a portion of the corpus luteum (CL) and the differentiated theca interna cells (TI).
H & E. X 200.



6a

6b

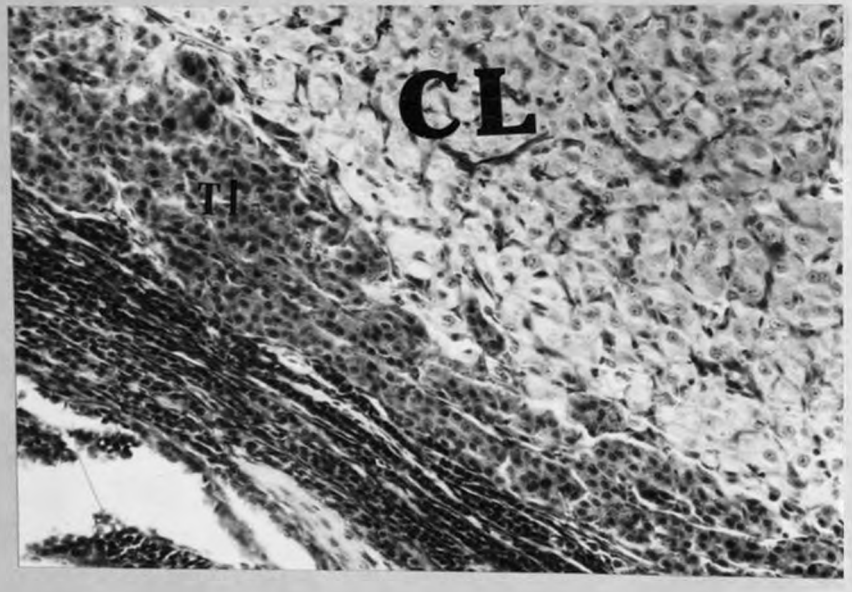
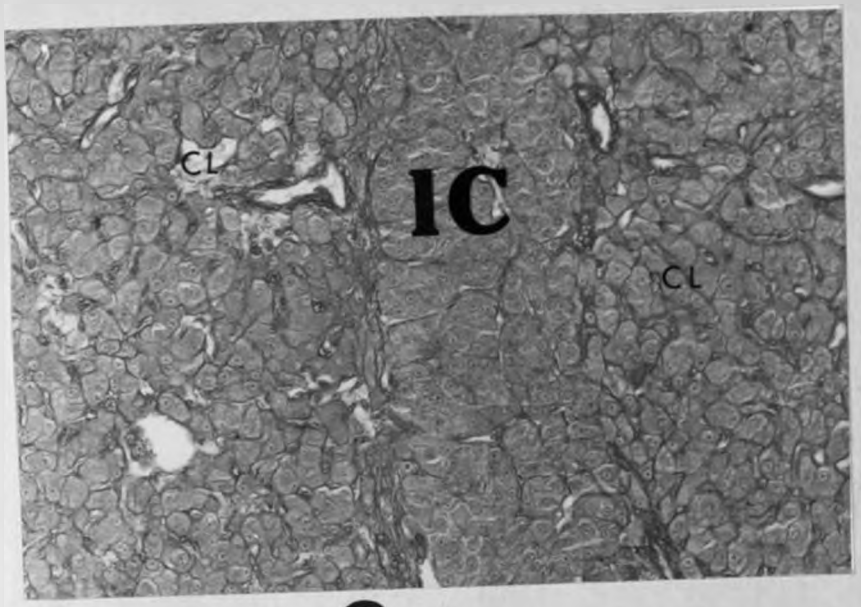


Fig. 6c

A section of the cane-rat ovary during pregnancy. Note the luteinisation of the interstitial cells (IC) intervening between the two corpora lutea (CL).
PAS-stain. X 200.

Fig. 6d

A section of the cane rat ovary towards the end of pregnancy. Note the increase in amount of the theca interna cells (TI) and the concomittant decrease in the size of the corpus luteum (CL).
H & E. X 75.



6c

6d

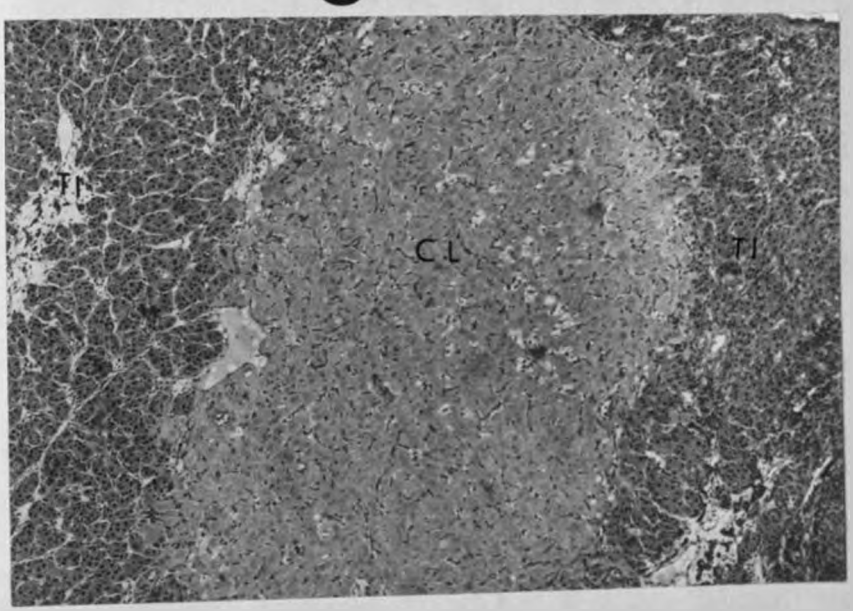


Fig. 7a

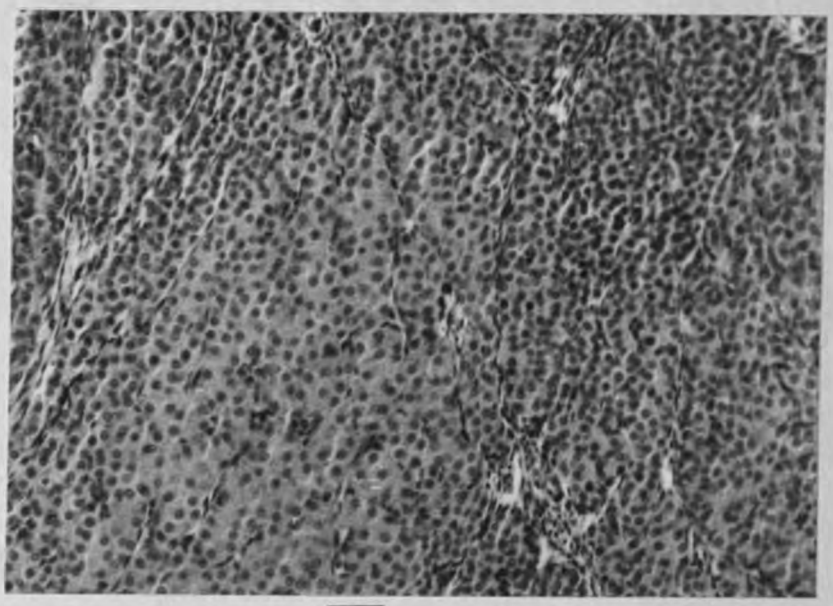
A section of the post-partum cane rat ovary to show the increased amount of interstitial gland tissue. Note that the cells seem to have dedifferentiated.

H & E. stain X 200.

Fig. 7b

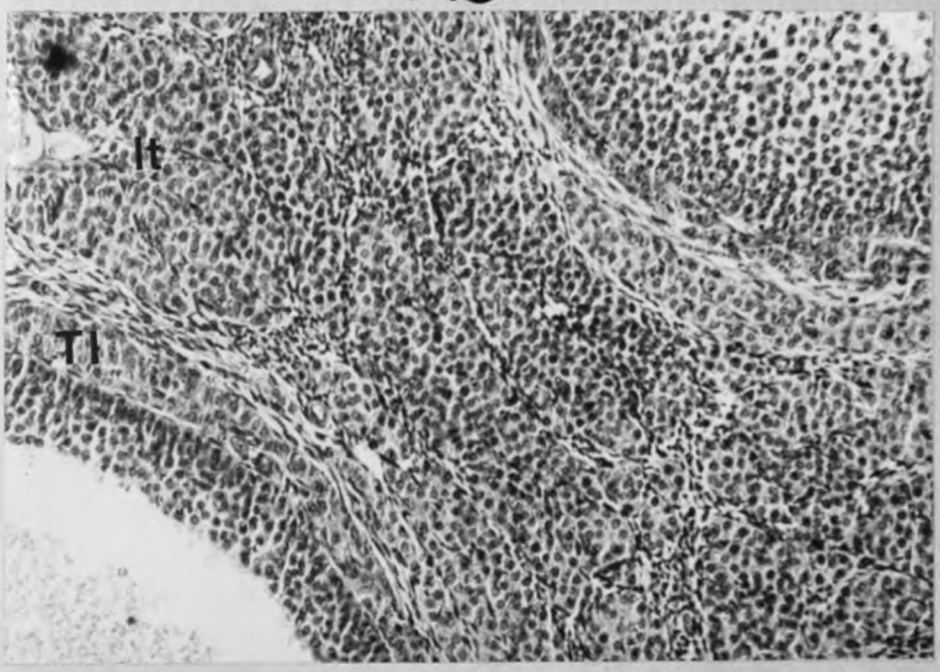
A section of the post-partum cane rat ovary. Again it shows extensive amount of interstitial gland tissue (It). The theca interna (TI) has diminished in size.

H & E. X 200.



7a

7b



4. THE NON-PREGNANT UTERUS

The non-pregnant adult uterus was a Y-shaped bicornual structure. The horns measured between 5 and 5.3 cm in length and ended up in a tortuous oviduct. The oviduct gave way to extensive fimbria. The broad ligaments suspends the uterine horns from the dorsal coelomic wall and carry with them blood vessels that supply the uterus and later the foetus via the maternal placenta.

The outer longitudinal muscle layer was thick and was separated from the inner circular layer (which appeared to be relatively thicker,) by a zone of loose connective tissue and spiralling muscle trabeculae. This intervening zone was richly vascularised and the muscle fibres of the trabeculae formed sheaths around the vessels. The uterine mucosa was thrown into folds and was lined by a simple cuboidal epithelium. The submucosa contained uterine glands that opened into the uterine lumen.

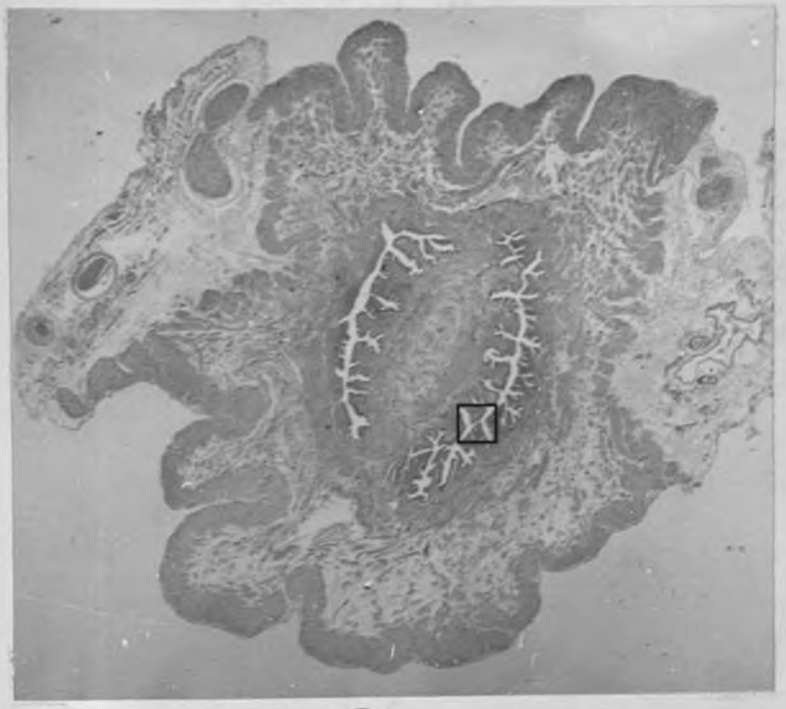
The uterus of the cane rat is duplex and the horns open into the vagina by two lateral ora cervicis lined by stratified columnar epithelium (Fig. 8, 9).

Fig. 8

A low power picture to show the two lateral ora cervicis opening into the vagina. H & E X 10.

Fig. 9

A higher magnification of the inset in Fig. 8. Note the stratified columnar epithelium. H & E X 200.



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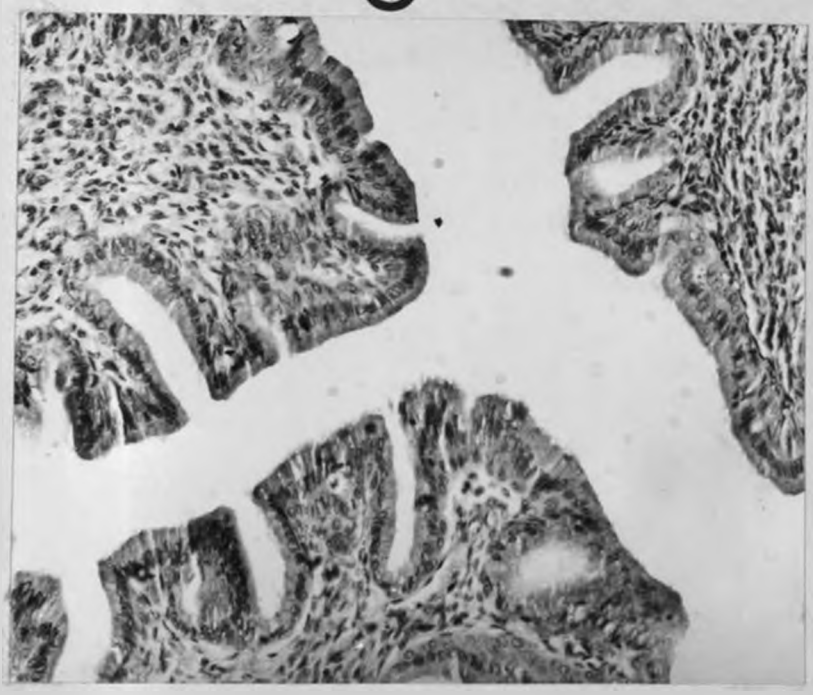


5. THE PREGNANT UTERUS

All the female cane rats that were studied were polyovular and carried from two to four embryoes per pregnancy. Even in the earliest stage pregnancy, the pregnant uterus was obvious by conspicuous, mesometrially placed locular enlargements (Fig. 10 a, b). The mesometrial blood vessels, which in the non-pregnant uterus were tortuous and small in calibre became less tortuous and quite large. In the regions of the locular enlargements both the longitudinal and circular myometrial muscular layers appeared to have been disrupted and replaced instead by rather large mesenchymal cells. There were no uterine glands in these enlargments. However, in the interlocular sections the structure and appearance of the uterus were similar to those of the non-pregnant uterus.

The conceptuses were often unevenly distributed in the two uterine horns (Table 1). They may all be confined to a single horn or they may be evenly distributed.

6. IMPLANTATION

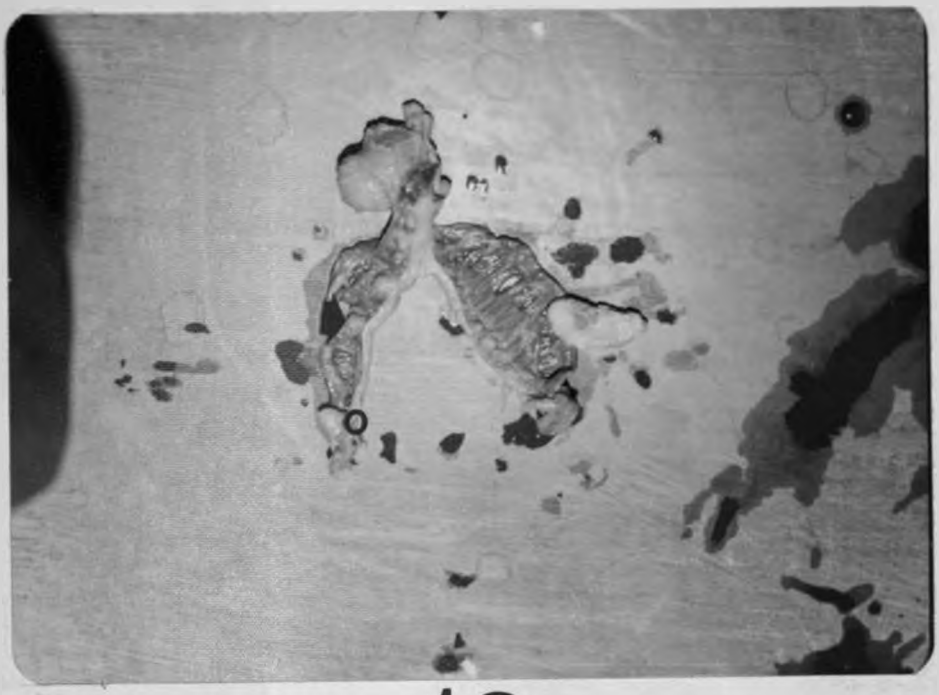
The earliest pregnancy available was represented by a uterus (CR12) with three locular enlargements in which the blastocysts were implanted. The loculi, two on the right horn and one on the left were essentially at identical stages of development.

Fig. 10a

A gross picture of the early pregnant uterus of the cane rat CR 12. Note the enlargements (arrow) along the mesometrial border. These mark the implantation sites. O = ovary X 10.

Fig. 10b

A gross picture of three conceptuses all confined to the right uterine horn of CR 1. The enlargements (E) mark the placental site. X 10.



10a

10b

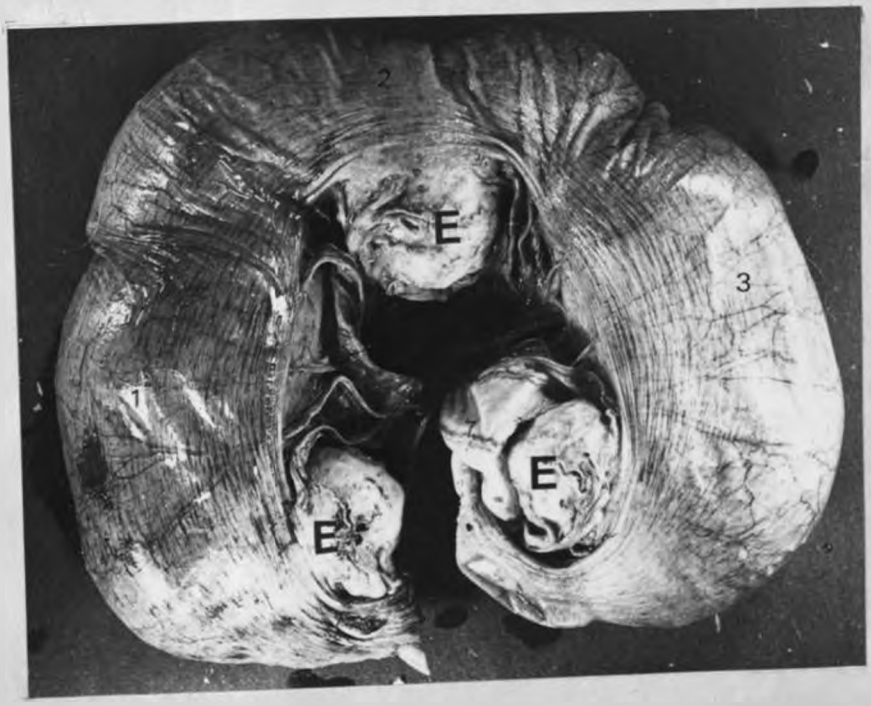


Fig. 11a

A low power photomicrograph of the arcon-shaped swelling of specimen CR 12. Note the position of the blastocyst (arrow) situated mesometrially. The arrow shows the mesometrium. AM = antimesometrial border. H & E. X 10.

Fig. 11b

A photomicrograph of the arcon-shaped swelling (CR 12) to show the hypertrophy of the endometrial cells. Some cells are binucleate (arrow). Note the oedema of the connective tissue.
H & E. X 200.

11a

11a

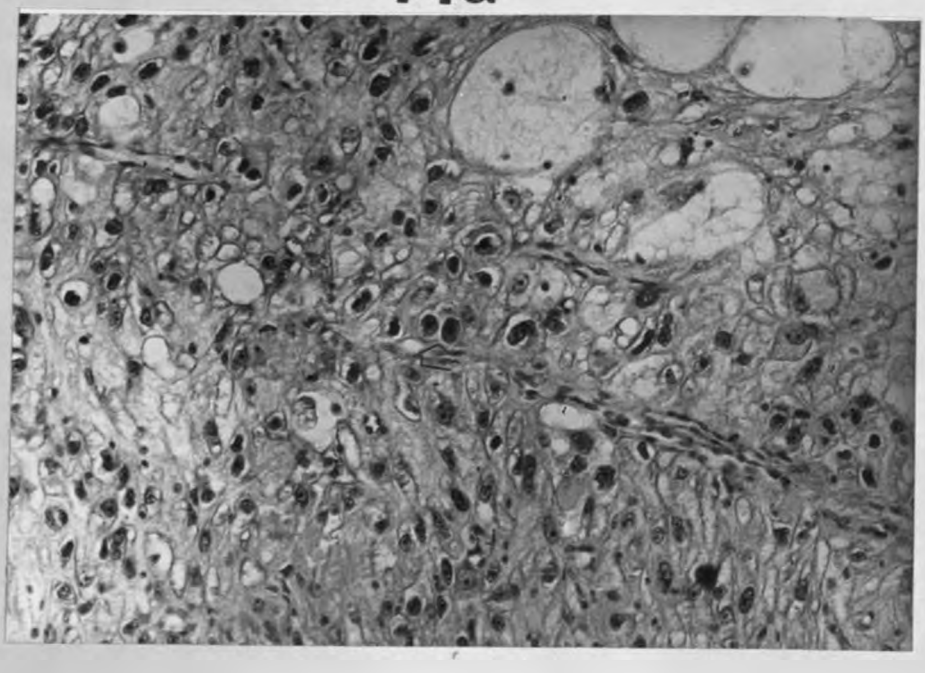
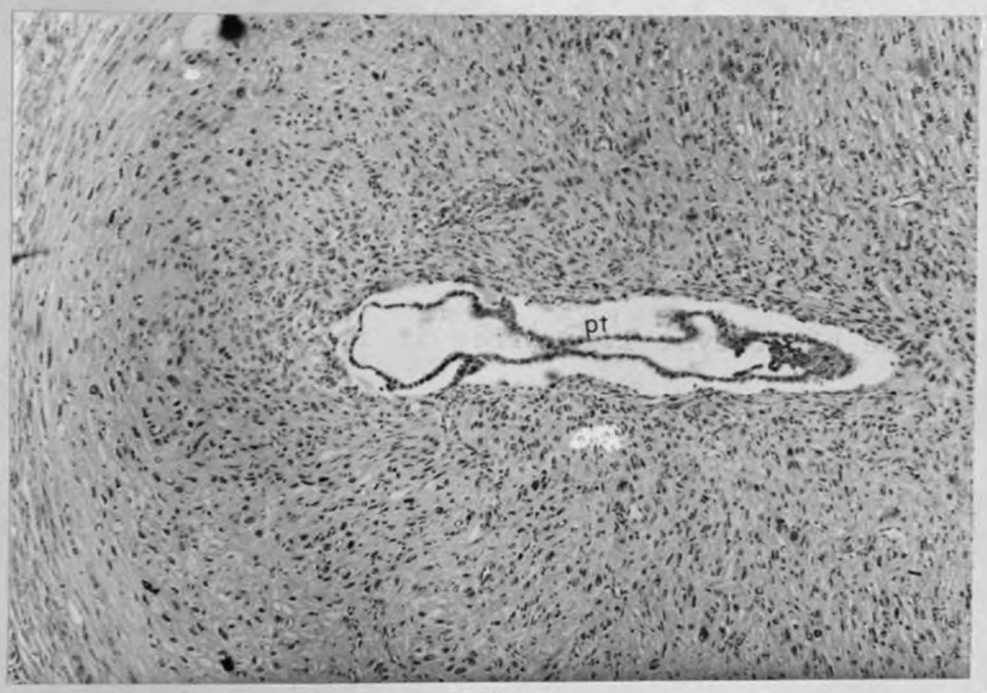


Fig. 11c

A photomicrograph of the implantation cavity showing the blastocyst lying free in it except at the abembryonic pole. The cavity has no epithelial lining. Note the tubular elongate nature of the blastocyst. Note also the continuity of the parietal trophoblast (pt).
H & E. X 75.

Fig. 11d

A section of the implantation chamber showing the extensive folding of the parietal trophoblast. The amnio-embryonic cell mass is antimesometrially orientated.
H & E X 75.



11c

11d

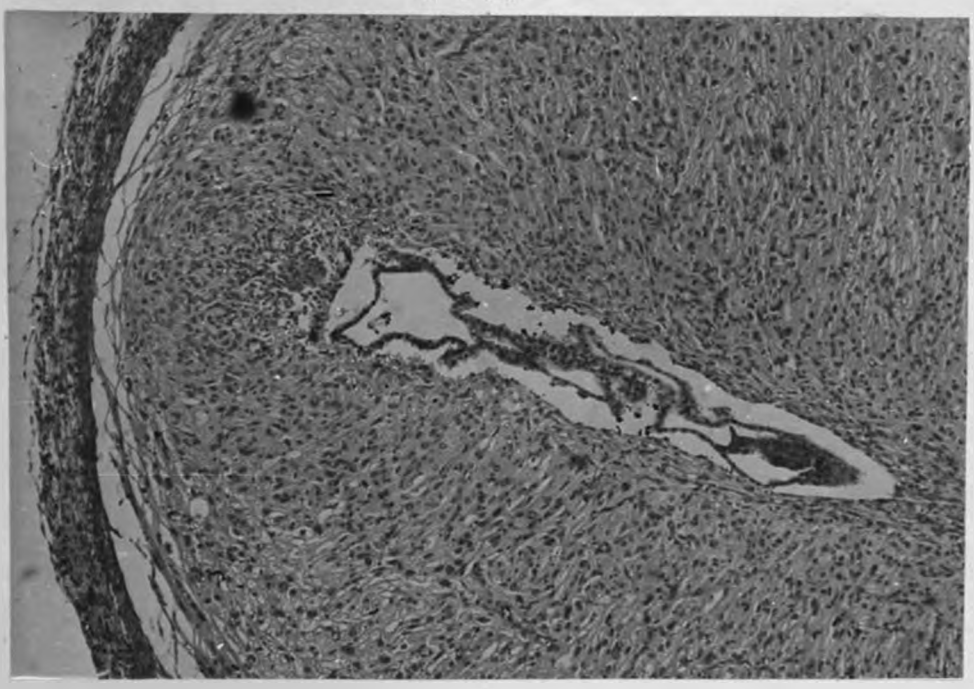


Fig. 12 b

A photomicrograph of the blastocyst in the implantation cavity. Note the trophoblastic pegs (arrows) attaching the blastocyst to endometrium. Note also a portion of the uterine lumen (ut).
H & E X 200. (CR 12).

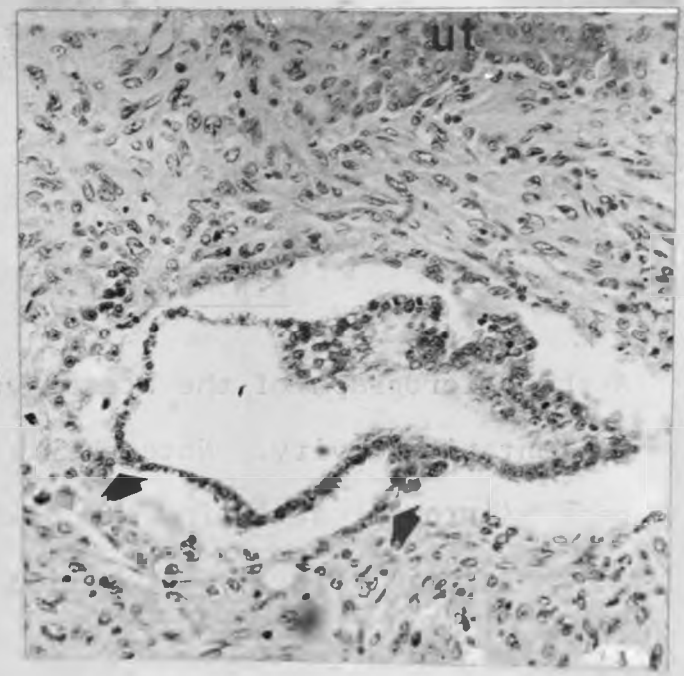
Fig. 12a

A section of the implantation cavity. Note the intact uterine epithelium marked ut.
H & E X 75 (CR 12).

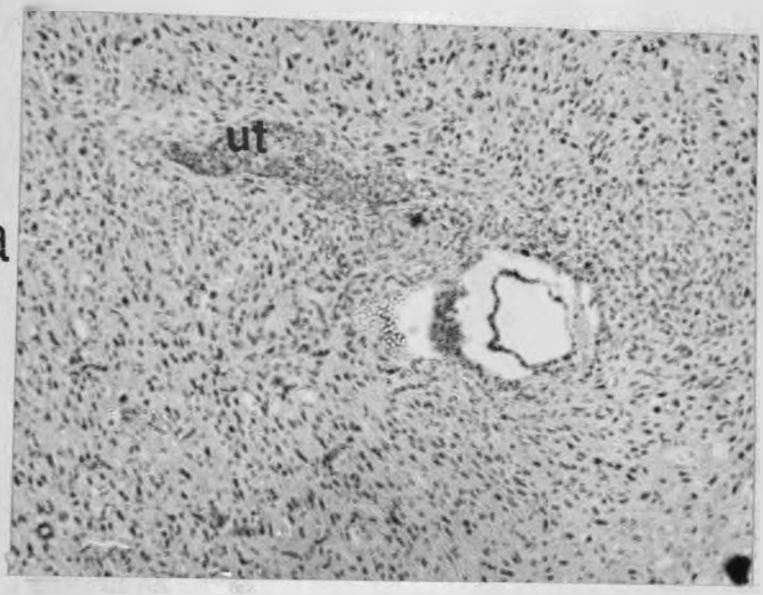
Fig. 13a

A photomicrograph of the embryonic pole of the blastocyst. The endoderm (arrow) has started to differentiate. pt = parietal trophoblast. H & E X 200 (CR 12)

12b



12a



13a

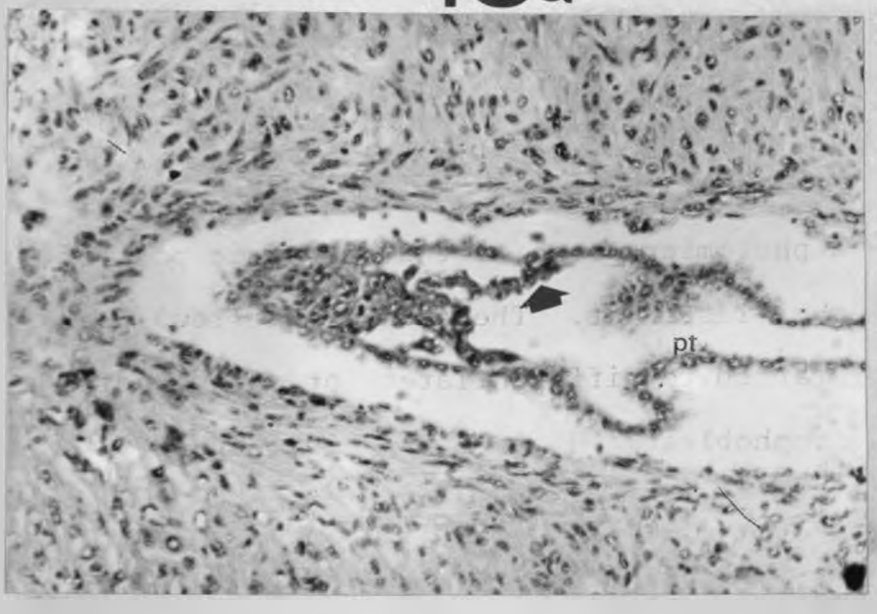


Fig. 13b

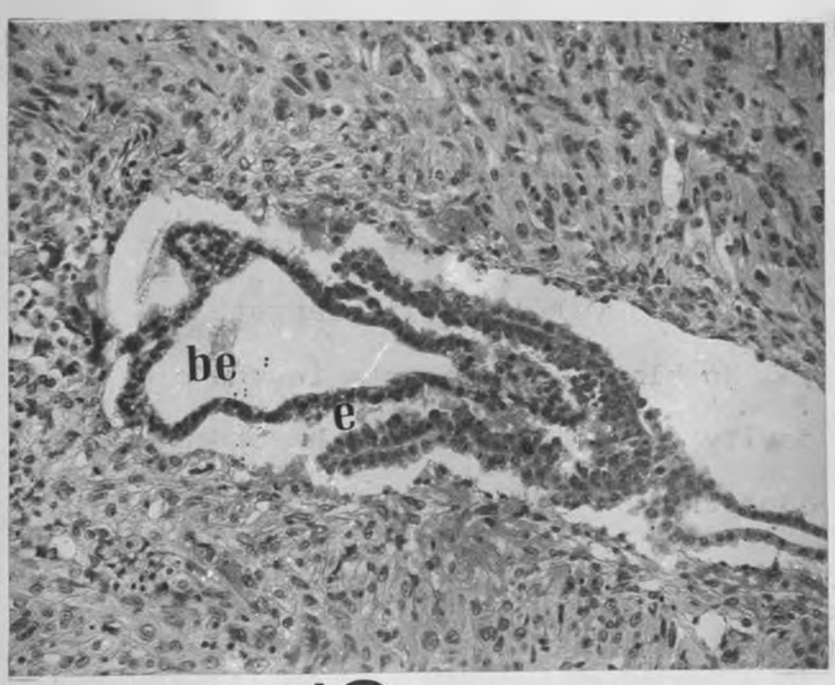
A photomicrograph of the abembryonic portion of the blastocyst. Note the extravasated maternal blood (e) in the implantation cavity. No blood was present in the blastocyst cavity (bc). Note also the oedema in the endometrium. H & E X 200 (CR 12).

Fig. 14

A photomicrograph of the amnio-embryonic cell mass showing the beginning of amnion formation (a). The endoderm is also differentiating. H & E. X 200. (CR 12).

Fig. 15

A low power section of the chorionic placenta of cane rat CR2. The chorionic placenta forms in a special chamber (arrow). Note the continuity of this chamber with the main uterine lumen (ul). H & E. X 10.



13b

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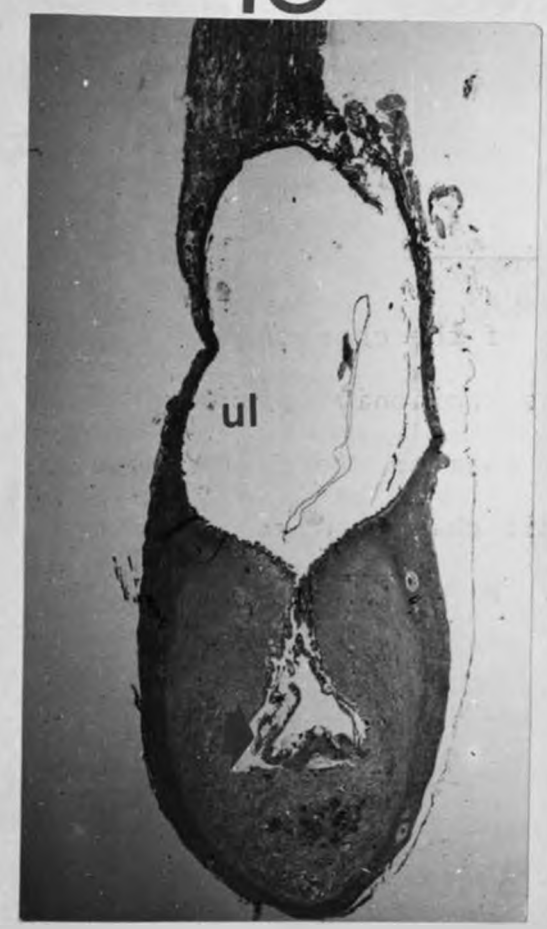

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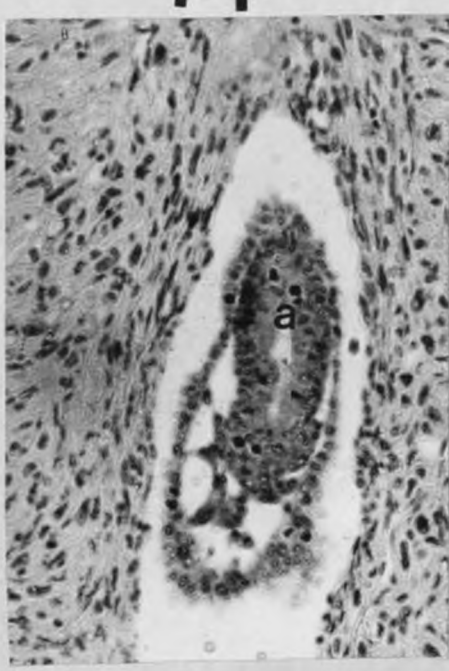


The uterine swellings (which were richly supplied with blood) were situated mesometrially. Histologically the enlargement of the swellings was due to hypertrophy of endometrial mesenchymal cells oedema of the connective tissue and a significant fatty infiltration. The hypertrophied mesenchymal cells were either round or oval and had large vesicular nuclei with prominent nucleoli. Some of these cells were binucleated (Fig. 11 a, b).

The blastocyst lay for the most part free in a large cavity, the implantation chamber, that was separate from the uterine cavity. The implantation chamber lay deep in the locular enlargment. It was devoid of epithelial lining (Fig. 11 c, d). Serial sections revealed an intact uterine epithelium to the lateral side of the chamber (Figs. 12 a, b). The blastocyst had elongated into a tubular structure with its long axis perpendicular to that of the uterus. It consisted of an inner cell mass (which was destined to differentiate into the amnion and the embryo proper) and an outer covering of unilaminar trophoblast. The unilaminar trophoblast was thrown into extensive foldings (Fig. 11 d). It enclosed a large blastocyst cavity. At the abembryonic pole the blastocyst was attached to the uterine connective tissue by peg-like outgrowths from the trophoblast (Fig. 12 b). The attachments were

mesometrial. It is important to emphasise here that the outer parietal layer of trophoblast was continuous and covered the inner cell mass. From the under surface of the inner cells mass the endoderm was beginning to differentiate. The endodermal cells lay close to the ectoderm (parietal trophoblast) and were beginning to spread as a single layer towards the abembryonic pole (Fig. 13 a). At the abembryonic pole there was loosening of connective tissue. The trophoblastic cells were already in the process of invasion; maternal blood vessels were opened up and the extravasated blood oozed into the implantation chamber (Fig. 13 b). It appeared that some red blood cells had been engulfed by trophoblastic cells thus emphasising the nutritive role of the early trophoblast. No blood cells were present in the blastocyst cavity.

Within the inner cells mass some clefts had began to form. When these later coalesce, they give rise to the amnion; hence amnion formation is by cavitation (Fig. 14).

Fig. 16

A low power photomicrograph of the chorionic placenta (p) in the special chamber. Note the continuity of the chorion (ch) into the main uterine cavity. H & E X 10.

Fig. 17

A high power section of the inset in Fig. 16. Note the intact epithelium (arrows) of the connecting channel. CH = Chorionic membrane. H & E X 500.

16

17

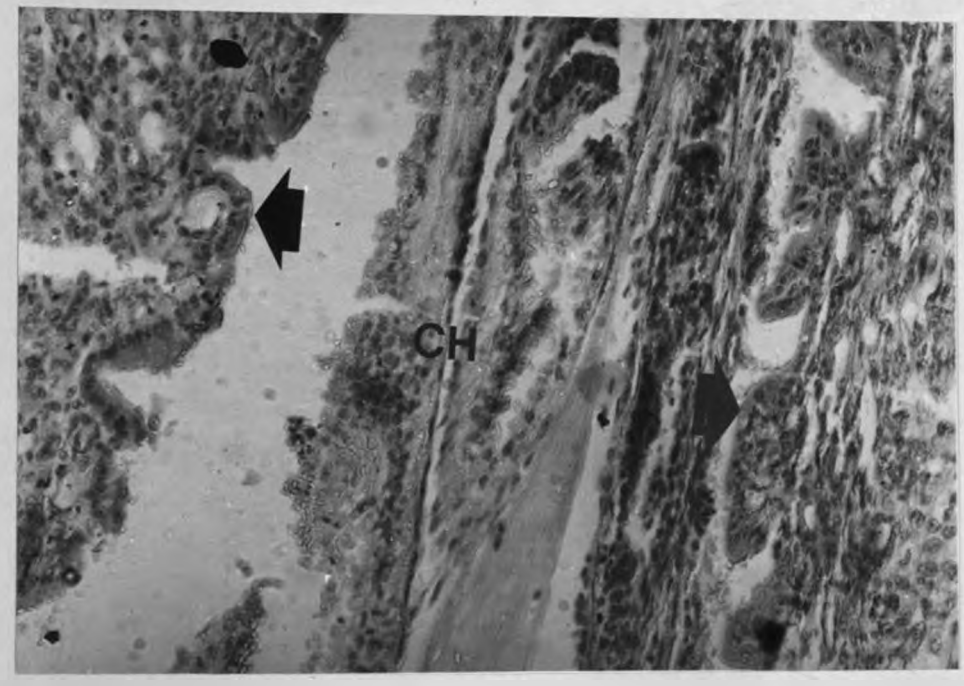


Fig. 18

A low power photomicrograph to show the amnio-embryonic cell mass (arrow) suspended antimesometrially in the main uterine lumen. pc = placental chamber; ys = yolk sac. H & E X 10.

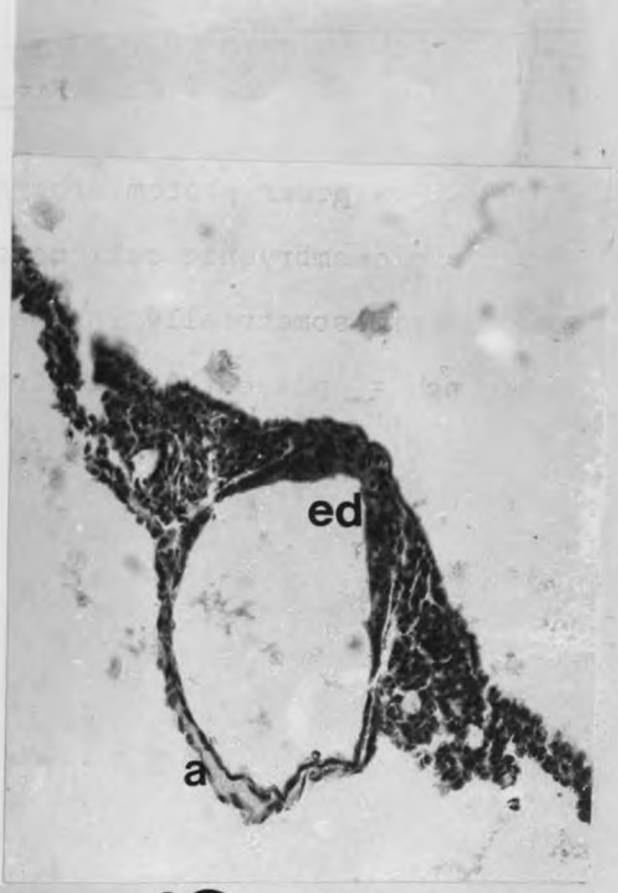
Fig. 19

A high power section of the amnio-embryonic mass. a = amnion; ed = embryonic disc. H & E X 200.

Fig. 20

A photomicrograph to show the chorion, CH: and the visceral yolk sac, ys. Note the arrangement of the germ layers.
H & E. X 75.

18



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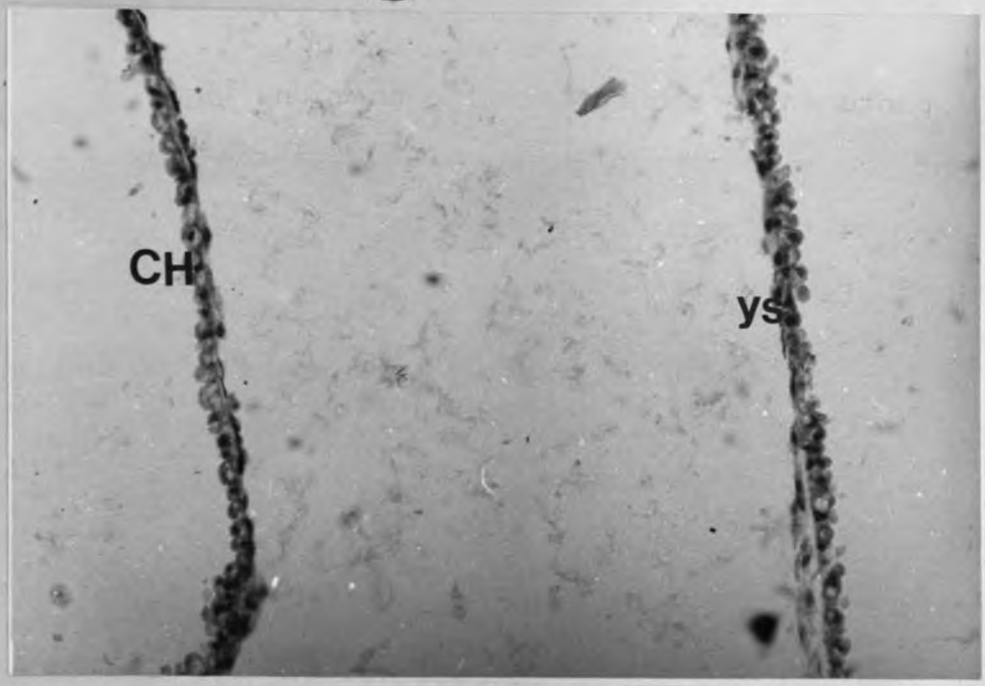
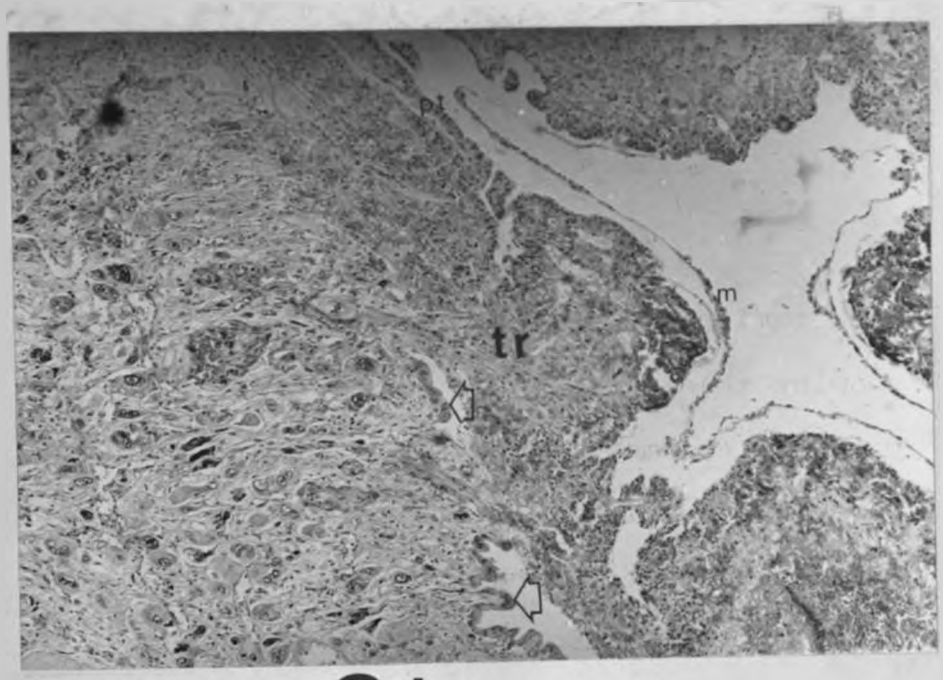


Fig. 21a

A photomicrograph of the chorionic placenta showing the invasion and destruction of maternal endometrium by the trophoblast (tr). Patches of intact maternal epithelium (arrows) are still present. m = myometrium. H & E X 75.

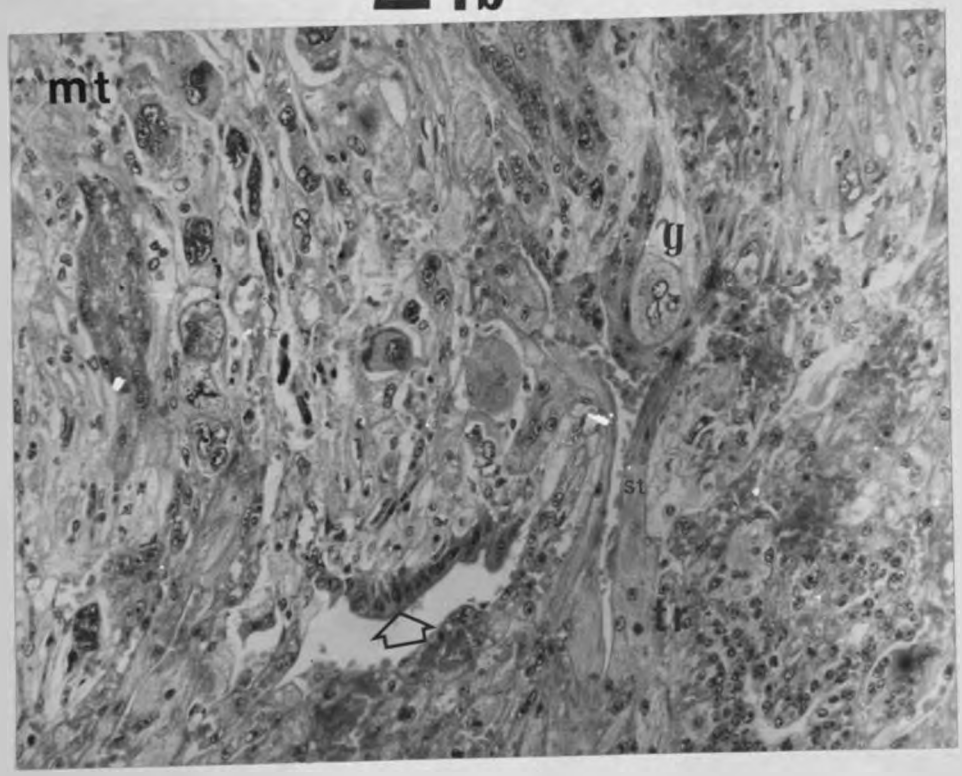
Fig. 21b

A high power picture of a portion of Fig. 21a. Note the invasive tongue of synctytial trophoblast (st) enclosing maternal blood. g = giant cell; tr = trophoblastic cells deep in the myometrium; arrow = remnant H & E X 200.



21a

21b



7. THE CHORIONIC PLACENTA

The next stage was represented by specimen CR 2 in which the placental architecture was already laid down. The chorionic placenta developed within an enlarged chamber in the uterine cavity (Fig. 15, 16). This chamber was lined by an epithelium except in those areas where the trophoblast had destroyed it and invaded the uterine tissues. The epithelium was eosinophilic and the cell boundaries had more or less disappeared. In the processes of invasion, some epithelial cells had remained amid the trophoblastic tissue and appeared in the form of large multinucleated giant cells. Maternal blood capillaries were closely related to this epithelium and came to lie just underneath its basement membrane.

The enlarged chamber within which the chorionic placenta developed was mesometrially situated and lay within the uterine enlargement. It was connected to the main uterine lumen by a narrow channel lined by epithelium (Fig. 16, 17). The epithelial lining of the special or "placental" chamber and its continuity with the main uterine lumen made it difficult to determine, accurately, the mode of implantation especially in the absence of

intermediate stages between CR 12 and CR 2 and stages earlier than CR 12. However, examination of CR 12 and CR 2 strongly suggested an eccentric or secondarily interstitial implantation. In the main uterine lumen of specimen CR 2, there was an extensive chorion consisting of outer layer of parietal ectoderm and an inner layer of mesoderm. This extensive chorionic membrane supported the amnio-embryonic mass at its antimesometrial pole whilst forming the chorionic placenta at its mesometrial pole (Fig. 16, 18). The amnio-embryonic mass was a signet-ring-shaped structure whose embryonic disc consisted of cells that were distinguishable by their tall columnar nature (Fig. 19). The amnion, with its ectoderm and mesoderm on the inside and outside respectively enclosed the amniotic cavity. The appearance and nature of the whole mass strongly suggest that the enclosed amniotic cavity was formed by coalescence of several smaller cavities within the original inner cell mass.

The true visceral yolk sac could be seen in Fig. 18. It was invested by an outer layer of mesoderm and an inner layer of endoderm enclosing the yolk sac cavity (Fig. 18, 20).

The trophoblast had proliferated mesometrially destroying maternal connective tissue and opening up maternal blood vessels. At this stage three types of trophoblast could be distinguished:-

a) trophoblastic giant cells: These consisted of aggregates of nuclei (sometimes as many as seven) and very little cytoplasm. These giant cells were present at the periphery of the chorionic placenta or deep in the endometrium. Those deep in the endometrium seemed to have been pinched off from the tips of invading syncytial trophoblastic tongues;

b) canalicular syncytio-trophoblast which lined the honey-comb of channels extending from the endometrial to the foetal surface of the placenta. These channels contained maternal blood which was in direct contact with the syncytial trophoblast;

c) primary trophoblast which lined a central cavity (Figs. 21 a, b).

8. THE CHORIO-ALLANTOIC PLACENTA (CRI, 3, 6,' 11, 13)

The chorio-allantoic membrane is attached to the placental surface by a peculiar fibrovascular ring which immediately surrounds a thin non-vascular collar. The fibrous stroma of ring was richly supplied with a network of capillaries (Fig. 22). This fibrovascular ring seems to be peculiar to and characteristic of hystricomorph rodents. The definitive placenta of the cane rat was a disc shaped structure situated mesometrially and its relative position within the uterine cavity could be surmised by the external uterine enlargement. The placental disc was raised above the surface of the uterus and was well supplied with umbilical blood vessels. Its foetal surface measured between 1.0 and 1.2 cm in diameter and it appeared to sit in a V-shaped excavation within the myometrium.

With the invasion of the chorion by the vascularised allantoic mesoderm the lobulation of the cane rat placenta became complex. In well stained H & E sections the lobulations were clearly visible with the naked eye. Each lobule consisted of a

labyrinthine zone interdigitating with a spongy zone - the trophospongium (Fig. 22 & 23).

It was in the labyrinthine zone that the foetal and maternal circulations were brought into close contact. Here the trophoblast with its supporting matrix of vascularised foetal mesenchyme was bathed by maternal blood. In the spongy zone, on the other hand only maternal blood was found contained within trophoblastic lacunae. (No foetal capillaries penetrated into this zone). In gluteraldehyde-fixed toluidine blue-stained sections the syncytial trophoblast immediately enclosing the maternal blood spaces stained darkly. One or two layers of cytotrophoblast internal to the syncytium stained lightly. The foetal and maternal blood streams were separated from each other by a layer of syncytial trophoblast, a layer or two of cytotrophoblast, foetal connective tissue and foetal endothelium such that in the cane rat placenta the three categories of the haemochorial type of placenta namely haemomono, haemodi- and haemotri-chorial relationship existed at the same time (Fig. 24).

The syncytial trophoblast was heavily studded with basophilic granules. The nuclei were large and spheroidal and had distinct nucleoli. The cellular

Fig. 22

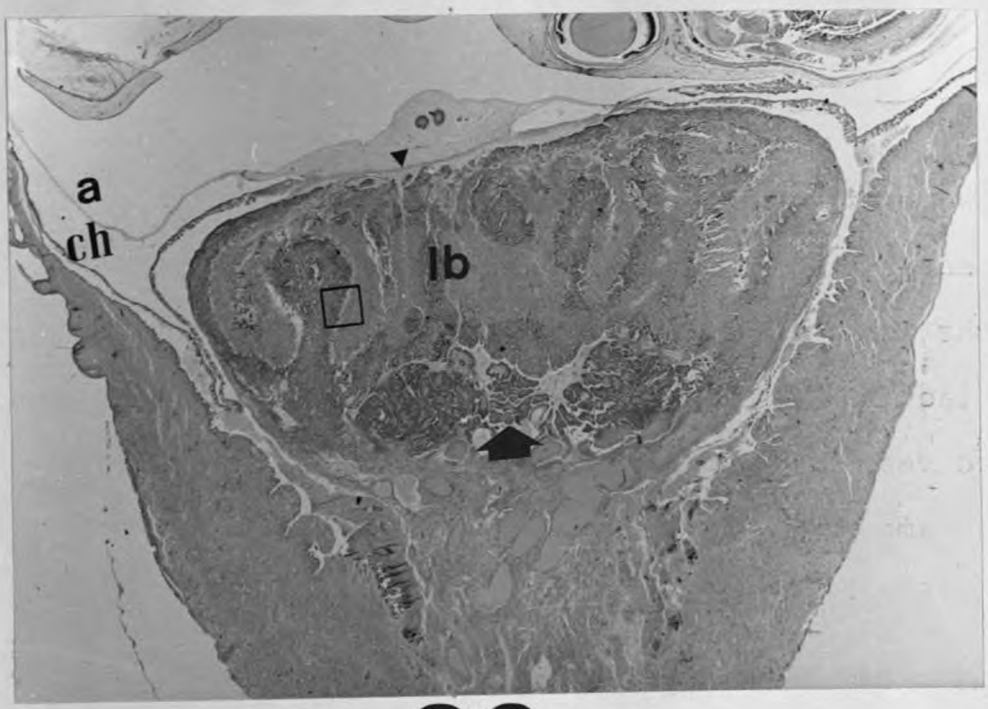
A low power photomicrograph of the definitive placenta of the cane rat. Note the lobulation of the placenta (lb); the well developed subplacenta (arrow). Note the large maternal blood vessels beneath the subplacenta.
a = amnion; ch = chorion.
H & E X 10.

Fig. 23

A high power section of the inset in (Fig. 22)
SZ = spongy zone; lz = labyrinthine zone; fv = foetal vessel.
H & E X 75.

Fig. 24

A photomicrograph of the cane rat placenta to show the relationship between the foetal (fc) and maternal (mb) circulations. Note the dark staining syncytial trophoblast and the light staining cytotrophoblast. Toluidine blue stain. X 500.



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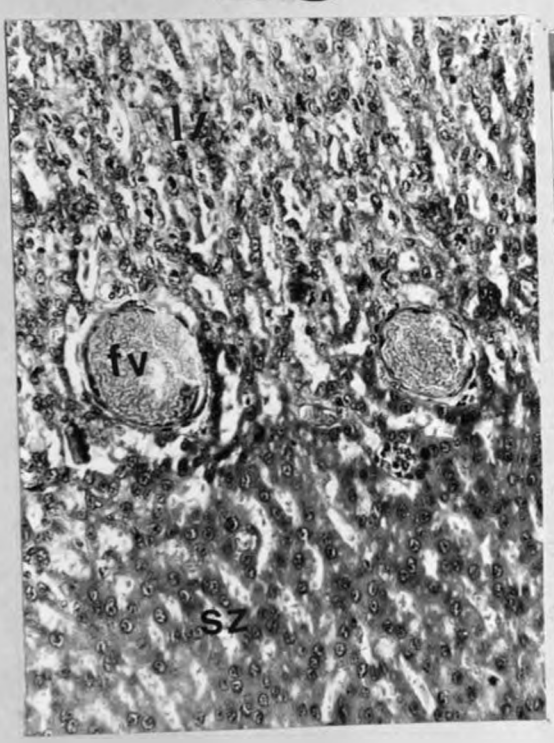

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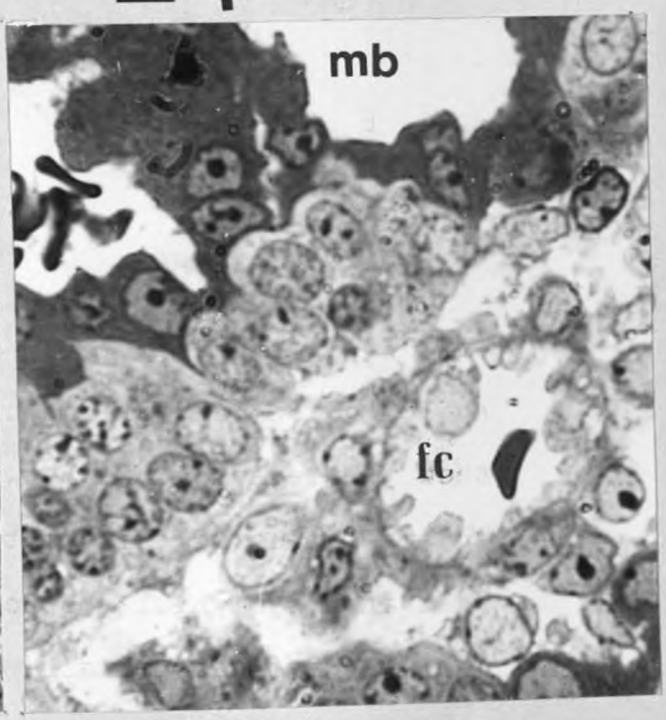


trophoblast was lighter and the cells had distinct nuclei with scattered chromatin and prominent nucleoli. Some of the cellular trophoblastic cells were undergoing mitotic division. Fig. 25 shows one of these cells in the metaphase stage. Whenever the syncytial trophoblast was missing, in places, the cytotrophoblast cells came into direct contact with maternal blood. At no stage was maternal blood observed to be in direct contact with the foetal capillaries.

9. ULTRA-STRUCTURE OF THE CHORIO-ALLANTOIC PLACENTA

The fine structure of the definitive placenta indicated that it was of the haemo-trichorial type, consisting of a layer of syncytial trophoblast immediately lining the maternal blood spaces, and one or more layers of cytotrophoblast (Fig. 26). The typical cytotrophoblast maintained clearly defined cell membranes with desmosomes. The nuclei were relatively large, round to oval in shape and were less electron dense than those of the syncytium. Dense patches of chromatin were found along the nuclear periphery. The cytoplasm of these cells was characterised by the paucity of cellular organelles. The Golgi complex, though present in some cells, was poorly developed (Fig. 27). The outstanding

cytoplasmic organelles were the free ribosomes scattered throughout the cytoplasm. Large membrane bounded vesicles were also present in the cytoplasm (Fig. 28).

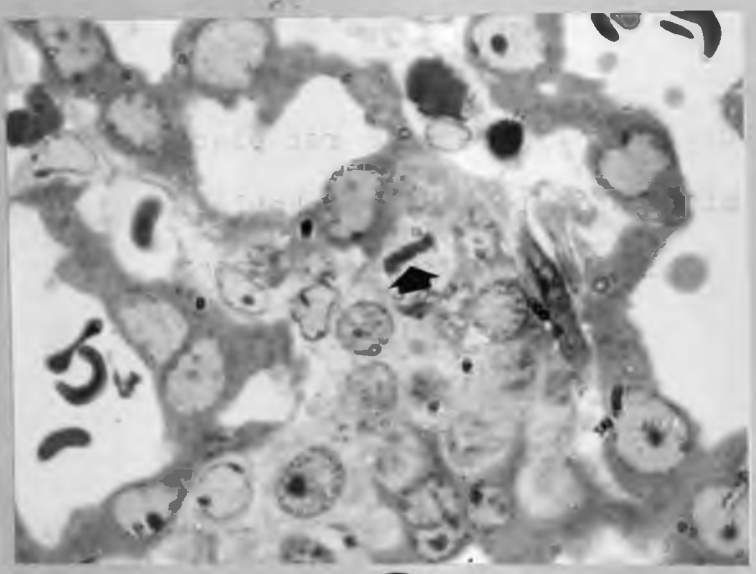
The syncytial trophoblast, on the other hand was characterised by irregularly shaped, chromatin-dense nuclei with prominent vesicular nucleoli. No cell membrane was observed between two adjacent nuclei but cell membranes dotted here and there with desmosomes were present between the syncytium and the cellular trophoblast (Fig. 29). The cytoplasm of the syncytium had abundant granular endoplasmic reticulum and well developed Golgi complex (Fig. 30). The granular ER was closely associated with numerous large membrane-bounded secretory droplets, the contents of which were similar to those in the cisternae of the ER. The syncytial surface was characterised by a profusion of microvilli protruding into the maternal blood space. These microvilli varied considerably in size and shape and at their bases there were numerous pinocytotic vesicles. In certain areas of the syncytium there were apparently deep recesses into which microvilli projected (Fig. 31). Whether these "intracellular canaliculi" were real or artifactural was difficult to ascertain. Large lipid droplets were also present in the syncytium although these were not numerous.

Fig. 25

A photomicrograph of the cane rat placenta to show mitosis in the cytotrophoblast (arrow). Toluidine blue stain: X 200.

Fig. 26

A low power electron micrograph of the cane rat placenta. ct = cytotrophoblast; st = syncytial trophoblast; mb = maternal blood space. X 4200.



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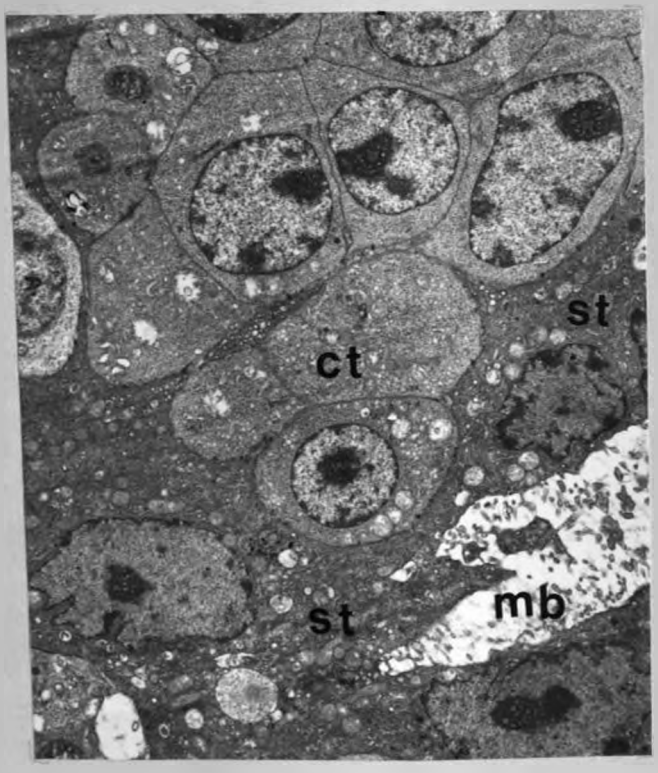
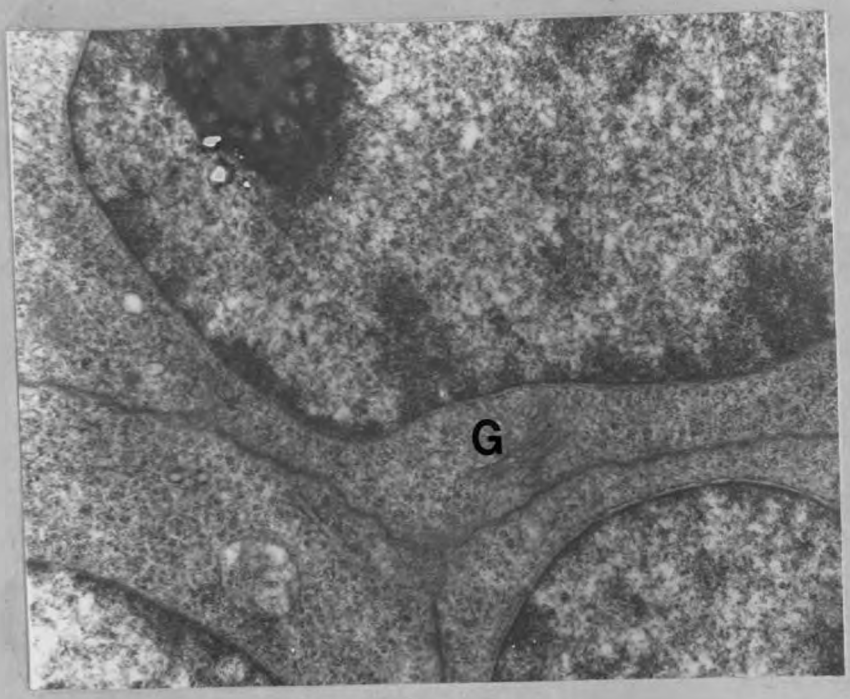


Fig. 27

An electron micrograph of the cytotrophoblast. Note the paucity of the cellular organelles. G = Golgi complex.
X 16,000.

Fig. 28

An electron micrograph of the cytotrophoblast to show large membrane bounded vesicles (v). G = Golgi complex. X 16,000.



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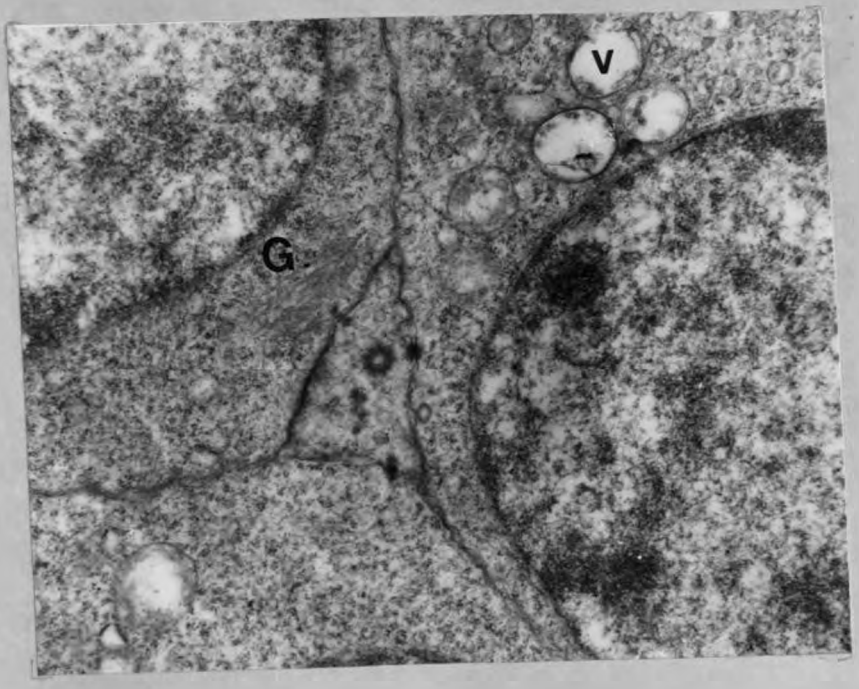
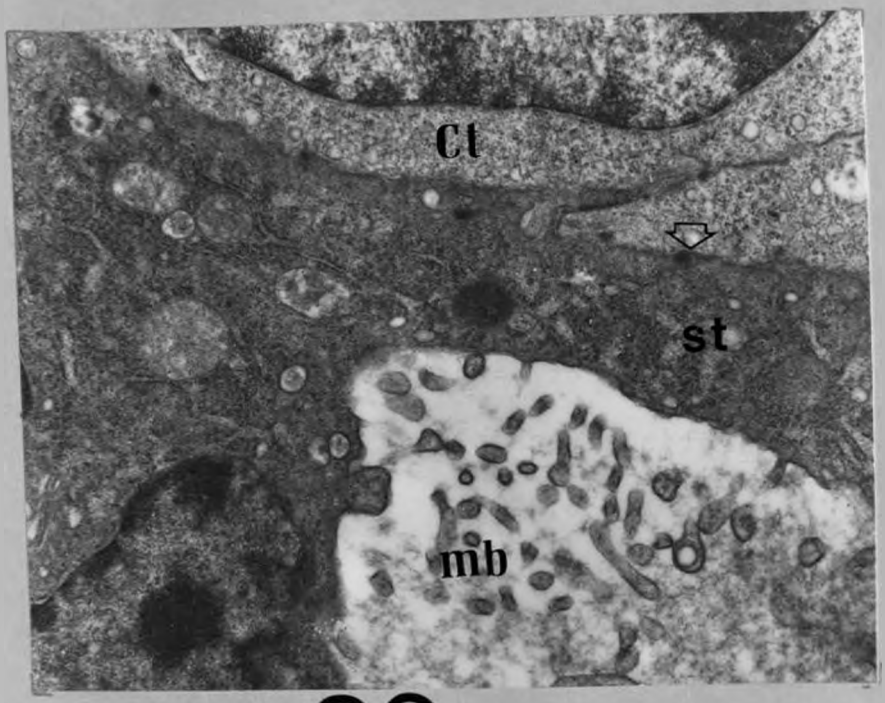


Fig. 29

An electron micrograph of cyto- and syncytial trophoblast. Note the desmosomes between the two (arrow). ct = cytotrophoblast; st = syncytial trophoblast: mb = maternal blood space. X 16,000.

Fig. 30

An electron micrograph of the syncytial trophoblast to show the well developed granular endoplasmic reticulum and numerous secretory granules. G = Golgi complex; mb = maternal blood space. X 16,000.



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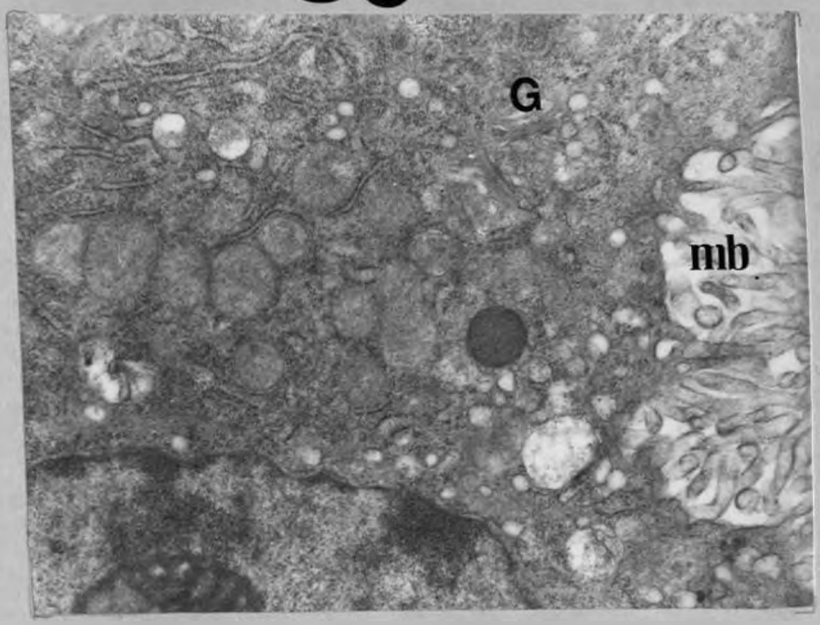
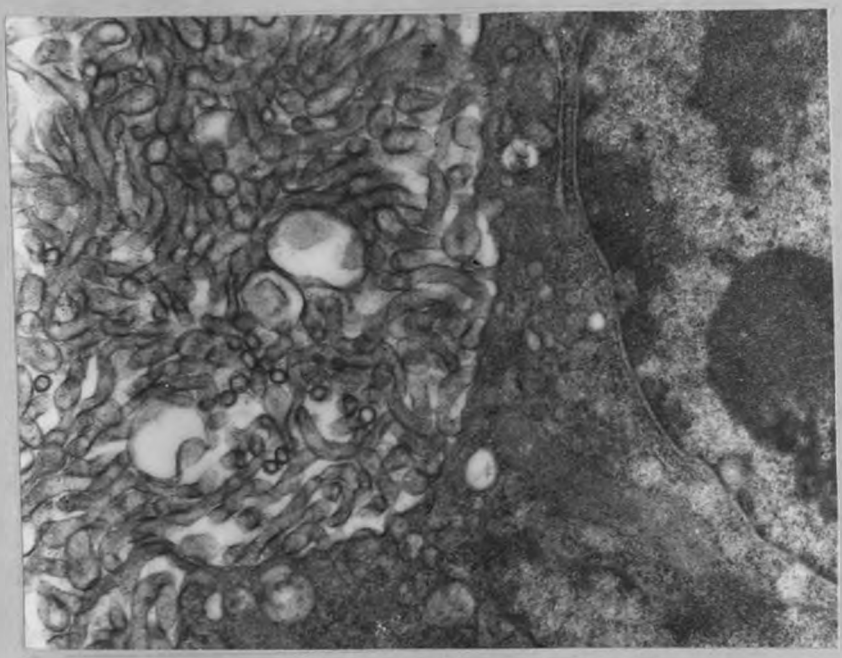


Fig. 31a

An electron micrograph of a portion of syncytial trophoblast to show the numerous microvilli protruding into the maternal blood space. X 16,000.

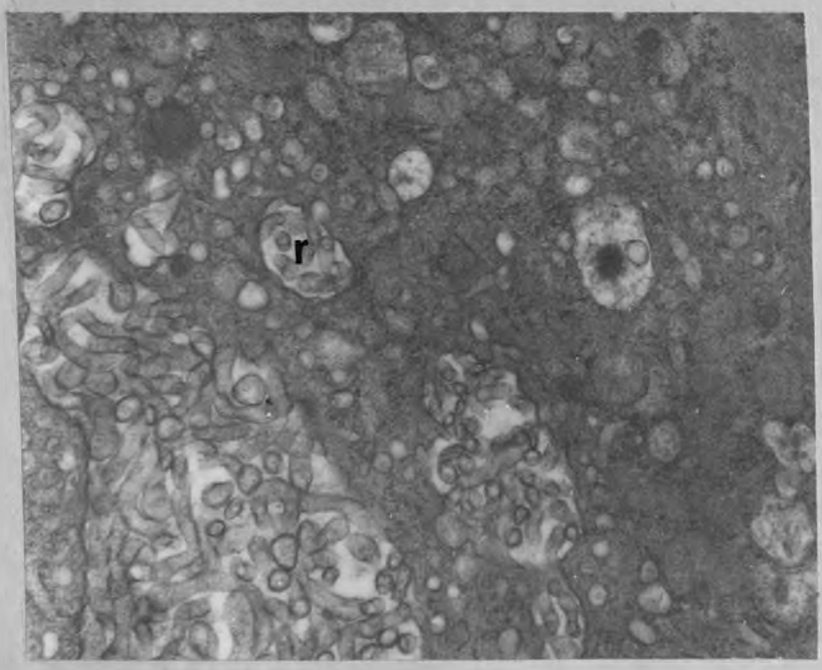
Fig. 31b

An electron micrograph of a portion of syncytial trophoblast to show deep recesses (r) into which microvilli project. X 16,000.



31a

31b



10. THE SUBPLACENTA

In tissues fixed in Bouin's and formalin solutions and stained in H & E the subplacenta in the definitive placenta of the cane rat stood out as a distinct region lying between the trophoblastic spongy zone and the junctional area. It was visible with the naked eye as a bluish staining area. The subplacenta was an extensive zone consisting of uniformly small chorionic ectodermal cells (Fig. 22). The cells occured in nests (or clusters) embedded in a matrix of chorionic mesenchyme that was richly vascularised by allantoic mesoderm. Large maternal blood vessels were also closely associated with the subplacenta. These maternal blood vessels consisted of arterial vessels coursing through the subplacenta on the one hand and venous channels collecting blood coming from the labyrinth on the other. At its periphery the subplacenta merged with the marginal syncytium of the chorio-allantoic placenta thus emphasizing the foetal origin of this accessory structure. Its cells were greatly modified by the condensation and fusion of their cytoplasm forming dense basophilic masses. In specimen CR 13 it was observed that the chorionic syncytium, accompanied by foetal mesenchyme, extended deep into the uterine enlargement, well below the

placenta (Fig. 32, 33). Trophoblastic tongues also extended deep below the subplacenta, enclosing maternal blood spaces and resting against the junctional zone. The chorionic syncytium resembled the subplacenta; it had small cells which stained strongly basophilic with H & E. The trophoblastic tongues on the other hand stained purple-reddish in H & E and ran in long columns (Fig. 33, 34).

Within the substance of the subplacental clusters there appeared droplets of diastase-resistant P.A.S. - positive material which formed a latice like pattern (Fig. 35, 36). The subplacenta was well vascularised. This P.A.S. - positive substance was in all probability a secretory product of the subplacental cells. In all probability the subplacenta is an endocrine organ. In the cane rat the subplacenta was present throughout gestation, showed no signs of degeneration towards term and was separat from the junctional zone by eosinophilic necrotic area with larger maternal blood spaces. Often the syncytial tongues from the subplacenta broke off and were found as aggregates of nuclei deep in the junctional zone and the decidua basalis where they stood out as trophoblastic giant cells. These cells had several nuclei with very little cytoplasm (Fig. 37 a & b).

Fig. 32

A low power photomicrograph of chorio-allantoic placenta of the cane rat, CR 13 to show the extent of trophoblastic invasion (tr) below the subplacenta (sp). H & E X 10.

Fig. 33

A higher magnification of the inset in Fig. 32. tr = trophoblast; fm = foetal mesenchyme. H & E X 200.

32

33

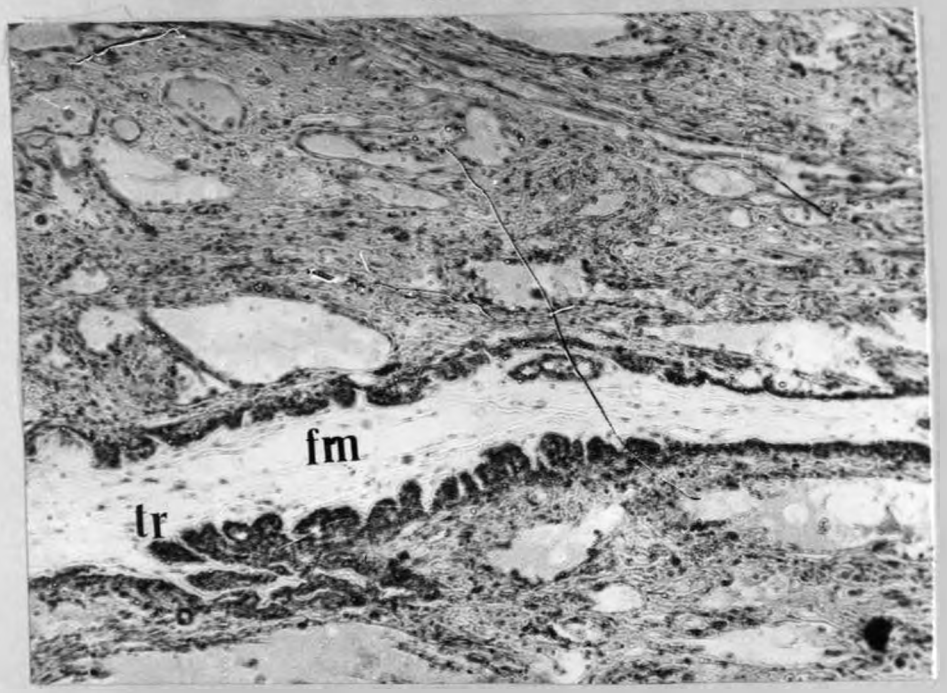
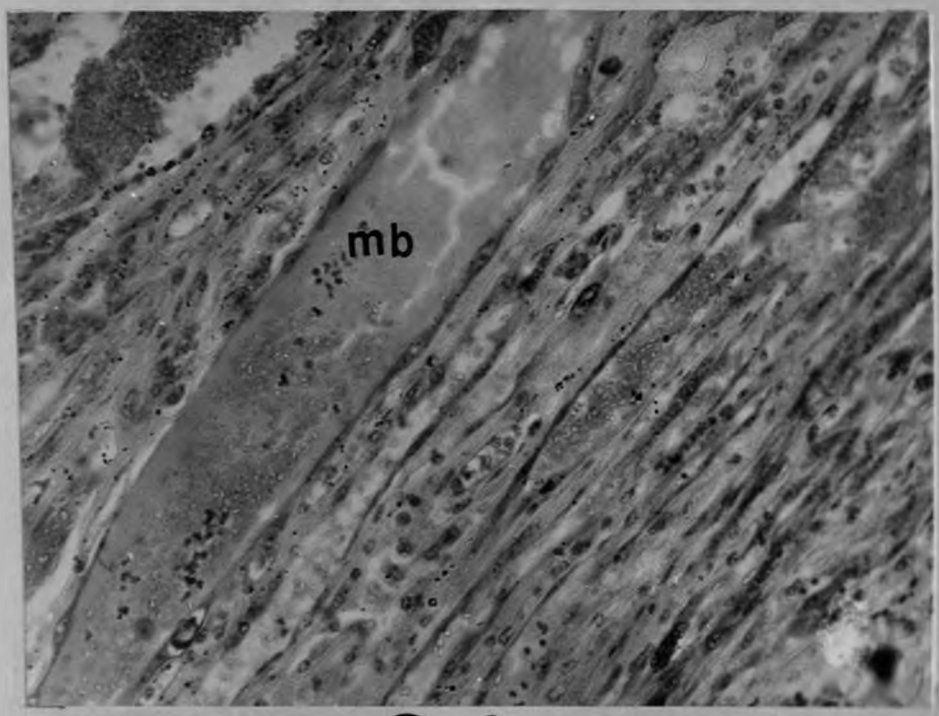


Fig. 34

A photomicrograph of dilated trophoblastic channels enclosing maternal blood. These occured below the subplacenta. mb = maternal blood space. H & E X 200.

Fig. 35

A photomicrograph of the subplacenta to show the clusters of cells.
H & E X 200.



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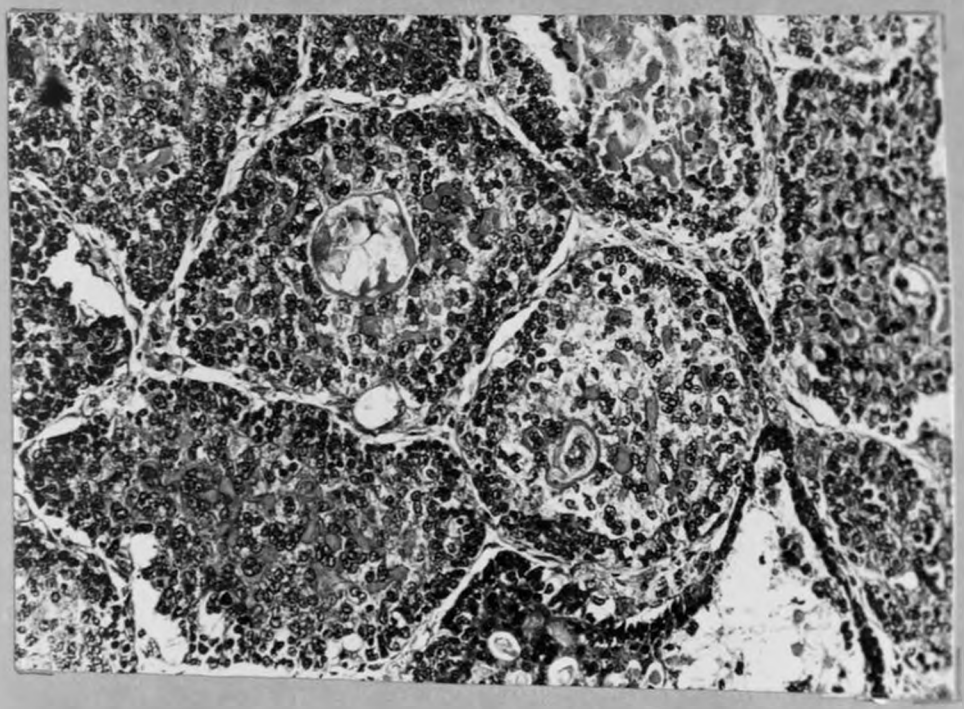
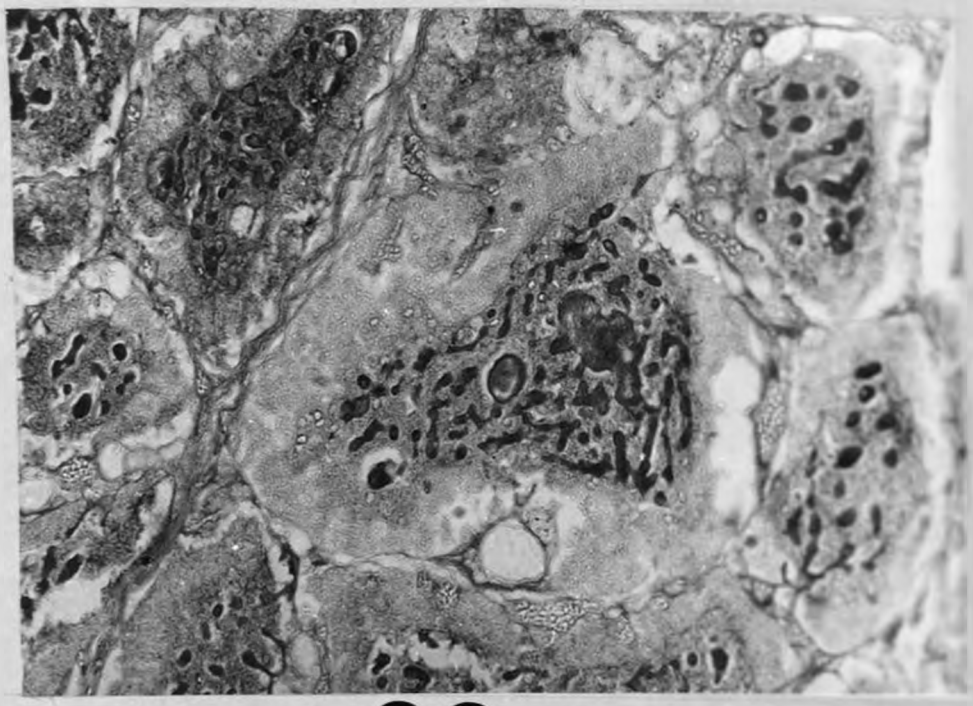


Fig. 36

A photomicrograph of the subplacenta to show the P.A.S.-positive, diastase resistant substance in the subplacenta. PAS-reaction. X 200.

Fig. 37a

A photomicrograph of the junctional zone to show the trophoblastic giant cells originating from the subplacenta (arrows).
sp = subplacenta; tg = trophoblastic giant cells. X 200.



36

37**a**

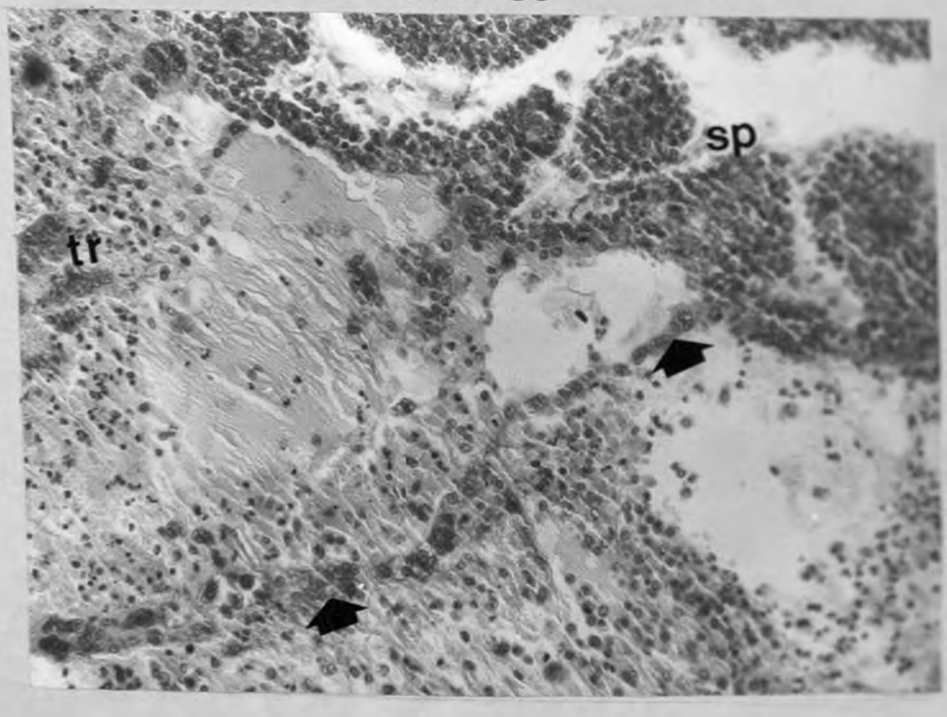


Fig. 37b

A photomicrograph of the junctional zone of the cane rat placenta to show the necrotic mass consisting of cellular debris.
H & E. X 200.

37b

11. THE CHORION

In the cane rat the presence of the parietal trophoblast was recognised. Fig. 11 c and d show the blastocyst in the "implantation chamber". It consisted of the inner cells mass and a single layer of trophoblast surrounding the blastocyst cavity. These figures obtained from CR 12 also showed clearly the beginning of endoderm formation from the inner surface of the solid inner cell mass. At a later stage of developmemt represented by Figs. 20 and 21 (CR 2) the chorion was clearly shown to consist of an outer trophoblast lined by an inner layer of somatic mesodermal cells. This chorion carried, at its antimesometrial pole the amnio-embryonic mass which was distinctly differentiated into the embryonic disc, the amniotic cavity and the amnion. Lying within the cavity of the extensive exocoelon was a structure which represented the splanchnic yolk sac. This structure consisted of an endodermal layer of cells lining a cavity. Outside the endodermal lining was a single layer of mesodermal cells. The parietal trophoblast and its mesodermal lining was clearly continuous with the chorionic placenta through the narrow channel (Figs. 16, 18 & 21).

The parietal trophoblast in the main uterine cavity was not invasive. After vascularisation by the allantoic mesoderm it formed a chorio-allantoic placenta of the epithelio-chorial type. At later stages the chorio-allantoic membrane was extensively folded and interdigitated with corresponding folds of the uterine mucosa (Fig. 38). Both the uterine and the chorio-allantoic folds were richly supplied with blood vessels.

In the space between the amnion and the chorio-allantoic membranes were many discrete pustules some of which were closely associated with the allantoic mesenchyme from which some originated (Fig.39 a, b, 40). Some, however, originated from the amniotic mesoderm (Fig. 41a). Histologically these pustules consisted of coiled layers of fibrous tissue with darkly staining nuclei. They were avascular and contained no glycogen granules.

12. THE UMBILICAL CORD

The umbilical cord of the full term cane rat foetus measured 2.0 to 2.5 cm. in length and consisted of five major blood vessels: two umbilical arteries (of nearly equal diameter) joining the

external iliac arteries; one large umbilical vein running into the foetal liver and two vitelline vessels, (an artery and a vein) which ran into the foetal intestine (Fig. 41c). There were a few blood vessels scattered in the stroma of the umbilical cord. These vessels were supplied and drained by the umbilical vessels. No nerves were observed in the umbilical stroma. The cord was covered by simple squamous epithelium on its outer surface. Where the amnion was reflected over the chord numerous discrete pustules were found.

13. THE AMNION

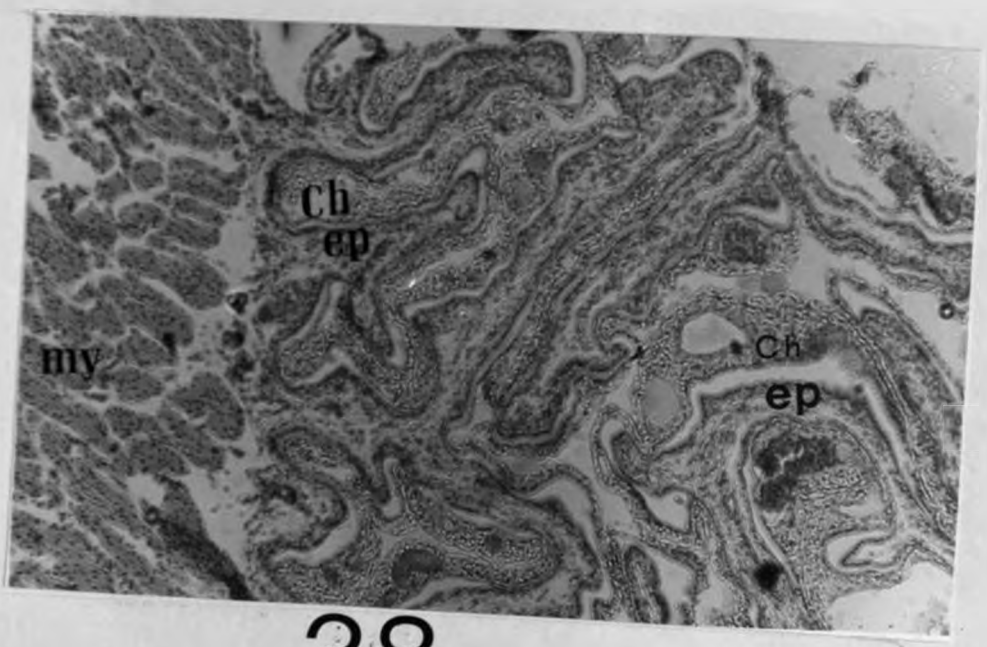
The amniotic membrane was avascular. In some areas it was closely apposed to the chorio-allantois. In such areas there appeared to be a fusion of the amniotic and chorio-allantoic mesoderm. But careful examination did not reveal any extensions of chorio; allantoic vessels to the amnion (Fig. 41b). On its mesenchymal surface the amnion awas also associated with discrete while pustules which were histologically similar to those of the chorio-allantois.

Fig. 38

A photomicrograph to show the extensive interdigitations between the chorio-allantoic membrane (ch) and maternal uterine mucosa. The uterine epithelium is intact.
ep = uterine epithelium; my = myometrium.
H & E X 200.

Fig. 39a

A gross picture of the conceptus of cane rat CR 3 in mid-pregnancy. Note the white pustules on the foetal membranes (arrow).
sp = region of subplacenta. X 10.



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39a

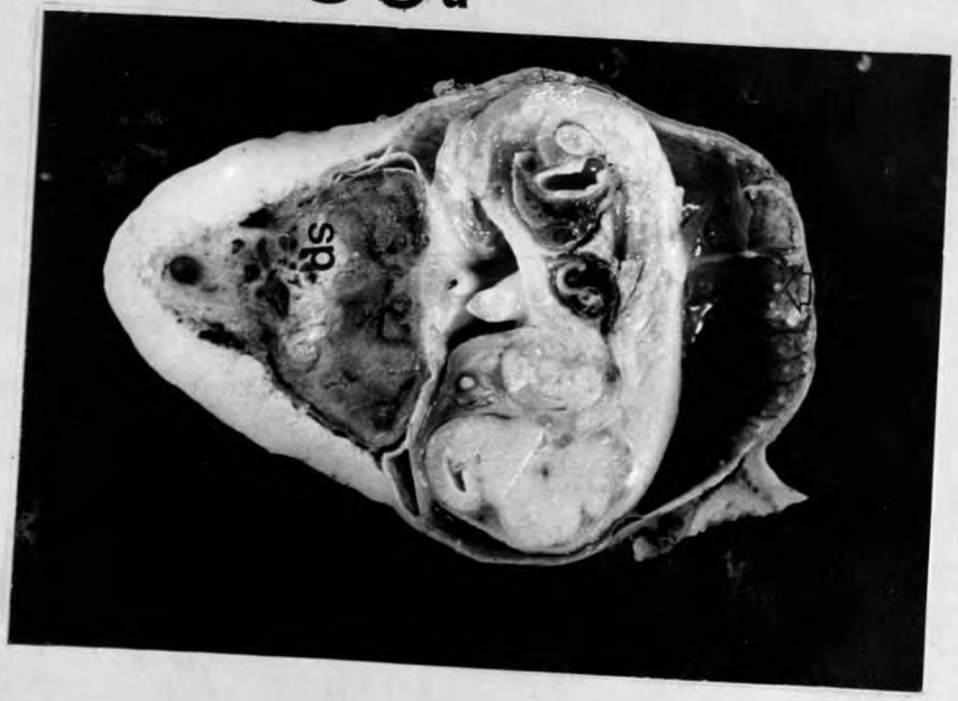
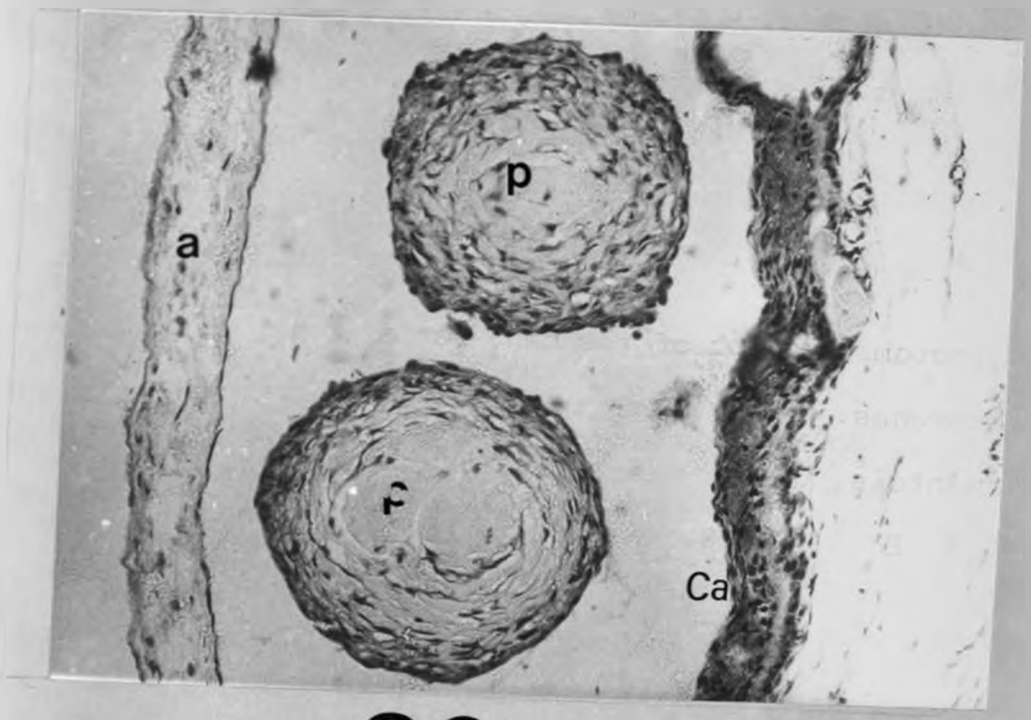


Fig. 39b

A photomicrograph of the cane rat foetal membranes. a = amnion; ca = chorio-allantois; p = pustules.
H & E X 200.

Fig. 40

A photomicrograph of the cane rat foetal membranes. Note the pustule (p) originating from the chorio-allantoic mesenchyme (ca). a = amnion. H & E X 75.



39b

40

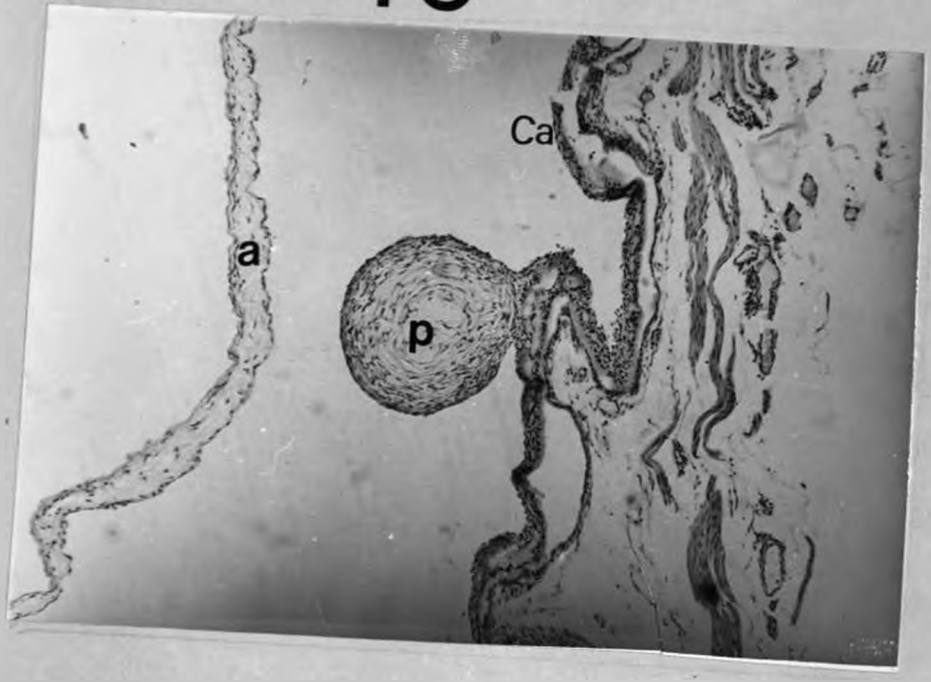


Fig. 41a

A photomicrograph of the cane rat foetal membranes. Note the origin of the pustule (p) from the amniotic mesenchyme (a).

ca = chorio-allantois

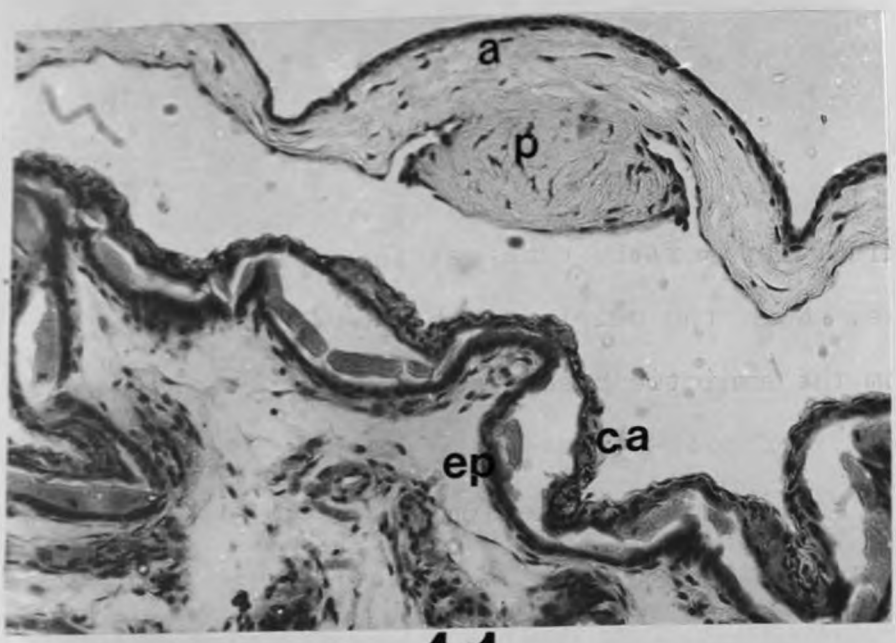
ep = uterine epithelium

H & E X 75.

Fig. 41b

A photomicrograph to show the close fusion between the amnion (a) and the chorio-allantois (ca). fv = foetal blood vessel.

H & E X 200.



41a

41b

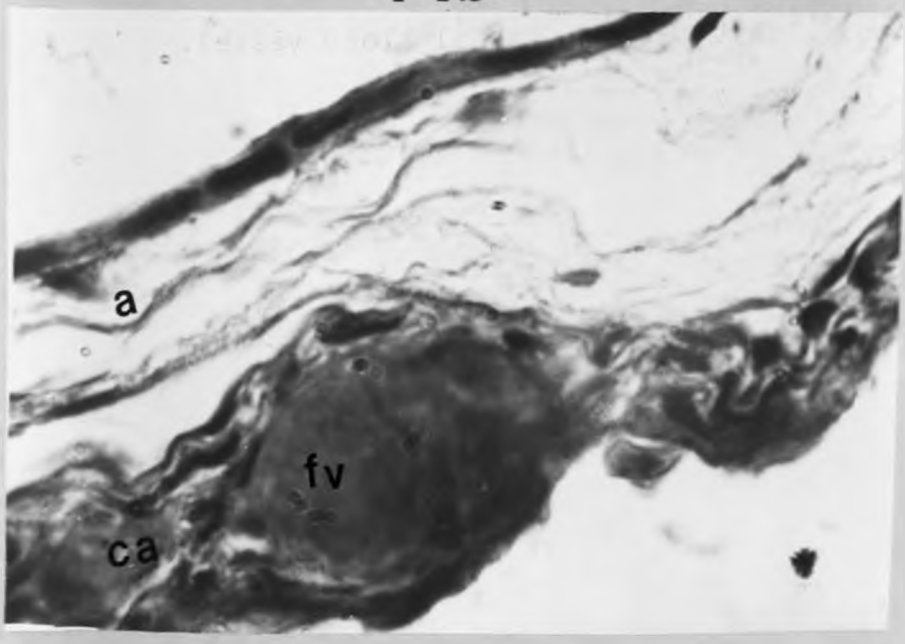


Fig. 41c

A section of the umbilical cord of the cane rat foetus showing five blood vessels:

ua = umbilical artery;

uv = umbilical vein;

vv = vitelline vessels;

PAS stain. X 75.

41c

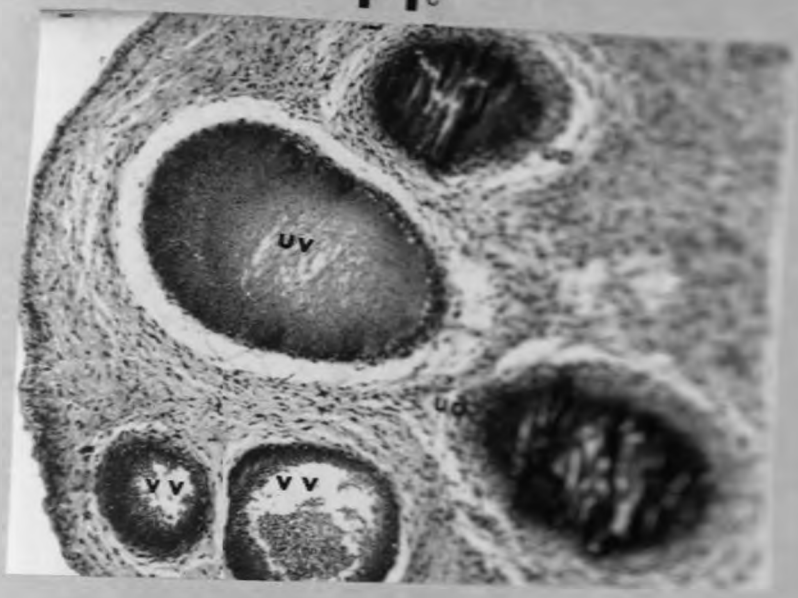


Fig. 41c

A section of the umbilical cord of the cane rat foetus showing five blood vessels:

ua = umbilical artery;

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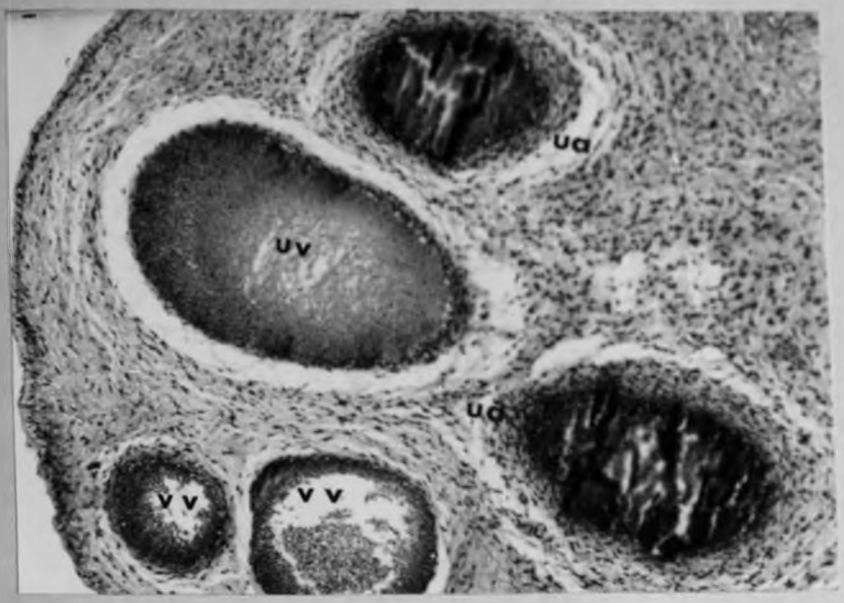
vv = vitelline vessels;

PAS stain. X 75.

41c



14. THE DECIDUA BASALIS AND THE GIANT CELLS

In the cane rat, a definite decidua capsularis was not observed but the reaction in the decidua basalis was remarkable. There were two types of giant cells in the decidua. One type consisted of aggregates of darkly staining nuclei numbering up to five or more - with very little cytoplasm. These cells were confined to the junctional zone and immediately below it. They resembled the trophoblastic cells of the subplacenta from which they seemed to originate (Fig. 37a, b). The second type of giant cells were found deep in the uterine enlargement but cells were not present in the anti-mesometrial uterine wall. It has already been mentioned that even at the implantation stage the stromal cells of the maternal decidua had become hypertrophied and some were binucleated (Fig. 42a). In later stages numerous multinucleate giant cells could be observed throughout the myometrium of the mesometrial and lateral uterine wall. Similar giant cells were also present in the mesometrium (Fig. 42b, c). These giant cells had large nuclei and prominently vacuolated cytoplasm. They did not seem to contain any PAS-positive granules in their cytoplasm. In Goldner and toluidine blue stained sections their cytoplasm contained fibre-like structures that were not arranged in

any organised manner (Fig. 42d, e). These large cells were so many that they musked the muscularis. Towards end of pregnancy as shown in specimen of cane rat I these cells could be seen intermingled with smooth muscle fibres of the muscularis (Fig. 42f). Many of them had reduced in size and no longer contained the fibre-like structures of the earlier stages.

15. THE JUNCTIONAL ZONE

The junctional zone lay between the placenta and the decidua basalis. It was composed largely of amorphous necrotic material that was acidophilic and PAS-positive. In this necrotic mass were numerous remnants of nuclear material and other cellular debris (Fig. 22 & 37b). Large maternal blood vessels passed through the junctional area. The junctional zone formed and intimate foeto-maternal contact. There were trophoblastic giant cells in the junctional zone as already mentioned.

16. THE PATTERN OF CIRCULATION

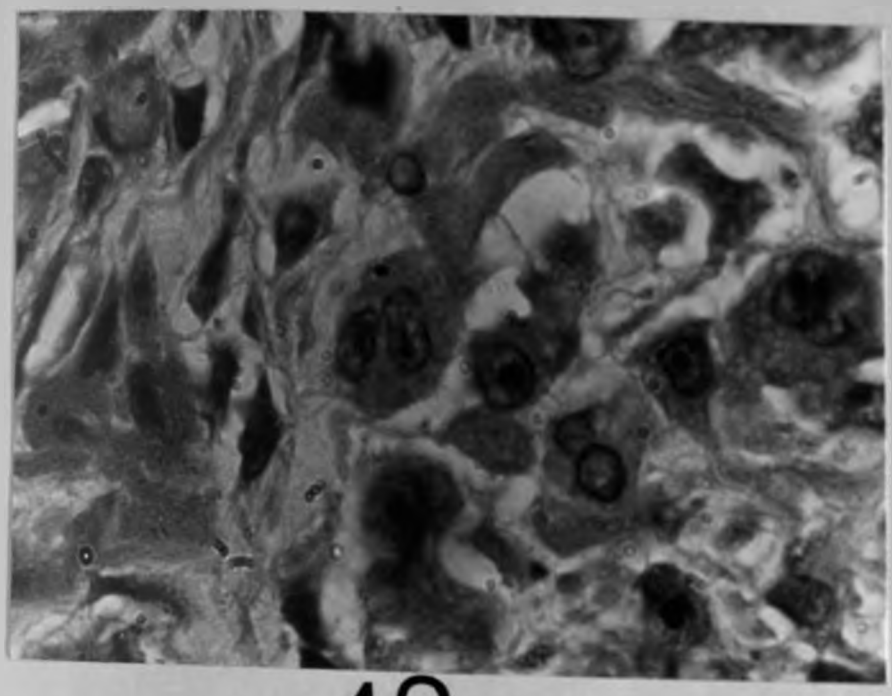
Arterial blood from the uterine artery passed through the decidua basalis, running through the subplacenta as a single vessel. As it approached the foetal surface of the placental disc the artery

Fig. 42a

A photomicrograph of the decidual reaction in early pregnancy of cane rat CR 12. Note the large binucleate giant cells.
H & E. X 1250 (oil emersion).

Fig. 42b

A photomicrograph of the decidual reaction in midpregnancy of cane rat CR 3. Note the numerous giant cells. H & E X 200.



42a

42b

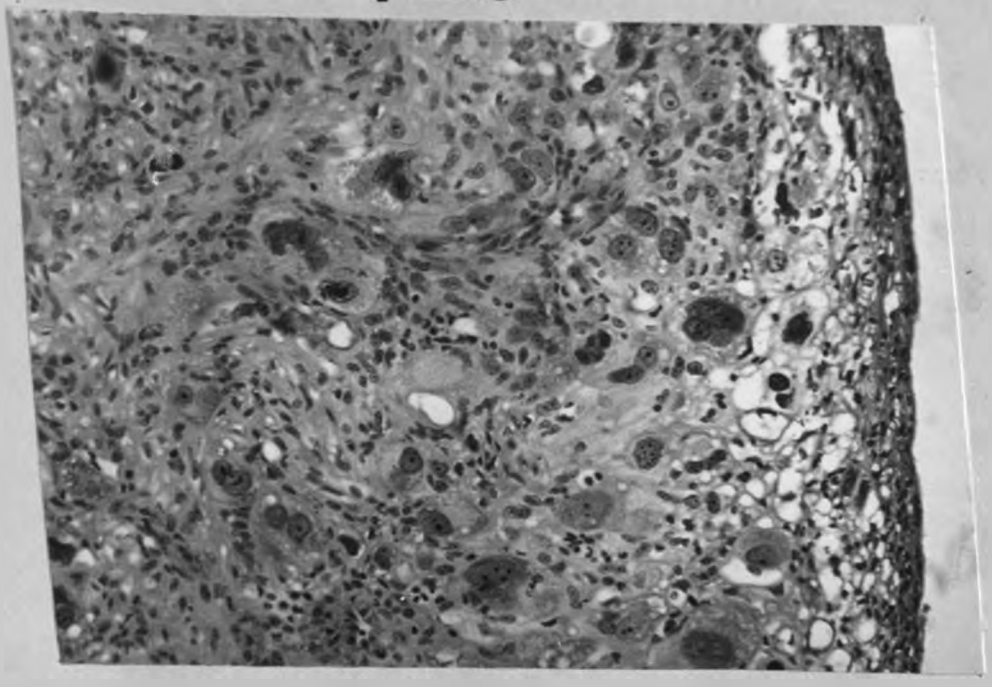
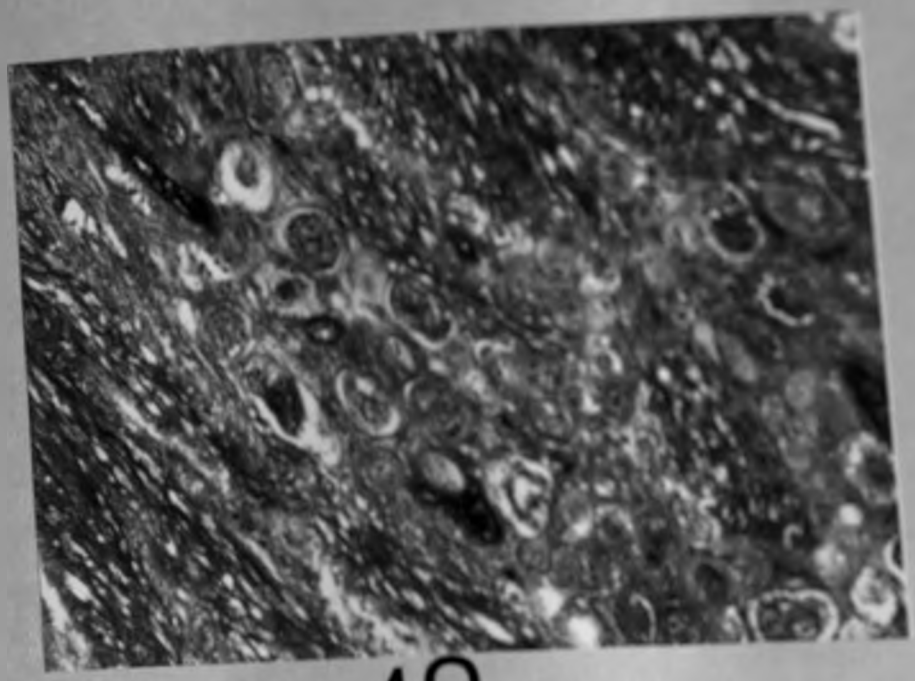


Fig. 42c

A section of the decidual reaction in pregnant cane rat showing the large giant cells. Intermingled with smooth muscle cells.
Goldner stain. X 2000.

Fig. 42d

A section of the decidual reaction as in 42c, showing the large giant cells with fibre like structures in their cytoplasm.
Mason trichome. X 1,250 (oil emersion).



42c

42d

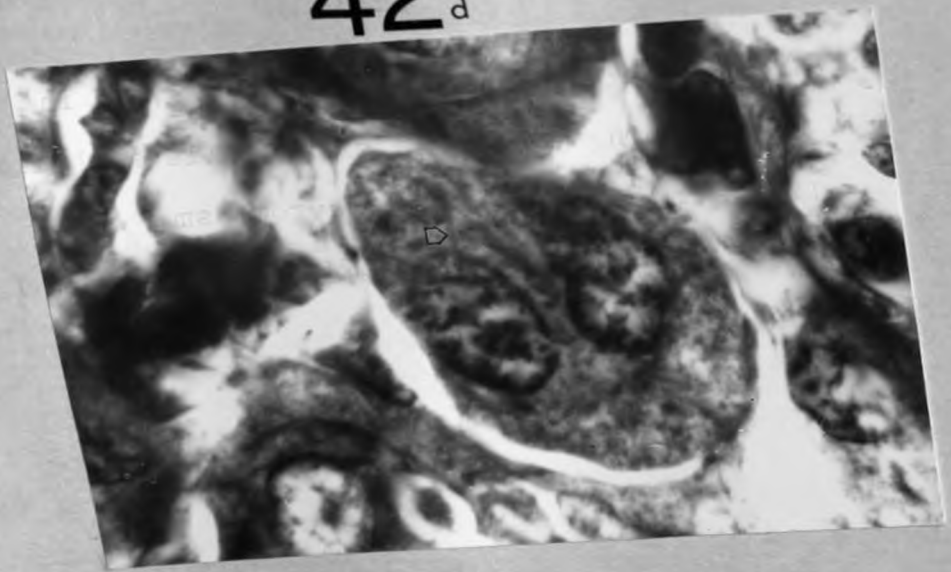


Fig. 42e

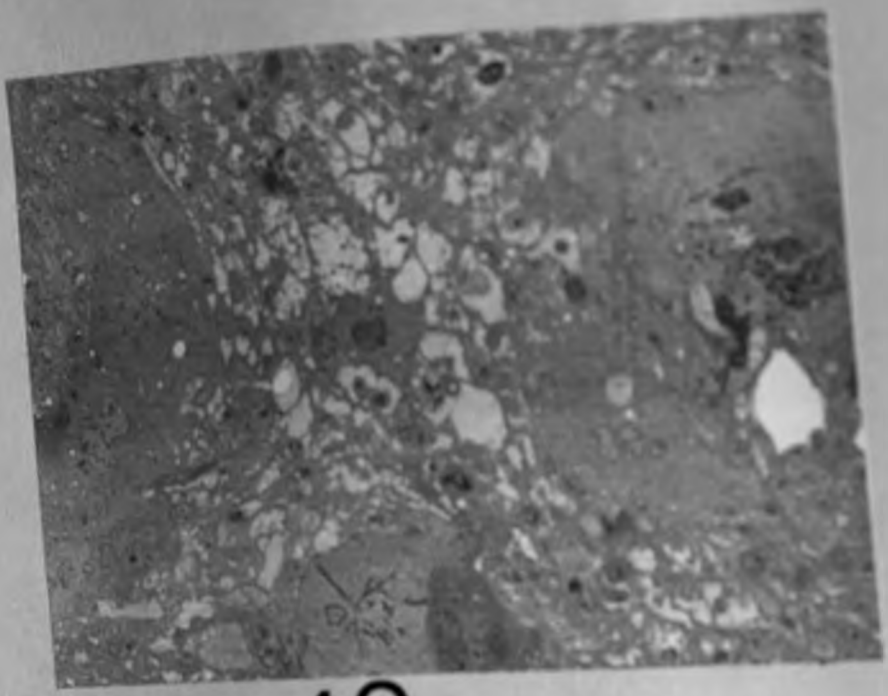
A photomicrograph of the decidual cells with prominent fibre like structures in their cytoplasm.

Toluidine blue stain. X 200.

Fig. 42f

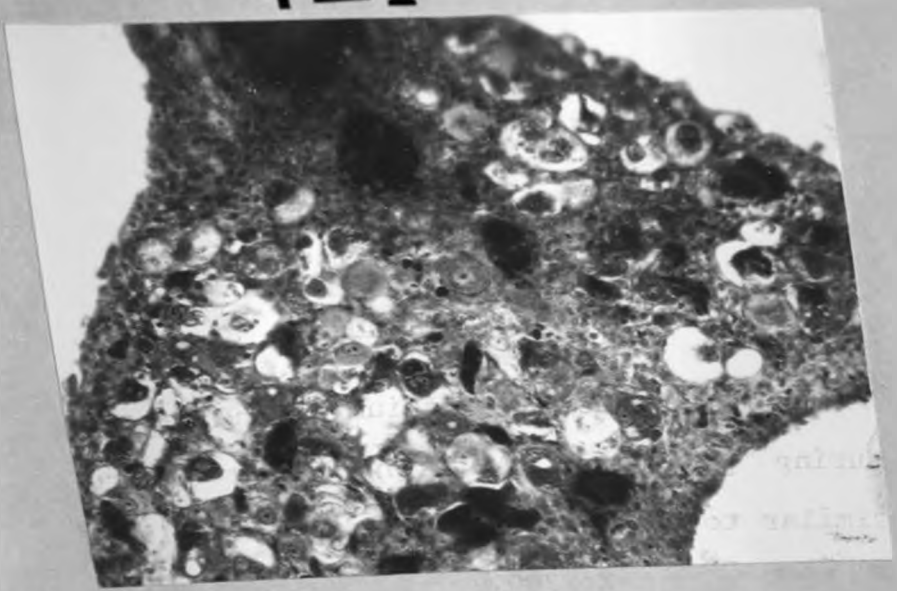
A section of the mesometrium of the cane rat during pregnancy. Note the giant cells similar to those in the decidua basalis.

H & E X 200.



42e

42f



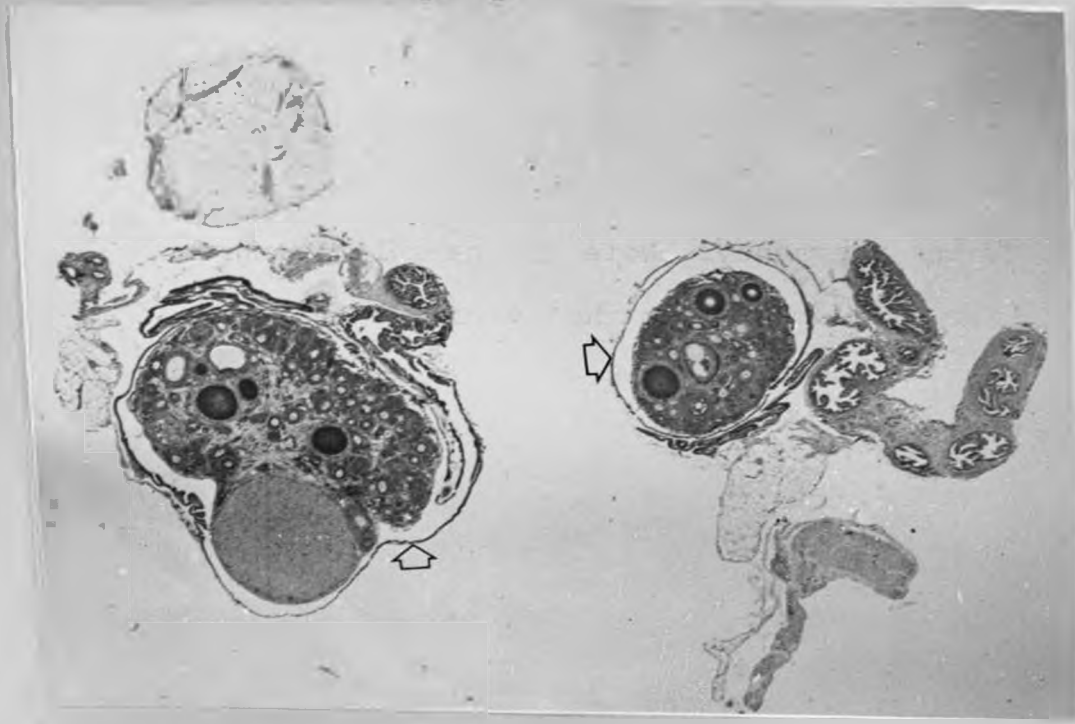
divided into left and right branches (Fig. 43). These branches then gave off smaller branches which supplied each placental lobe as the maternal arterial channel. Flowing through the trophoblastic labyrinth the maternal blood came into close contact with the foetal capillaries from which it was separated by one to three layers of trophoblast (Fig. 24). The foetal vessels first ran into the central zone of each lobe whence capillaries were given off in the opposite direction to the maternal blood. That is, while the maternal blood flowed towards the base of the placenta, the foetal blood flowed towards the top of the placenta thus establishing a "counter-current" flow. The maternal blood filtered through the spongy zone and ran towards the periphery where it was collected in large veins. Such veins could be seen in the vicinity of the subplacenta. Back in the decidua basalis these venous channels coalesced into one or two veins that passed into the mesometrium.

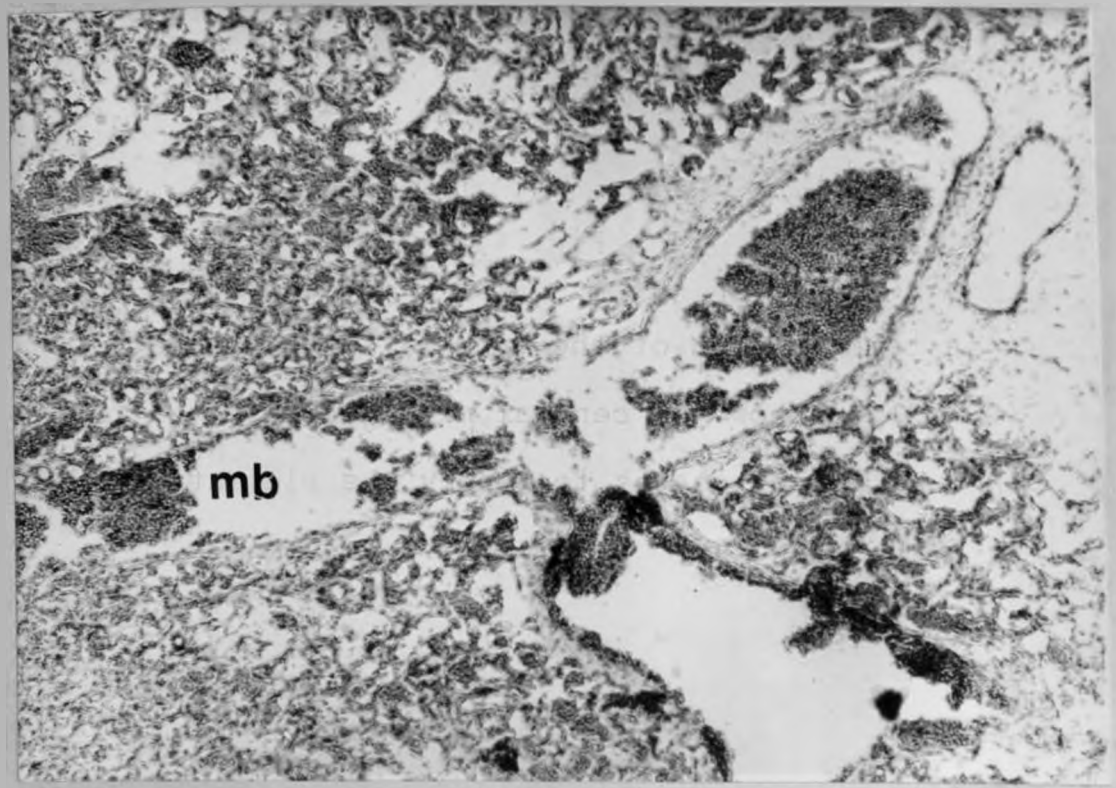
Fig. 43

A photomicrograph of the cane rat placenta (CR 13) to show the central maternal artery (mb) as it bifurcates to supply the placenta. H & E X 75.

Fig. 44

A low power picture of the ovaries of R. chrysopygus. Only one CL was present during pregnancy. Note the capsular membrane (arrows) surrounding the ovaries.





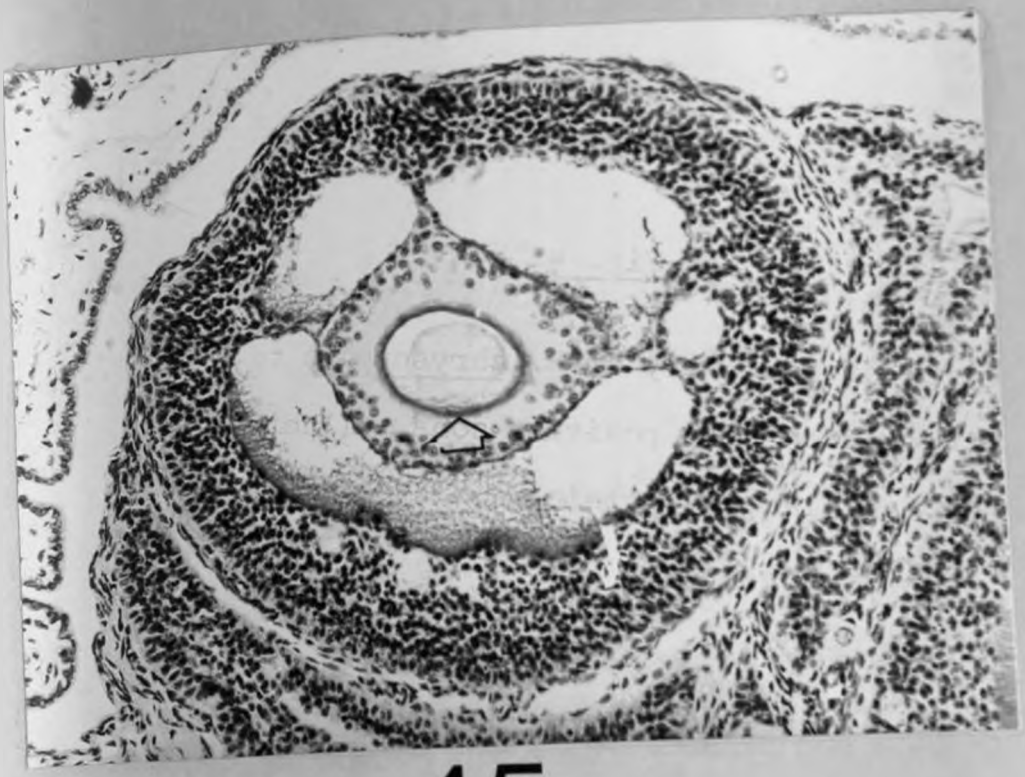
43

Fig. 45

A section of the ovary of R. chrysopygus to show the alcian blue positive zona pellucida (arrow). Alcian Blue stain. X 200.

Fig. 46

A low power photomicrograph of the ovary of E. rufescens to show the two corpora lutea in the right ovary (arrows). There was no CL in the left. The animal had two conceptuses. Note also the lobulation of the ovary.
H & E X 10.



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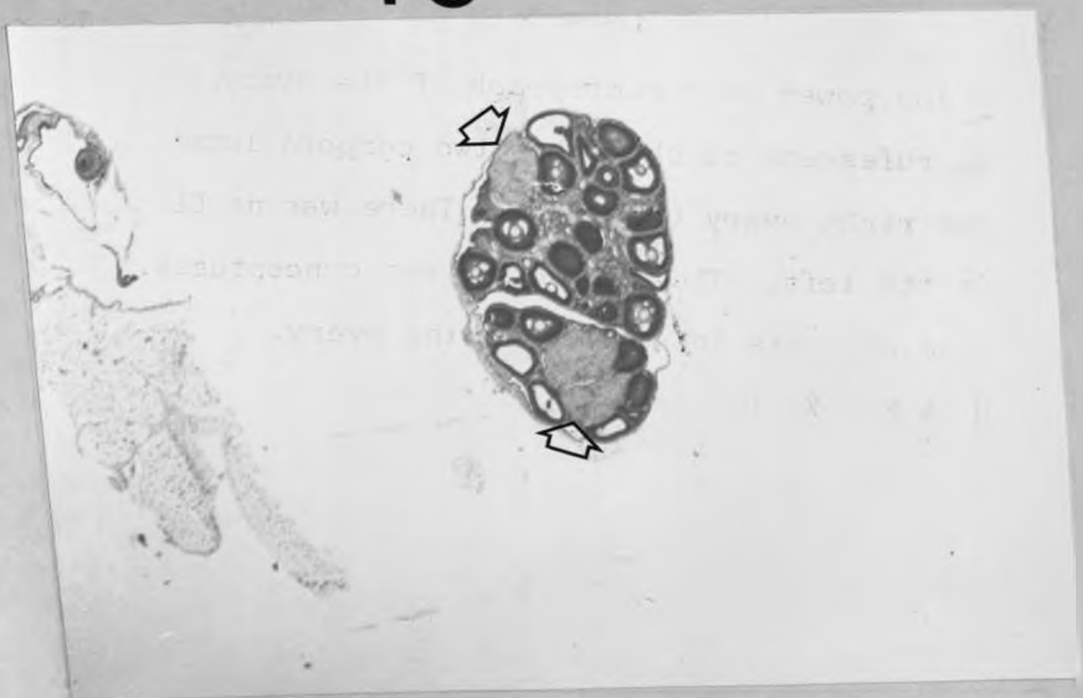


PART II. THE ELEPHANT SHREWS (MACROSCELIDIDAE)

The three species of elephant shrews namely Rhynchochon chrysopygus, Petrodromus tetradactylus and Elephantulus refuscens studied in this thesis are characterised by their long thin legs, large eyes, long tails and long flexible proboscis-like noses. However, they differ in their colour, sizes, body weights and habitats. R. chrysopygus is dark amber in colour, has black leg and feet and a distinct gold coloured rump patch. It is the largest of the three species under study having a mean total length of about 50 cm and a mean body weight of about 540 g. (Rathbun, 1976). It lives in forest areas along the Kenyan North Coast. P. tetradactylus is sandy brown in colour, has a well defined spectacles of white around the eyes and a soft fur. It has a mean total length of about 36 cm and a mean body weight of 210 g. (Rathbun, 1976). P. tetradactylus and R. chrysopygus live in the same locality. E. rufescens is a reddish in colour, has a white ocular ring which contrasts sharply with the black eyes ± hence it is also called the "spectacled elephant shrew". It is the smallest of the three species with a mean total length of about 25 cm and body weight of about 55 g. (Rathbun, 1976). Unlike the other two, E. rufescens lives in dry

bushland areas away from the coastal strip. For more details of their habitats, Rathbun (1976) should be consulted.

1. THE OVARIES OF ELEPHANT SHREWS

The ovaries of elephant shrews studied were compact and lobular. They were surrounded by the ovarian capsule which was formed from the expanded infundibulum and was lined by a ciliated cuboidal epithelium. In pregnant females the ovary with the corpus luteum was much larger than its opposite number and tended to fill the capsular cavity so that the capsular epithelium was closely applied to the germinal layer. In the three species studied the ovaries contained numerous follicles. In mature follicles, the oocytes measured 65 to 70 um in E. rufescens and 70 to 80 um in R. chrysopygus and P. tetradactylus. The zona pellucida was strongly alcian blue and PAS-positive while the cells of the corona radiata were only PAS-positive. The oocyte cytoplasm was vacuolated. These vacuoles may represent extracted yolk or lipid droplets. In R. chrysopygus and P. tetradactylus only one egg was ovulated at each oestrus cycle as evidenced by the invariable presence of only one corpus luteum in one of the ovaries (Fig. 44, 45). In E. rufescence a maximum of two eggs were ovulated,

one from each ovary. Fig. 46 shows two corpora lutea in one ovary. This particular anímal, E.r. 550 had two conceptuses, one in each horn: the two corpora lutea were in the right ovary while the left had none. This demonstrated an instance of ovum migration to the opposite uterine horn.

2. THE UTERINE HORNS

The uterine horns of R. chrysopygus were short stout bodies. Externally they seemed to be joined but a distinct groove down the middle indicating that the two horns were separated internally. In the non-pregnant state, they measured between 9 and 15 mm at their widest cross section.

The uterine horns of P. tetradactylus were long slender bodies which measured on the average 10 mm. The horns did not taper gradually into the oviduct. There was an abrupt and clear demarcation at the utero-tubal junction.

3. THE PREGNANT UTERINE HORN

Rhynchocyon and Petrodromus species invariably carried one embryo per pregnancy but with no preference to one horn or the other. Implantation was restricted to a localised area at the caudal end of each uterine horn. In late gestation, the pregnant

Fig. 49a

A higher magnification of the inset in Fig. 48 to show the growth of the allantois (al) and its spread over the chorionic placent (p). H & E X 75.

Fig. 49b

The chorionic placenta of Rc 353. Note the giant cells (g) in the trophoblastic lacuna. H & E X 200.

Fig. 49c

A high power section of the zone of proliferati Note the cell in anaphase.
H & E stain. X 1,250 (oil emersion)

Fig. 47a

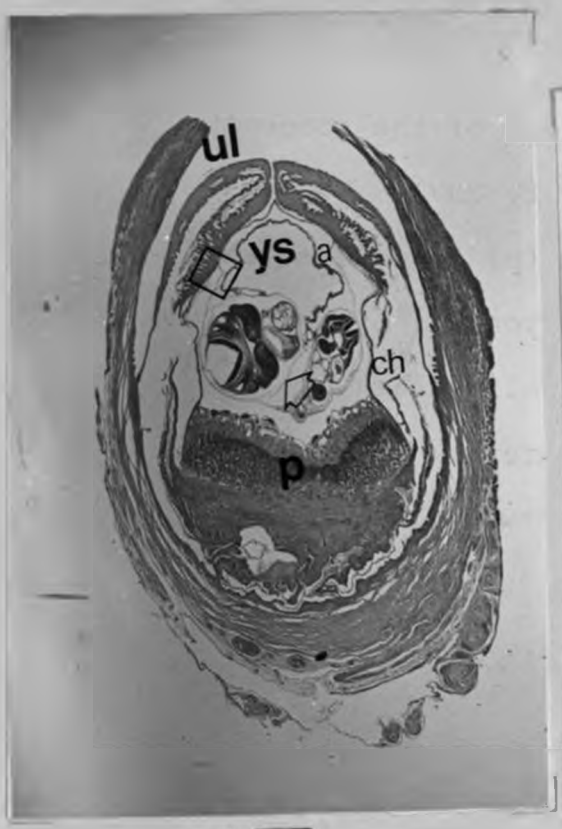
A low power photomicrograph of the conceptus of P. tetradactylus - (p.t. 367). The chorioallantoic placenta (p) is well established in the bursa embryonica.
ys = yolk sac; a = allantois;
ch = chorion; ul = uterine lumen.
Note the communication between the bursa embryonica and the main uterine lumen.
Amnion is shown by the arrow.

Fig. 47b

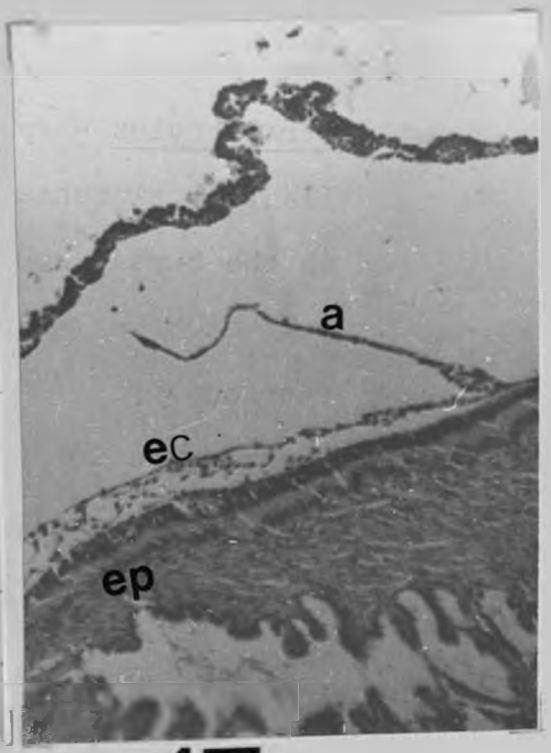
A higher picture of inset in Fig. 47a. Note the close relationship between the uterine epithelium (ep) and the chorio-allantois (ec) constituting an epithelio-chorial placenta.
a = allantois. H & E X 200.

Fig. 48

A low power photomicrograph of the bursa embryonica of R. chrysopygus, (Rc 353).
Embryo is in the primitive streak stage.
ul = uterine lumen. H & E X 10.



47a



47b

48

blood spaces there were aggregates of darkly staining cells. These cells had very large nuclei and very little cytoplasm. Their nuclei, which occupied almost the entire cells, had compact chromatin material that completely masked the presence of nucleoli.

Many cells in this zone were actively undergoing various stages of mitotic division (Fig. 49c). It was a center of active proliferation and probably these cells gave rise to the syncytial trophoblast of the spongy zone.

4. THE DEFINITIVE CHORIO-ALLANTOIC PLACENTA

The architecture of the definitive placenta in the three species of elephant shrews was basically similar. The placenta was discoidal and lobulated and histologically could be divided into four main zones:-

a) a prominent and well differentiated columnar zone;
b) a zone of proliferation;
c) a spongy zone;
d) an attachment zone (Fig. 50a, 51).

a) Columnar zone;

The columnar layer comprised about one half of the placenta. It was thicker in the centre but narrower at the margins. The tall columns were composed of syncytiotrophoblast separating the maternal blood from the foetal allantoic vessels (Fig. 52 a, b). In gluteraldehyde-fixed materials the maternal and foetal circulations were separated only by one layer of syncytial trophoblast, foetal connective tissue and the foetal or allantoic endothelium; hence the placental organisation is of the haemo-monochorial type (Fig. 53). The allantoic mesenchyme came to an abrupt end at the junction with the zone of proliferation. In near term placenta when the zone of proliferation had disappeared, the junction between the columnar and spongy zones was marked by numerous well defined foetal collecting capillaries (Fig. 54). It was only in the columnar zone that the foetal and maternal blood streams ran side by side and this therefore was the main area for physiological exchange. The syncytial trophoblast of the columnar layer was continuous with that of the spongy layer, but the marked divergence in the structure of the two layers was so profound that even at low power magnification the distinction between the two was obvious.

b) The Zone of Proliferation

The zone of proliferation was already well defined when the embryo was in the primitive streak stage (Fig. 49 a). It was a distinct zone of darkly staining cells that intervened between the columnar and spongy zones (Fig. 52 a). It has already been mentioned that without the oil immersion, this region gave the impression that the nuclei were embedded in cytoplasm thus forming a syncytium. But under oil immersion these were individual cells which had exceptionally large nuclei and very little cytoplasm. It was an active center of cell proliferation. In the later stages of gestation this zone disappeared (Fig. 54).

c) The Spongy Zone

This zone comprised the other half of the placenta. It consisted of a maze of relatively broad cords that were directly bathed by the maternal blood. The syncytial cords were continuous with the syncytium of the columnar zone. The cytoplasm contained eosinophilic granules and the nuclei were prominent and vesicular. The blood spaces in the spongy zone got larger towards the zone of attachment (Fig. 52a, b).

d) The attachment Zone

There was a marked difference in the attachment zone between Rhynchocyon on the one hand and Petrodromus and Elephantulus on the other. In Rhynchocyon, the attachment zone consisted of a basal layer of trophoblast resting against a distinct layer of hyaline connective tissue (Fig. 50a, b). The basal layer was composed of tall columnar cells with elongate and vesicular nuclei. The cytoplasm contained PAS-positive, diastase labile granules which were interpreted as glycogen. That this basal layer was not part of the uterine epithelium was borne out by the observations that:

a) the tall columnar cells rested on a well defined PAS-positive basement membrane situated on the placental side and

b) the basal layer was continuous with the chorion at the periphery of the placental disc (Fig. 50c).

Aggregates of cells were interposed at intervals between the basal layer and the maternal hyaline connective tissue. These cells had dark staining nuclei and eosinophilic cytoplasm. Some were seen deep in the basal layer and also deep to the maternal hyaline connective tissue. Uterine glands

Fig. 49a

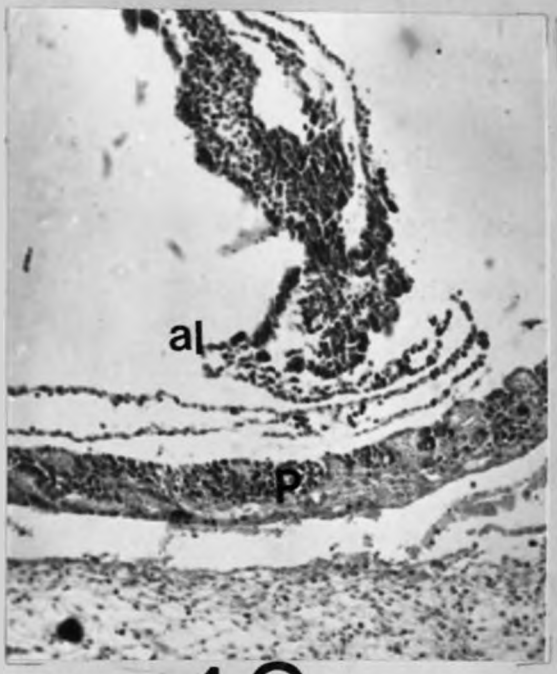
A higher magnification of the inset in Fig. 48 to show the growth of the allantois (al) and its spread over the chorionic placenta (p). H & E X 75.

Fig. 49b

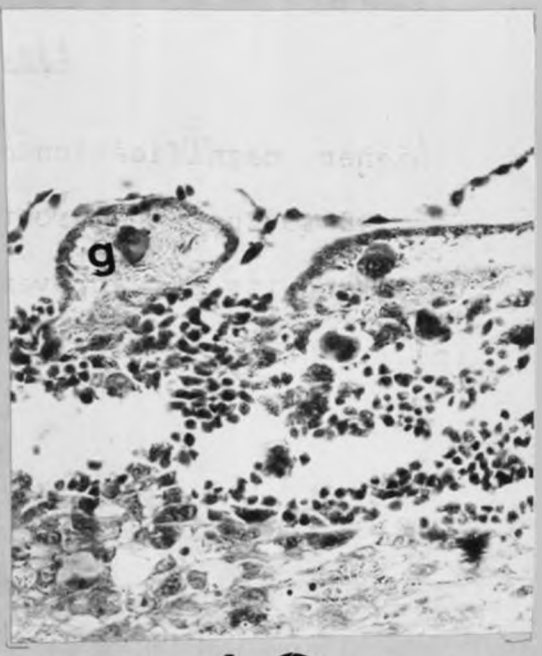
The chorionic placenta of Rc 353. Note the giant cells (g) in the trophoblastic lacuna. H & E X 200.

Fig. 49c

A high power section of the zone of proliferation. Note the cell in anaphase.
H & E stain. X 1,250 (oil emersion)



49a



49b

49c

Fig. 50a

A section of the chorio-allantoic placenta of R. chrysopygus to show the various zones. cz = columnar zone; sz = spongy zone; bt = basal trophoblast; mh = maternal myeline. layer.

H & E X 75.

50a

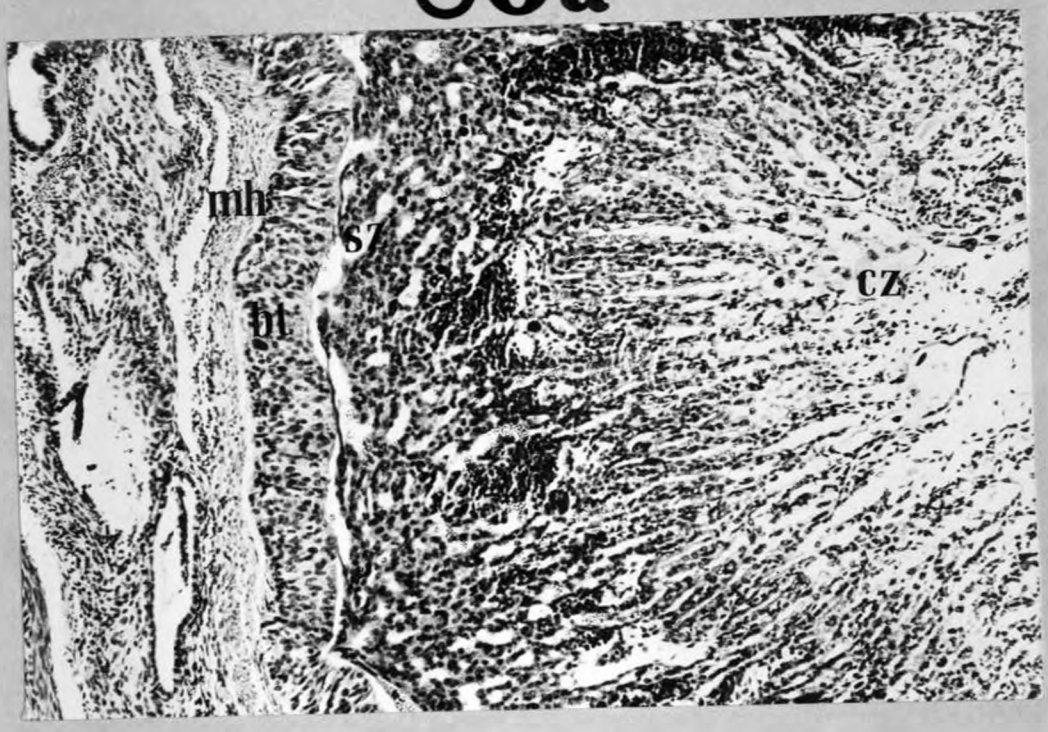
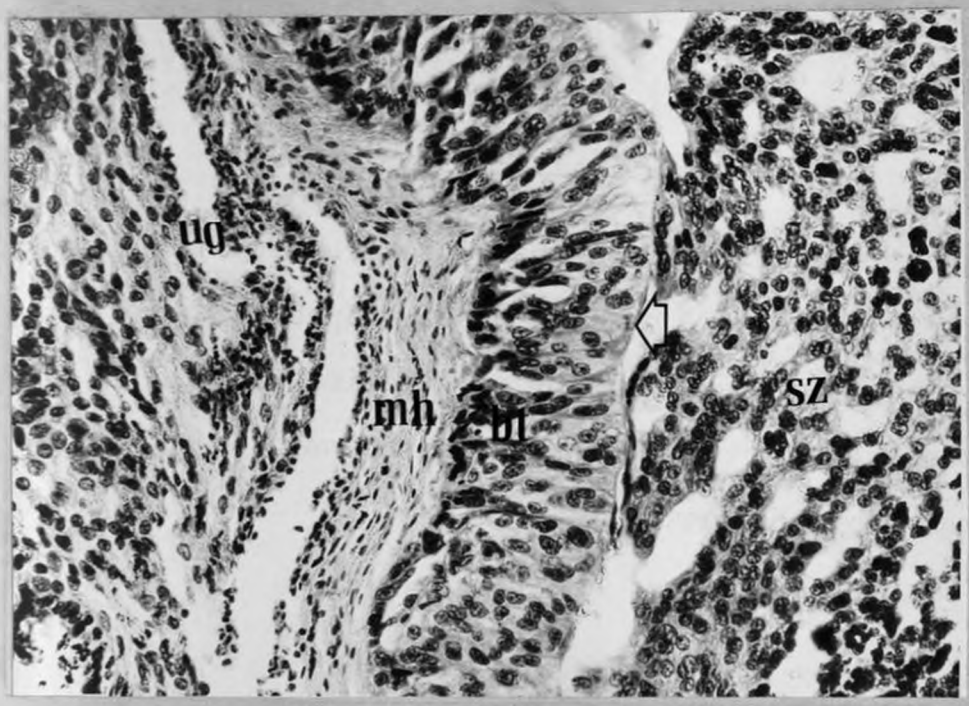


Fig. 50b

A higher magnification of basal layer, region: sz = spongy zone; bt = basal trophoblast; mh = maternal hyaline; ug = uterine gland. The basal lamina of the basal trophoblast is indicated by the arrow.

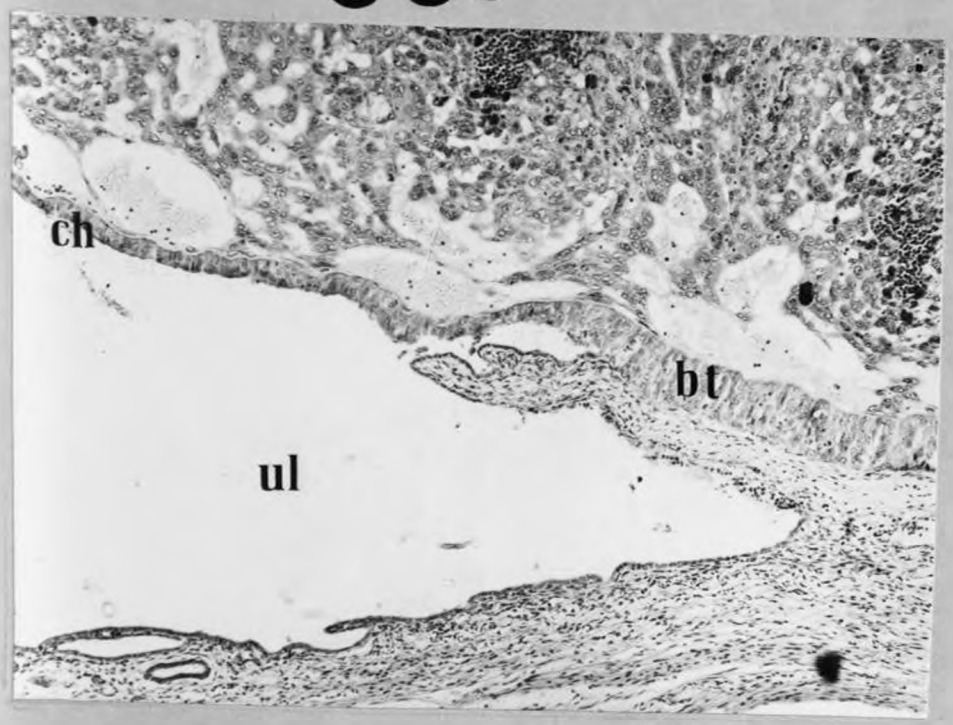
Fig. 50c

A section of the chorio-allantoic placenta of R. chrysopygus to show the continuity of the basal trophoblast (bt) and the chorion (ch) at the placental periphery. ul = uterine lumen. H & E X 75.



50b

50c



persisted throughout gestation. These glands were extensively dilated and lined by a simple cuboidal epithelium. They contained fibrinoid material that was Alcian blue positive. Apart from the formation of the hyaline layer, there was no significant decidual reaction in the uterine mucosa after the embryo had developed beyond the primitive streak stage.

In Petrodromus and Elephantulus, the attachment region presented a different picture. Like Rhynchocyon, the definitive placenta could be divided into the columnar, proliferative and spongy zones. Instead of the basal layer however, they had a necrotic zone consisting of degenerating trophoblastic cells: These cells had lost their cellular integrity. There was extensive vacuolation of the cytoplasm and pyknosis of the nuclei (Fig. 55). The zone of degeneration phased out into the junctional zone consisting of necrotic material embedded in an amorphous, eosinophilic, PAS-positive substance.

5. THE YOLK SAC

The specimens available to me showed that the yolk sac in the Macroscelididae was separated from the uterine mucosa by the chorioallantois (Fig. 47, 48, 56). The yolk sac was a prominent structure in the early stages of gestation but did

not exhibit "entypy" which is so characteristic of the hystricomorph rodents. It was richly vascularised and consisted of cuboidal to tall columnar epithelium which in some instances seemed to be pseudostratified; the nuclei being placed more towards the apical zone. The yolk sac was surrounded by the allantois on the upper and amnion on the lower sides. Adistinct circular line marked the point of contact between the yolk sac and amnion (Fig. 47, 56a, b).

6. THE CHORION

In the three species of elephant shrews that were studied, the chorion, surrounded the embryo completely. In Rhynchocyon the chorion was contiuous with the basal layer while in Petrodromus and Elephantulus it came into contact with the placenta at the zone of degeneration (Fig. 47a,57). The chorion was richly vascularised in the three species. In Elephantulus the chorion was multilayed and was characterised by numerous aggregates of nuclei constituting massive giant cells (Fig. 57). In Petrodromus the vascularised chorionic epithelium was in such close apposition to the uterine lumen between them (Figs. 47 a, b). The chorionic

cells in such places were tall and the nuclei were concentrated towards the basal region. A high degree of physiological exchange was probable and the relationship constituted an epithelio-chorial placenta. The uterine glands in the endometrium of the decidua pseudocapsularis were extensively dilated. This particular specimen also showed an area adjacent to the epithelio-chorial relationship where the non-vascularised chorion was in intimate appcsition to the uterine epithelium, a situation which could be termed "chorionic placenta". This particular specimen of Petrodromus presented three very interesting relationships:

a) the haemochorial placenta which must, of necessity, have been the main physiological exchange area;

b) the epitheliochorial placenta which persisted to later stages of gestation;

c) the chorionic placenta which was temporary and was soon replaced by the epithelio-chorial placenta.

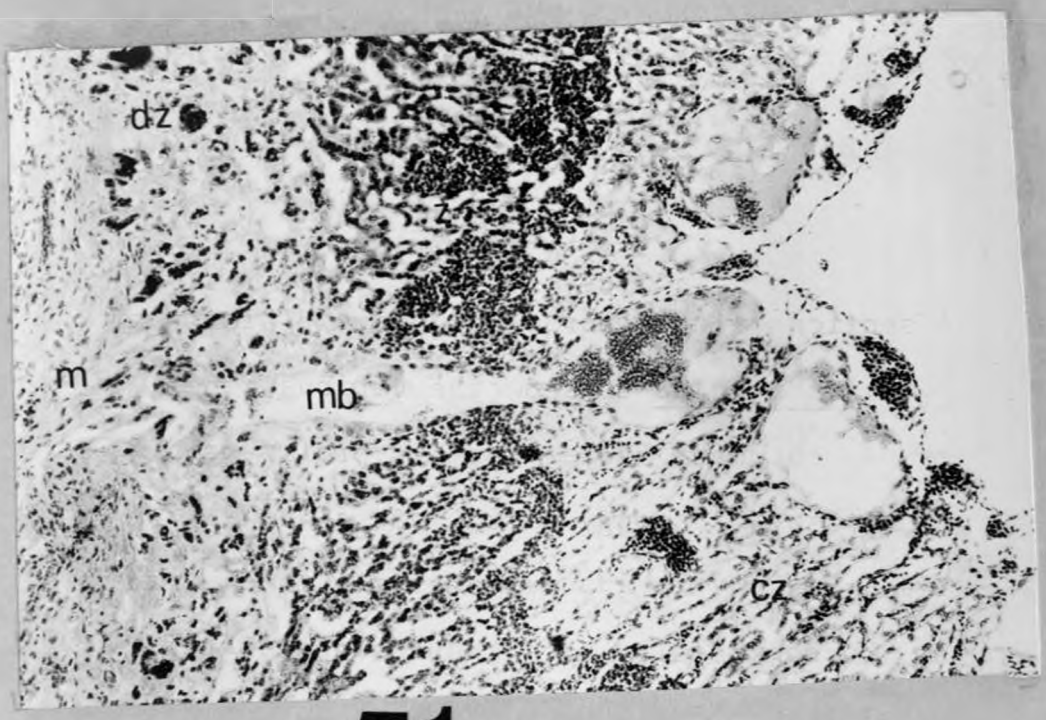
The embryo was still in the bursa embryonica but the chorion was beginning to make its way through the communicating channel between the main uterine cavity and the bursa embryonica (Fig. 47a). The

Fig. 51

A section of the chorio-allantoic placenta of P. tetradactylus to show the various zones. cz = columnar zone; pz = proliferative zone; sz = spongy zone; dz = zone of degeneration; m = myometrium; mb = maternal blood vessel. Note the absence of the basal trophoblast. H & E X 75.

Fig. 52a

A section through the placenta of R. chrysopygus. cz = columnar zone; pz = proliferative zone; sz = spongy zone. H & E X 75.



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52a

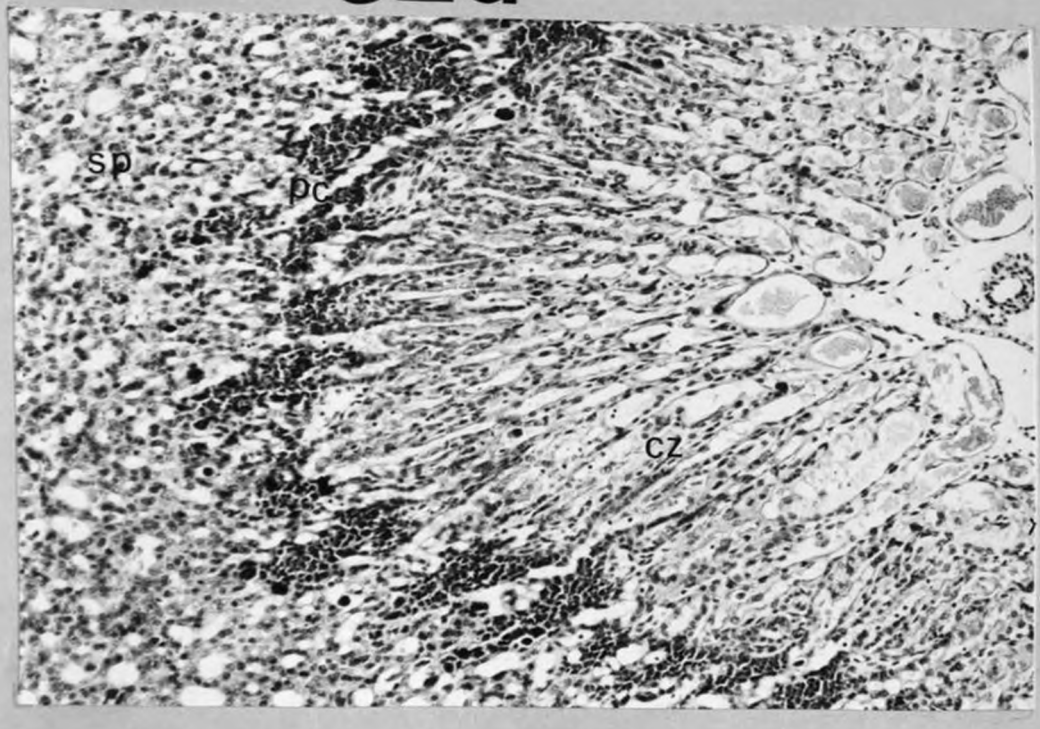


Fig. 52 b

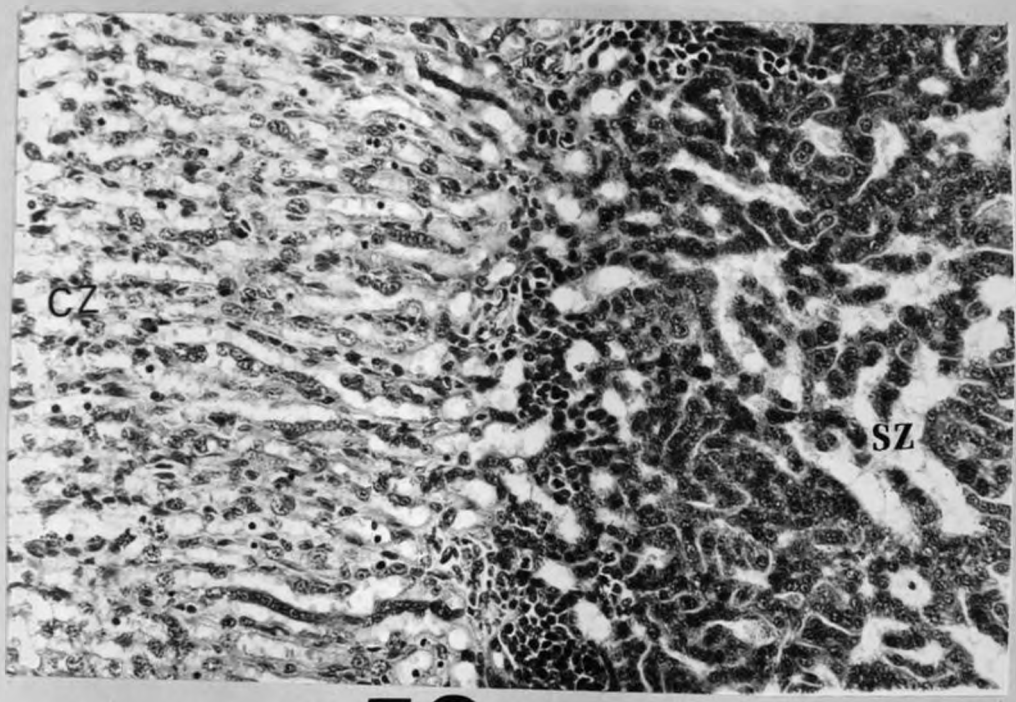
A high magnification of Fig. 52a.

H & E X 200.

Fig. 53

A photomicrograph of the placenta of *R. chrysopygus* to show the haemo-monochorial condition. mb = maternal blood space; fc = foetal capillary.

Toluidine blue stain. X 200.



52b

53

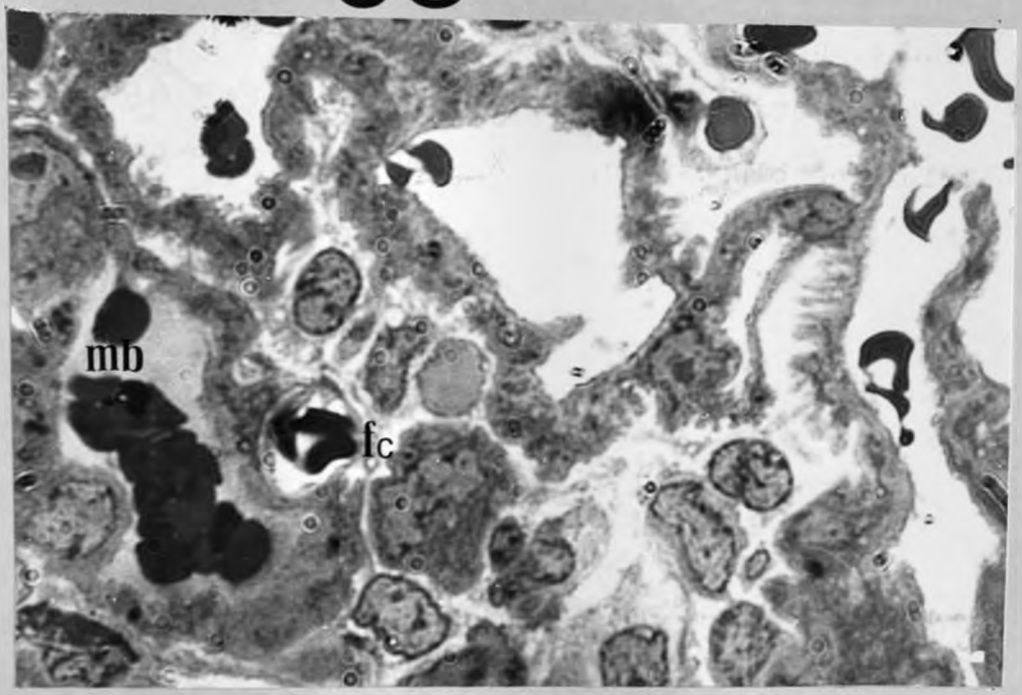


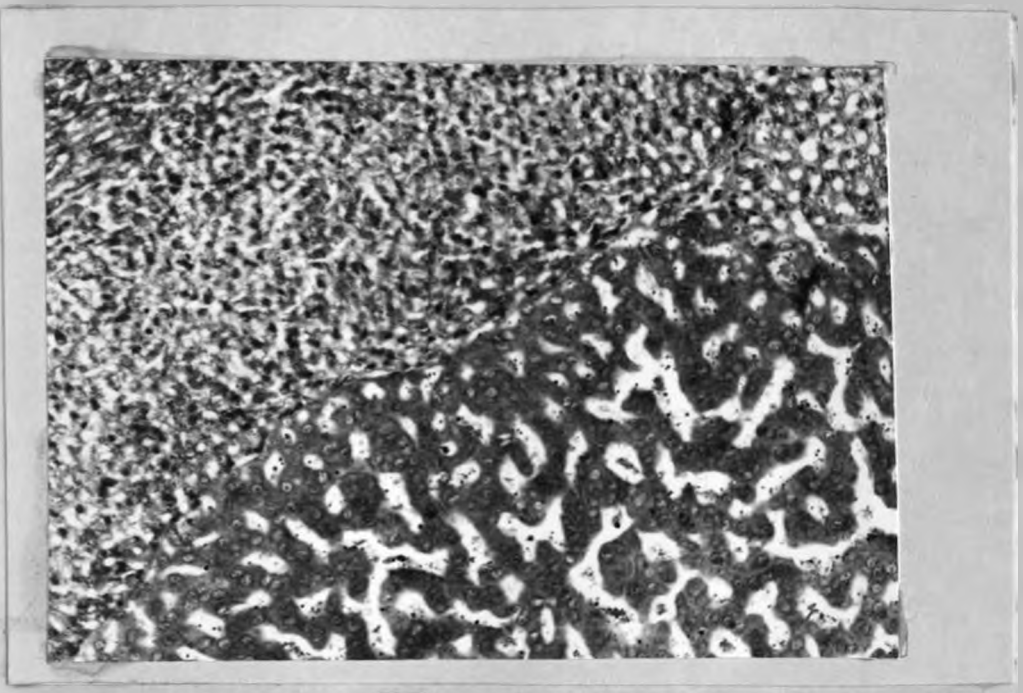
Fig. 54

A photomicrograph of the chorionic placenta of *R. chrysopygus* near term.

cz = columnar zone; sz = spongy zone. Arrow indicates how far the foetal capillaries go. Note the absence of the zone of proliferation. X H & E X 200.

Fig. 55

A section of the chorionic placenta of *E. rufescens*. sz = spongy zone; dz = zone of degeneration; mp = mesoplacentarium (arrow). Also note the absence of the basal trophoblast. H & E X 200.



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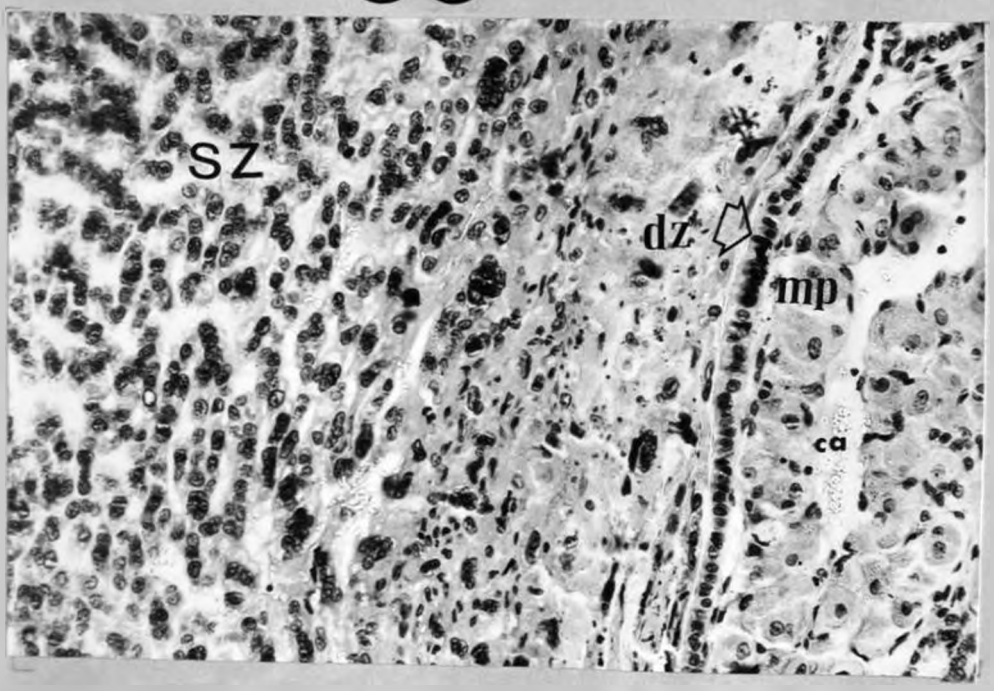


Elephantulus specimen E.r. 553 showed the embryo within the main uterine cavity; the decidua pseudo-capsularis had disappeared. The chorion was richly vascularised by allantoic vessels and was stratified. It was characterised by numerous multinucleated giant cells some of which had vacuolated cytoplasm (Fig. 57). The uterine epithelium was intact and although no intimate apposition between the chorion and the epithelium was observed in this particular specimen, it was believed that the seperation between the two was artificial rather than real. An epithelio-chorial placenta was therefore implicated. The apical villi of the chorion and the uterine epithelium and the fibrinoid substance in the uterine lumen all gave positive PAS and Alcian blue reactions respectively (Fig. 58). This secretion which coated both epithelia may have been a glycoprotein secreted by the chorionic cells.

7. THE AMNION

The early stages to show the mode of amnion formation were not available so the type of amnion formation, whether by cavitation or folding was not observed. When fully established the amnion was in intimate contact with the allantois in many areas forming an allanto-amniotic membrane

(Fig. 56). In such areas the amnion may have been vascularised by the allantoic blood vessels.

8. THE ALLANTOIS

Apart from participating in the formation of the definitive chorio-allantoic placenta of the haemochorial type, the allantois in the elephant shrews displayed an interesting arrangement as portrayed in Figs. 47 and 56 c. The yolk sac was interposed between the amnion and allantois. The allantoic cavity was very large and the allantois sent branches which "anastomosed" with the chorio-allantois thus dividing the cavity into compartments and in many places it was in very close contact with the amnion. The vascularisation of the chorion was initiated by the allantoic mesoderm at the periphery of the placental disc.

9. THE UMBILICAL CORD

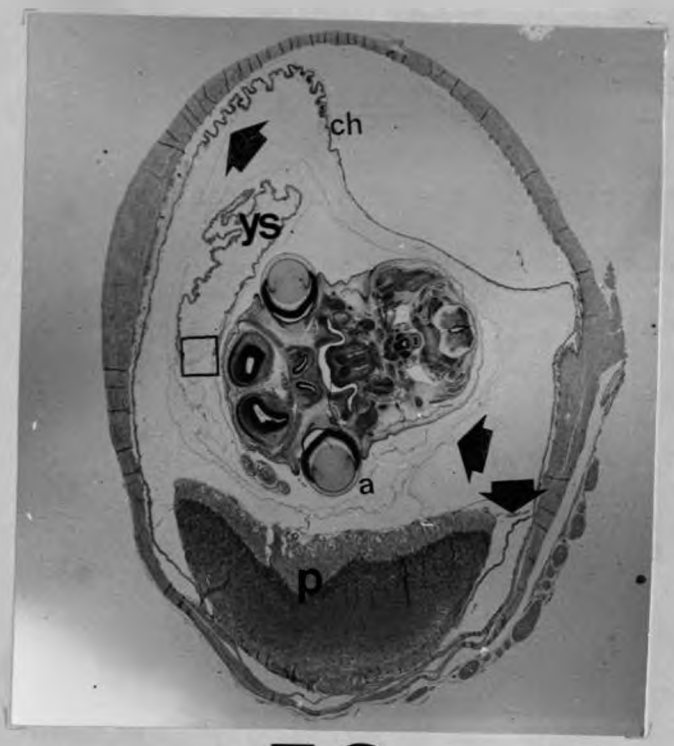
The umbilical cord in the three species of elephant shrews was a long twisted structure which measured about 10 cm. in near term foetuses. Unlike the cane rat, the umbilical cord of these animals consisted of one large vein and two arteries of equal size. The allantoic vesicle lay between the two arteries (Fig. 59). The umbilical arteries

Fig. 56a

A low power section of the placenta and foetal membranes of *E. rufescens*. p = placenta; a = amnion; cha = chorio-allantois; yc = yolk sac. The extensive allantois is indicated by the arrows. H & E X 10.

Fig. 56b

A high power picture of the inset of Fig. 56a. Note the abrupt end of the yolk sac epithelium and its continuation as thin monolayer with the amnio-allantoic membranes (arrows). H & E X 200.



56a

56b

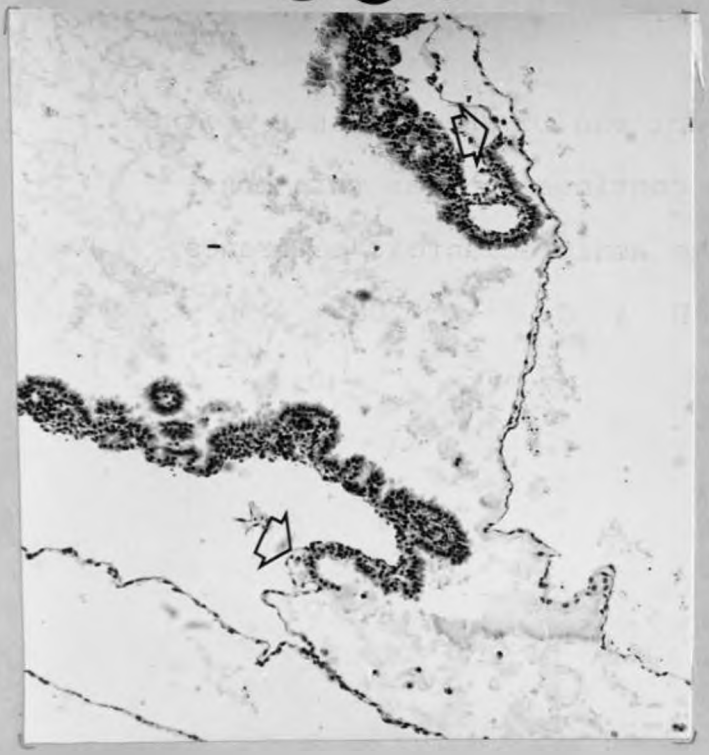


Fig. 56c

A low power section of the placenta and foetal membranes of E. rufescens. The arrows indicate the extensive allantois.

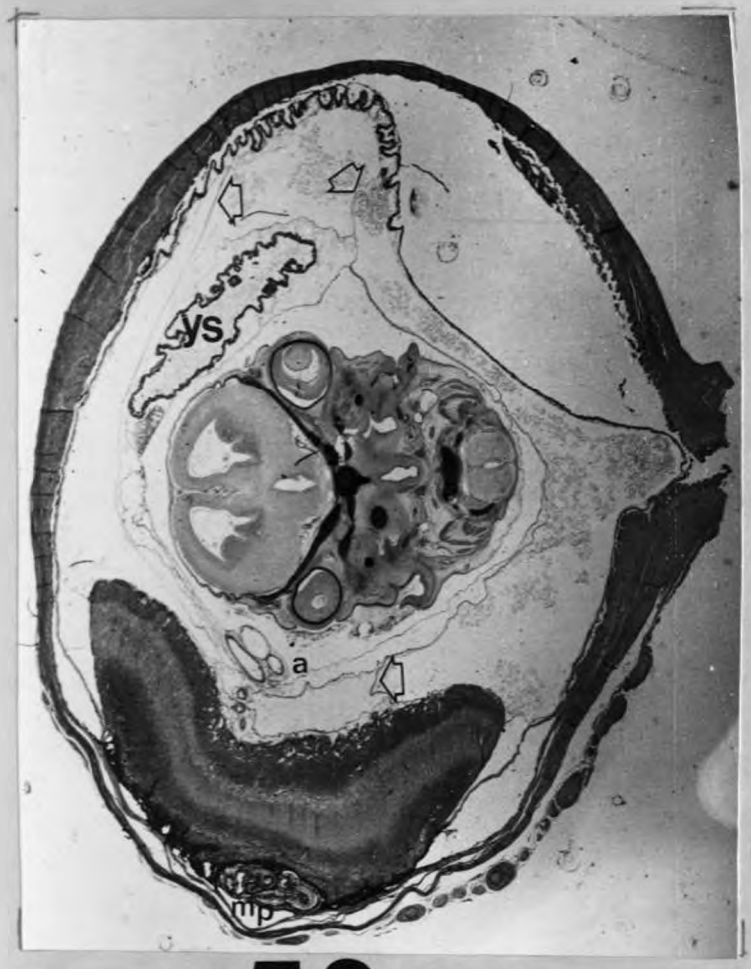
mp = mesoplacentarium; ys = yolk sac;

a = amnion. H & E X 10.

Fig. 57

A section of the placenta of E. rufescens. Note the abrupt end of the stratified chorion (ch) at the zone of degeneration (dz).

Note also the chorionic giant cells (arrow).



56c

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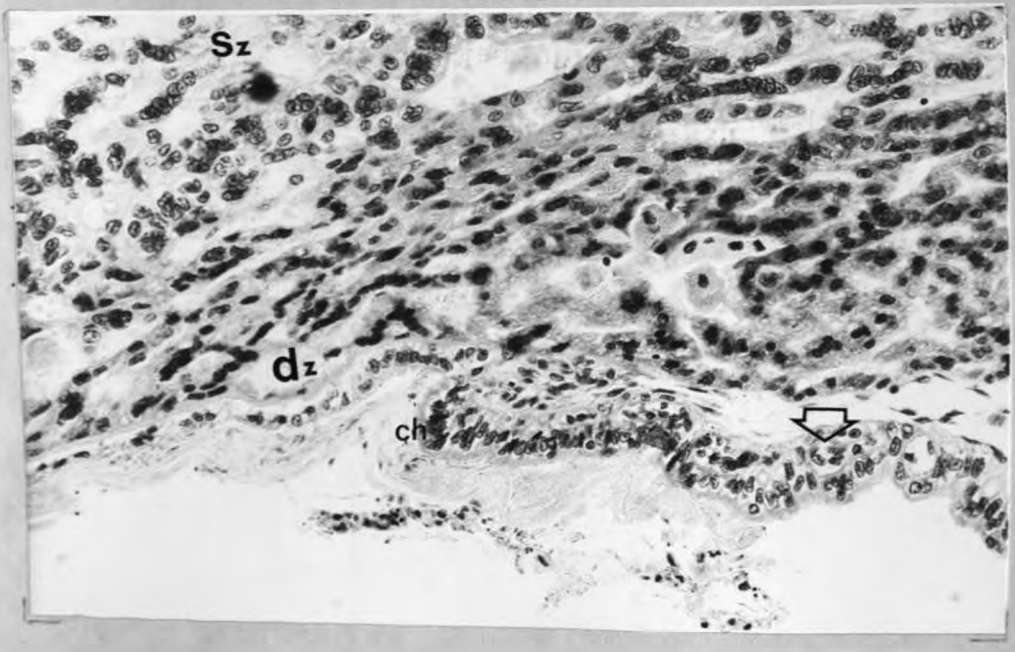
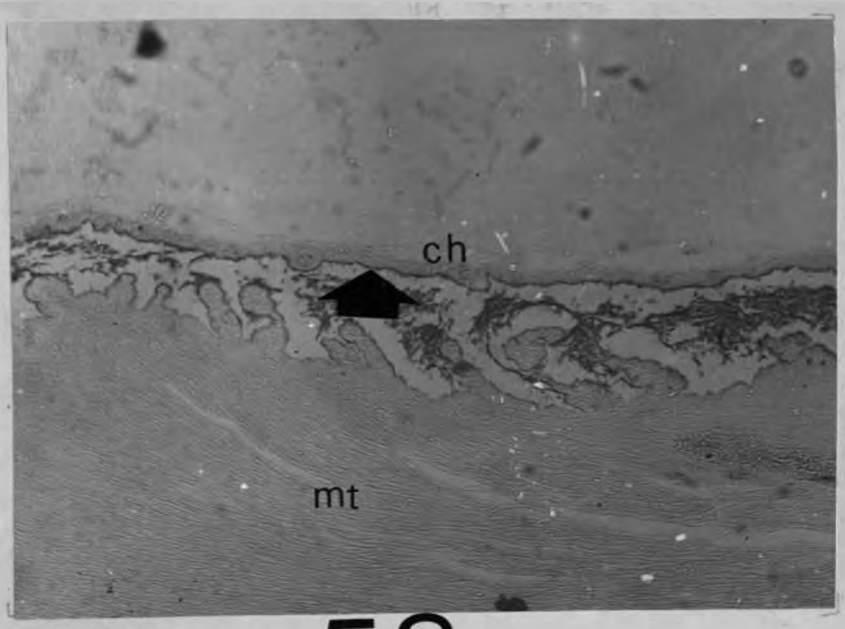


Fig. 58

A photomicrograph of the chorio-allantoic membrane (ch) to show the Alcian blue positive brush border (arrow).
mt = maternal tissue. Alcian blue stain.
X 200.

Fig. 59

A section of the umbilical cord of
R. chrysopygus. ua = umbilical artery:
uv = umbilical vein; av = allantoic
vesicle. H & E X 75.



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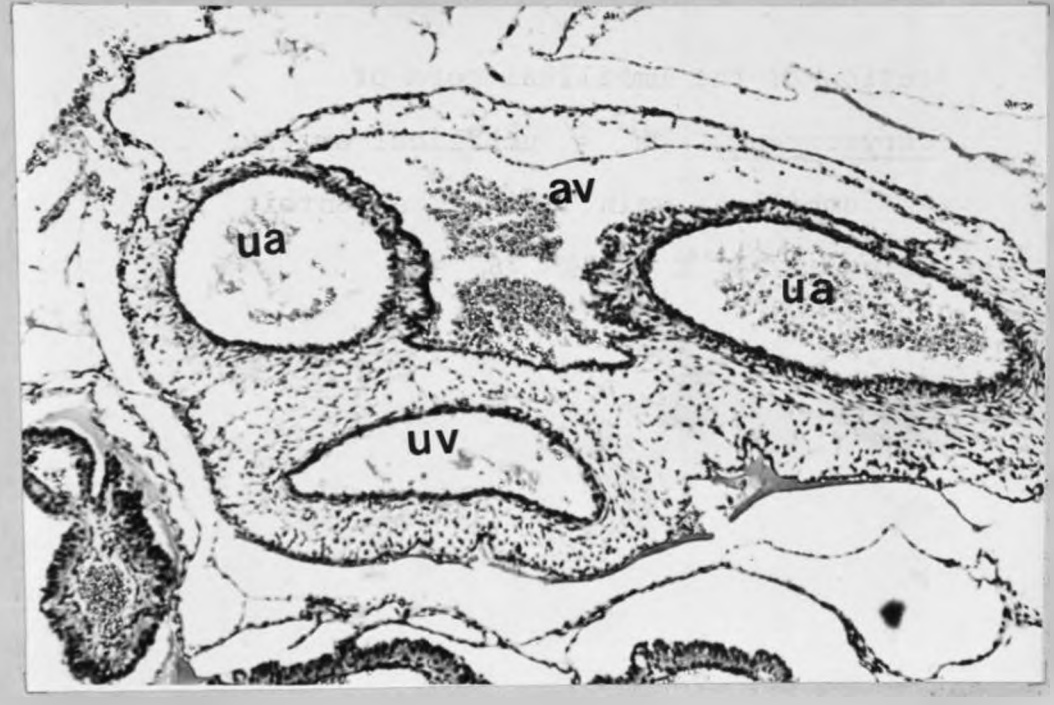


branched off extensively to supply the discoid placenta. As the allantoic vessels ramified on the surface of the discoid placenta, they were lined by an endothelium on the inside, a layer of loose connective tissue in the middle and a layer of tall columnar cells on the outside (Fig. 60a, b). These columnar cells were transformed allantoic mesenchymal cells, a fact which was reinforced by their actual continuity with the squamous allantoic mesoderm cells. The nuclei were located in the basal region while the apical region was vacuolated. The junction between the outer tall cells and the middle loose connective tissue zone was very indistinct. These allantoic blood vessels were interconnected by squamous allantoic mesoderm cells which eventually spread out over the placental surface.

10. THE DECIDUAL REACTION

The decidual reaction in the very early stages of gestation (up to the primitive streak stage) was marked by a pronounced oedema in the bursa embryonica particularly in the area below the implantation and future placental site (Fig. 48 a). The uterine glands though few, were significantly dilated. The cells were transformed from being typical fibroblast with elongate nuclei to spherical cells with round nuclei. Their

cytoplasm was eosinophilic.

In the later stages of development when the definitive chorio-allantoic placental was fully established, the reactions in the decidual regions differed markedly between Rhynchocyon on the one hand and Petrodromus and Elephantulus on the other. In Rhynchocyon, there was the definite layer of maternal fibrous connective tissue lying against the basal layer of trophoblast (Fig. 50 a, b). Below the fibrous connective tissue uterine glands persisted their lumina being markedly dilated and containing a fibrinous secretion. Numerous giant cells were present in the submucosa and some were present even in the myometrium (Fig. 61). The origin of these giant cells was difficult to determine; they could be hypertrophied endothelial cells, migrating giant cells of stromal origin or trophoblastic giant cells. Some of these giant cells were present in the lumen and walls of the blood vessels (Fig. 61 and 62). It is possible these cells could be deported to the lungs to be sieved out.

In Petrodromus and Elephantulus the decidual reaction portrayed a different picture. Below the junctional zone the maternal coiled blood vessels, with distinct endothelial linings were surrounded

by thick walls consisting of giant cells many of which were binucleated. These giant cells stood out very clearly because of the large bifringent, PAS-positive diastase-resistant granules in their cytoplasm (Figs. 56c, 63a, b). These giant cells had completely replaced the intima of the blood vessels. Externally the whole structure, which resembles a gland, was surrounded by a simple cuboidal epithelium that was in continuity with either the epithelium of the uterus or that of the greatly dilated uterine glands (Fig. 63c). These glandular structures resembled the "mesoplacentarium" of Starck (1949). Similar giant cells loaded with PAS-positive granules were also found in the arterial walls of the mesometrial arteries in all the three species of elephant shrews studied but were absent from the walls of the veins. (Fig. 64 & 65). The endothelium of the coiled arteries in the "mesoplacentarium" sometimes underwent marked hypertrophy and grossly narrowed the arterial lumen (Fig. 65). Some of the hypertrophied, seemingly endothelial cells were sometimes found lying free in the arterial lumen. The secretory granules found in cytoplasm of the giant cells were probably glycoprotein in nature.

Fig. 60

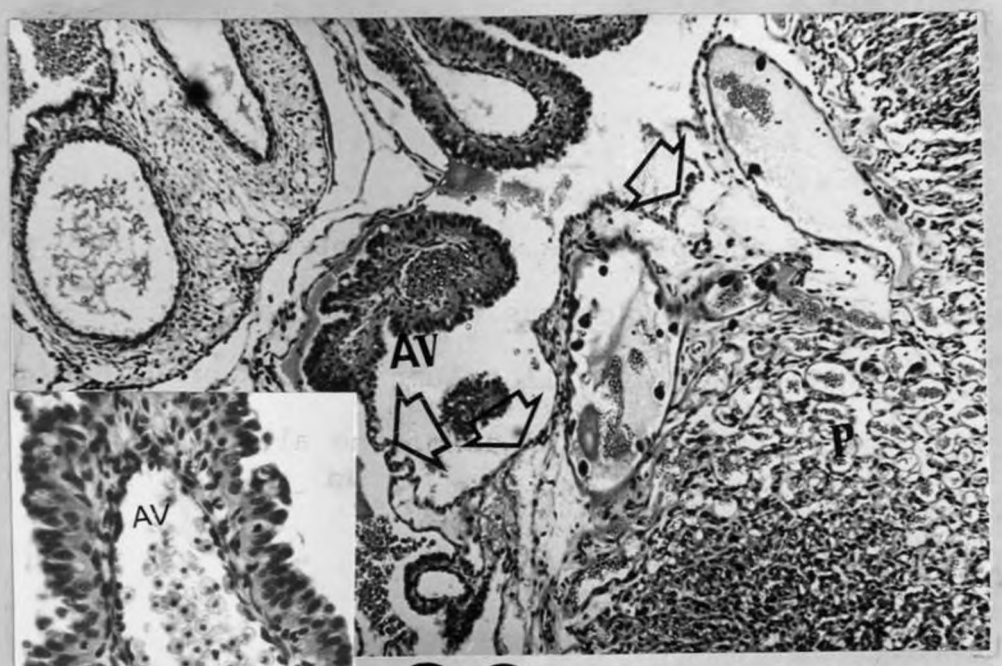
A section of the placenta (p) and foetal membranes of R. chrysopygus to show allantoic vessels (AV) lined by tall columnar cells on the outside. Note the spread of the allantoic mesoderm over the placenta (arrows).
H & E X 75.

Fig. 60a

A magnification of allantoic vessel (AV).
H & E X 200.

Fig. 61

A section of the maternal artery in the decidua of Rc. Note the large giant cells that have replaced the endothelial lining. Note also the binucleate giant cells in the connective tissue (arrow). H & E X 200.



60

60a

61

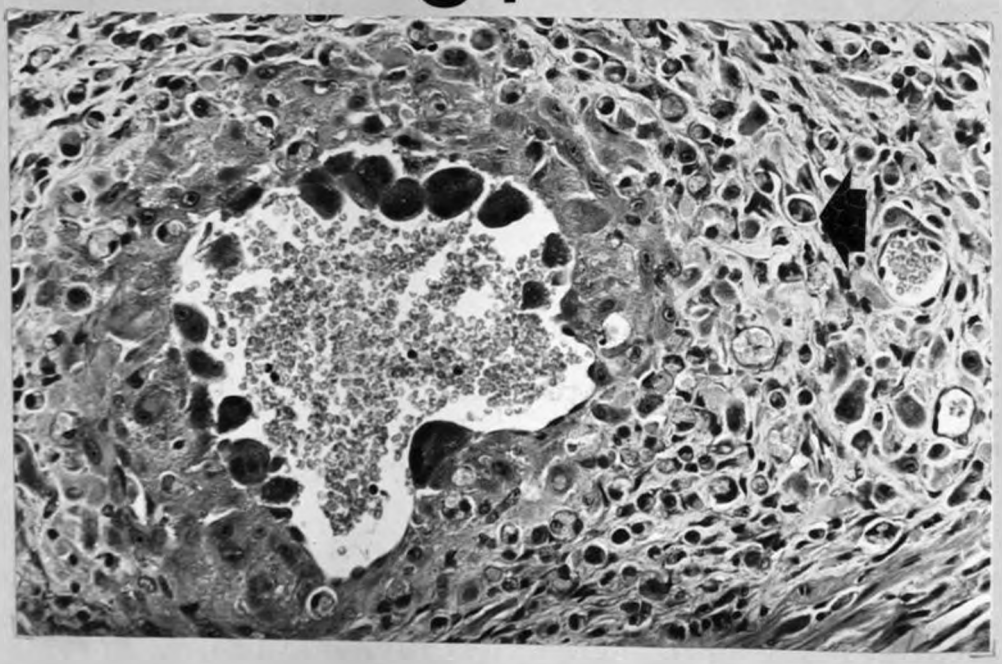
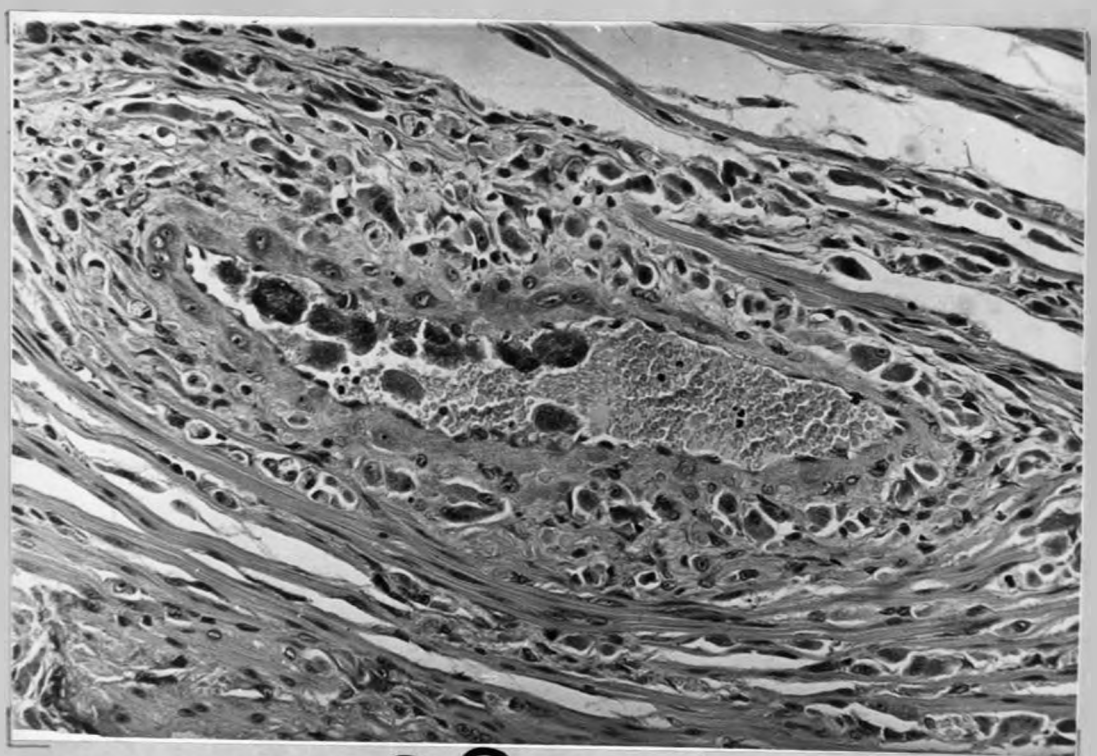


Fig. 62.

A section of maternal artery in the decidua of R.c. Note the giant cells in the lumen of the vessel. Also note the giant cells with eccentrically placed nuclei in the vessel wall. H & E X 200.

Fig. 63a

A section of the mesoplacentarium of E.rufescens to show the coiled artery and the large binucleate giant cells with bifringent granules in their cytoplasm. Note the epithelium of the mesoplacentarium (arrow). H & E X 200.



62

63a

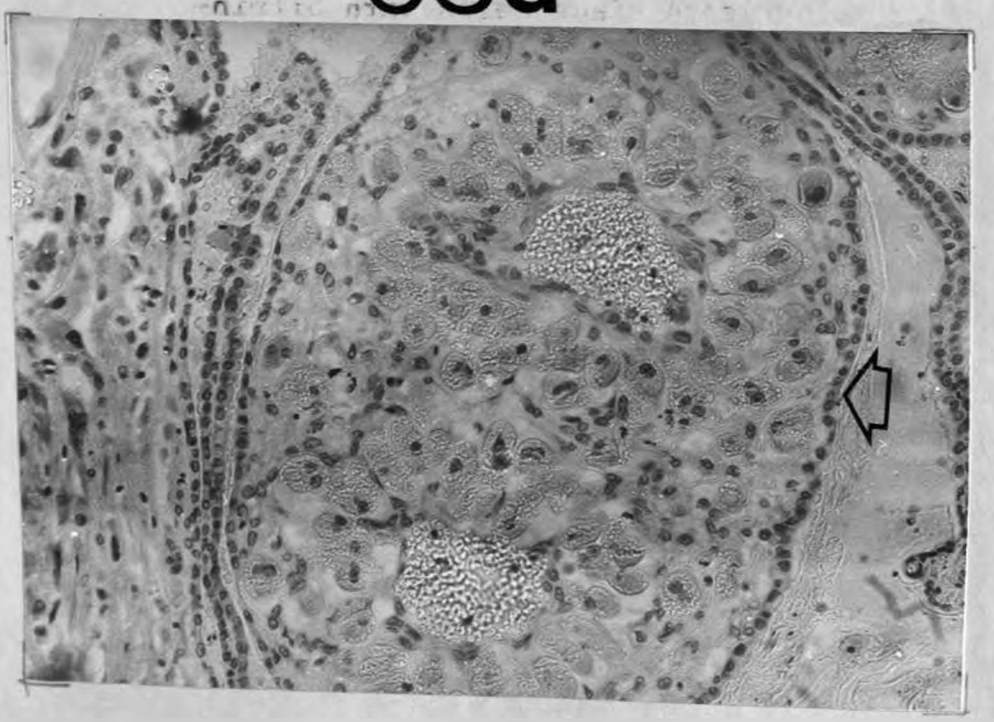


Fig. 63b

A section of the placenta of E. rufescens to show the PAS-positive granules of the meso-placentarial giant cells. mp = meso-placentarium; dz = zone of degeneration; sz = spongy zone. PAS stain X 75.

Fig. 63c

A section of the mesoplacentarium to show the continuity of its epithelium with that of the uterus (arrow). ca = coiled artery; sp = spongy zone. H & E X 75.

63b

63c

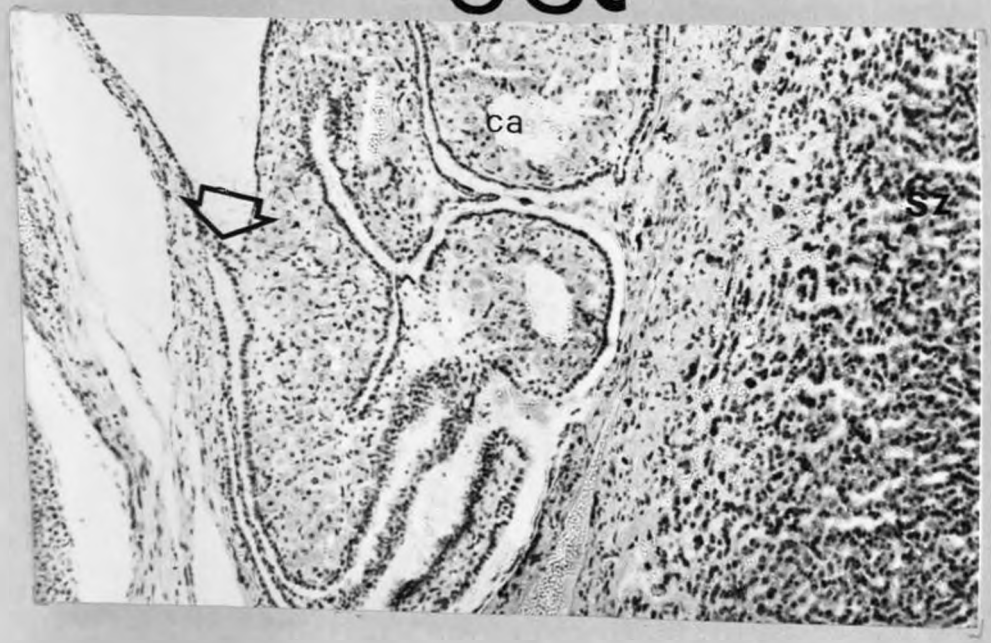
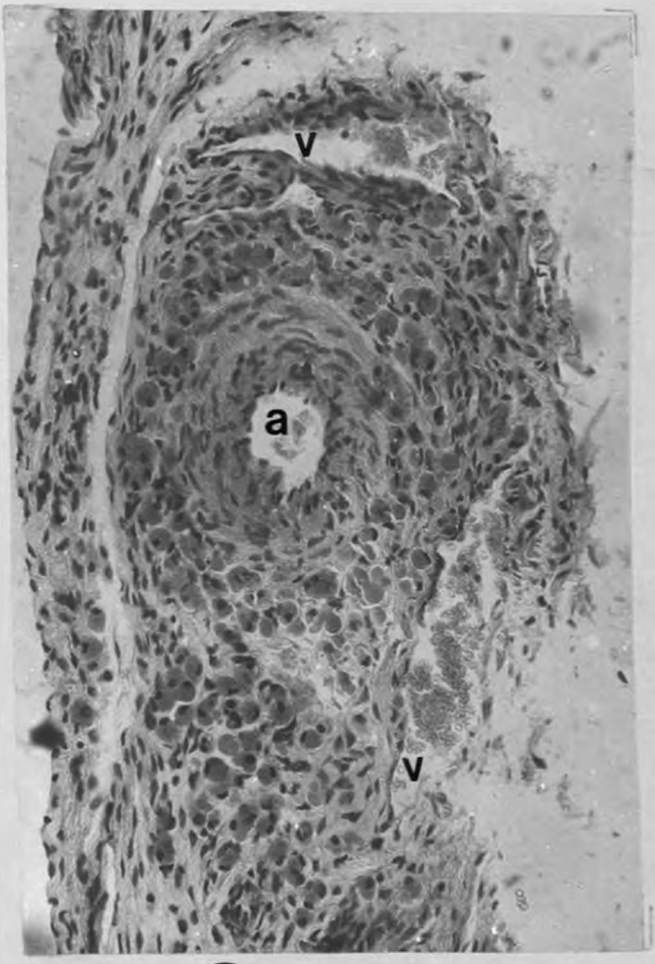


Fig. 64

A photomicrograph of the blood vessels of the mesometrium of R.c. Note the giant cells with PAS-positive granules in the cytoplasm, occupying the wall of the artery. Also note the close relationship between the artery (a) and the veins (v). PAS stain. X 200.



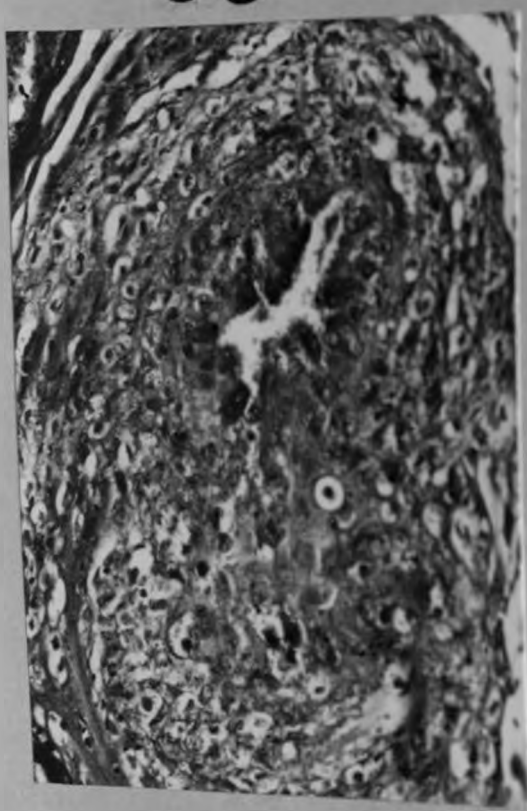
64

Fig. 65

A section of the placental artery in the decidual region of R. chrysopygus placenta. Note the swelling of the endothelial cells have greatly narrowed the arterial lumen.

H & E × 200.

65



11. THE PATTERN OF CIRCULATION

In all the elephant shrews examined, the pattern of blood supply to the placenta was similar. Several coiled arteries in the myometrium or decidua compacta converged to form one artery that entered the placenta more or less centrally as the central maternal artery (Figs. 51, 66). In the junctional region the artery was linned by greatly hypertrophied endothelial cells. These hypertrophied cells greatly narrowed the lumen of the vessels. The vessel passes through the junctional zone consisting of an amorphous, PAS-positive necrotic material in which was found cellular debris (Fig. 66). After entering the substance of the placenta the artery lost its endothelial lining and the wall now consisted of a dense reticulum of course fibres in which were enmashed two types of cells:

a) Small cells with very hyperchromatic nuclei

b) Large, multinucleated cells that resembled giant trophoblastic cells (Figs. 67 a, b & c).

Near the foetal surface of placenta the artery bifurcated into a left and right branches. Eventually the branches lost their reticulum of coarse fibres and maternal blood percolates through the trophoblastic channels (Fig. 67 d). The direction of flow in the lacunae was downwards towards the spongy zone. Maternal blood eventually collected in two veins, one from the left and another from the right (Fig. 66). The two veins converged to form one large vein that left the decidua compacta.

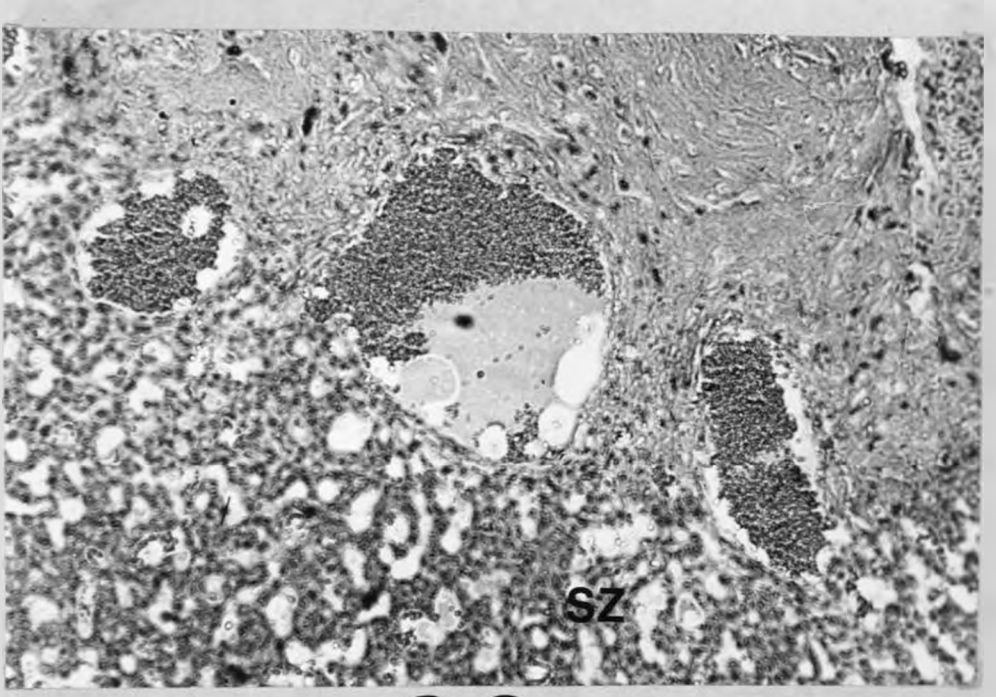
In the mesometrium, the veins and arteries displayed a special relationship. There was extensive contact between the walls of the veins and the walls of the arteries. The adventitia in the area of apposition of the two vessels formed a single stratum such that demarcation between the two vessels was poorly defined (Figs. 64, 68).

Fig. 66

A section of the placenta and the junctional zone of R.c. to show the maternal artery flanked by two veins. sz = spongy zone. H & E X 75.

Fig. 67a

A section of the placenta of P. tetradactylus to show the central artery (mb). Note the giant trophoblastic cells in the arterial lumen. H & E X 200.



66

67a

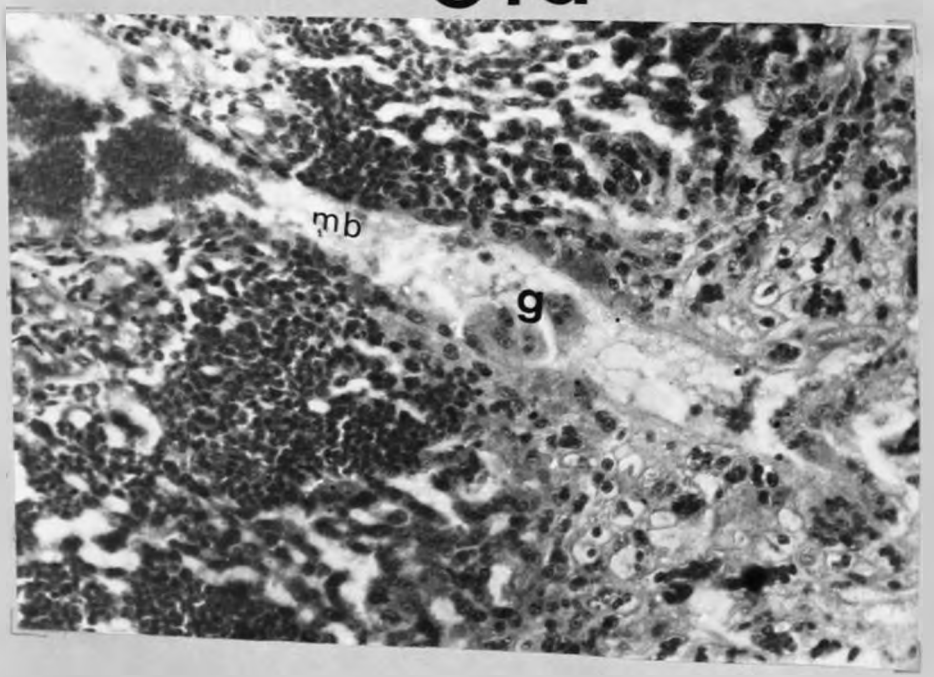


Fig. 67b

A section of the central placental artery of R.c. Note the absence of any epithelium which has been replaced by a thick hyeline layer. Note also the small cells embedded in the hyeline tissue (arrow).

H & E X 75.

Fig. 67c

A section of the placental artery of R.c. Note the hyeline layer (arrow). At "A" the hyeline layer has disappeared and maternal blood seeps into the trophoblastic channels.

H & E X 75.

67b

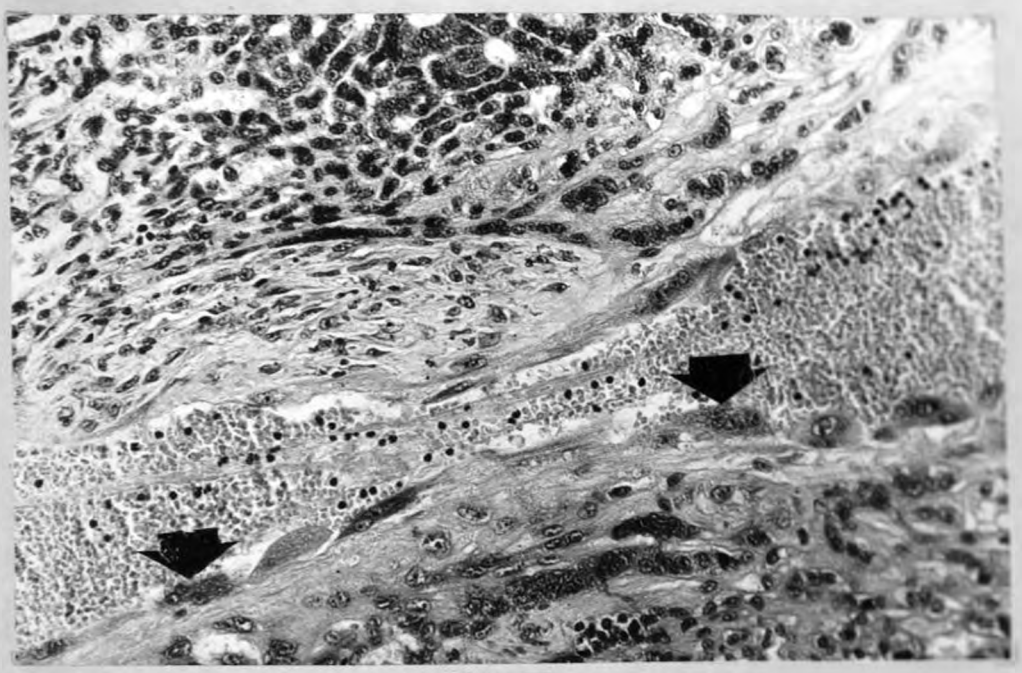
67c

Fig. 67d

A section of the placenta of R.c. showing the central placental artery. Note the endothelium has been replaced by large trophoblastic cells (arrows).
H & E. X 200.

Fig. 68

A section of the mesometrium of P. tetradactylus to show the close relationship between the artery (a) and the vein (v). Note the absence of any dermacation line.
H & E X 200.



67d

68

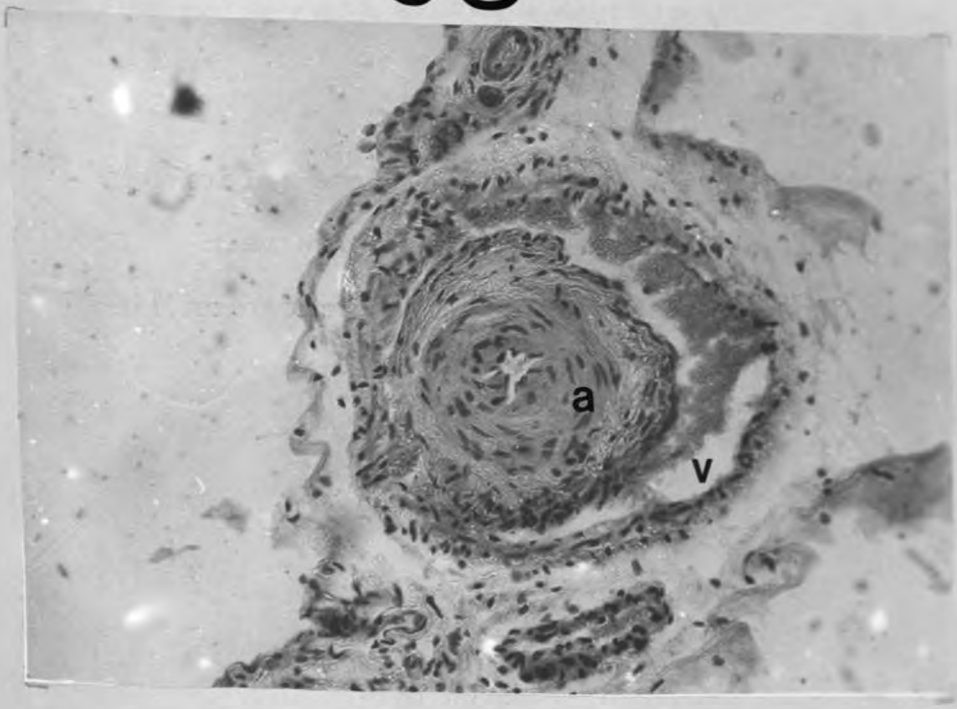
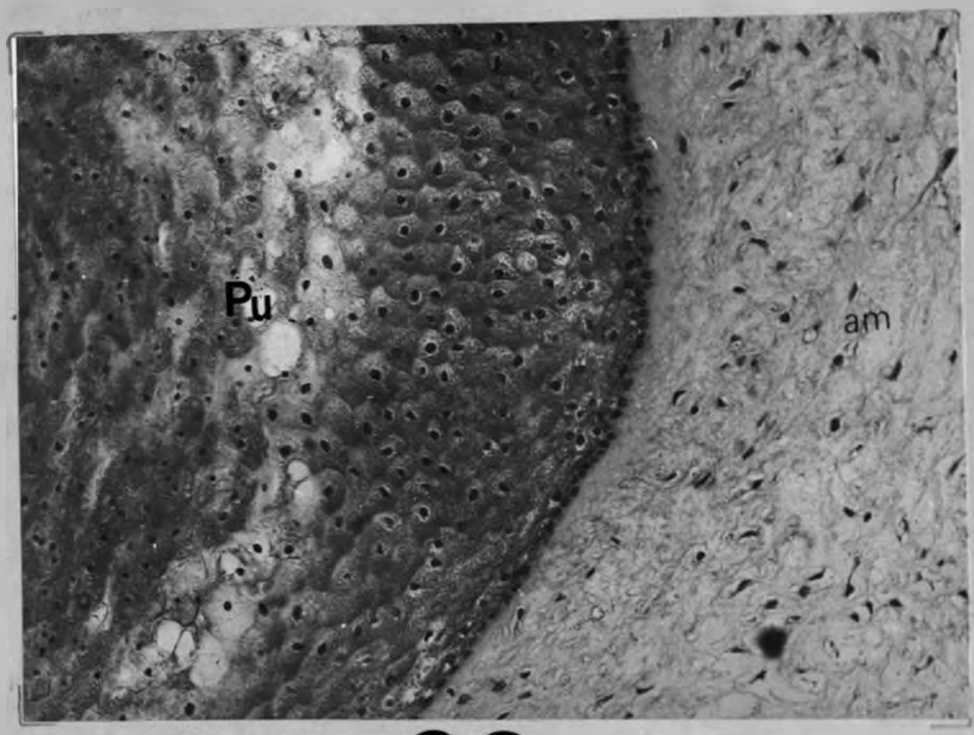


Fig. 69

A section of the amniotic pustule from an eland foetus. Note the cells are distinct and have prominent nuclei. The cytoplasm is ladden with PAS-positive, diastase labile granules. Also note that the pustule is ectodermal in origin (cf. pustules of cane rat). pu = pustule; am = amnion.
PAS-stain. X 200.

Fig. 70

A section through an amniotic pustule recovered from the stomach of an eland foetus. The cells are ladden with PAS positive, diastase labile granules.
PAS-stain. X 200.



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70

*"No man's knowledge can go beyond his experience." John Lock.

VII. DISCUSSION

PART I

A. The Cane Rat

Our knowledge of the hystricomoprh reproductive biology has been confined to the guinea pig and the American species. Very little attention has been focused on the African species of this interesting suborder of rodents. The African hystricomorph rodents include the cane rat, the porcupine, the grey mole rat and the naked mole rat. From the present study of the cane rat and the brief communication on the grey mole rat, Bathyergus janetta, by Mossman and Luckett (1968) it appears that the American and African hystricomorphs share many reproductive characteristics. Table III summarises some of the peculiarities characteristic of this interesting group of rodents.

1. BREEDING AND GESTATION LENGTH

Although pregnant females were not obtained every month during the course of this study (Table 1), the differences in gestation stages as

recorded is fairly indicative that these animals breed throughout the year. Asibey (1974) has reported that cane rats of Southern Ghana are continuous breeders throughout the year while those of Northern Ghana are seasonal breeders. A similar situation may prevail in various parts of East Africa but this has not been studied. The gestation length in the African cane rat has been recorded as about 155 ±9 days by Asibey (1974). This gestation length would put the cane rat within the hystricomorph range (Table III). Hystricomorphs have exceptionally long gestation periods among the rodents ranging from 53 days in the cuis to 285 days in the pacarana, Dinomys branickii (Weir, 1974). Unusually long gestation periods have also been recorded in a number of unrelated species such as the roe deer, Capreolus capreolus (Short and Mary, 1966; Aitken, Burton and Steven, 1971; Aitken, 1974), the Antarctic seal (Sinha and Erickson 1974) and the African fruit bat, Eidolon helvum (Mutere, 1967). However, in the roe deer, the fruit bat and others the long gestation period is partly due to delayed implantation either obligatory or facultative (Wimsatt 1975). Delayed implanation has not been recorded among the hystricomorph species. An interesting case is, however, offered by the plains viscacha, Lagostomus maximus, in which the fertilised

ova do not reach the uterus until day 18 post coitus (Roberts and Weir, 1973). In the majority of hystricomorph so far studied, including the guinea pig the fertilized ova reach the uterus between day 3 and day 6 p.c. (Amoroso, 1952; Roberts and Perry, 1974). Roberts (1971), Weir (1974) and Roberts and Perry (1974) have suggested that the long pregnancies in these animals may be attributed to two factors:

a) the first quarter of gestation is more concerned with the proliferation and establishment of the placenta; and

b) the rate of growth of the foetus is very slow. Holms (1966) reported the tendency for the duration of gestation to be inversely proportional to the number of conceptuses. He noted that in pure bred strain of guinea pig the mean gestation was 69.9 days for a litter size of one and 65.3 days for a litter size of six. The litter size in the cane rat ranges from one to four (Table 1) although Asibey (1974) has recorded a litter size of up to eight but with an average in the range of four. He has also calculated a foetal growth velocity of 0.0614 using the Huggett and Widdas formula (1951). This growth velocity falls in line with those of other hystricomorph rodents so far studied (see Roberts and Perry 1974 pg. 351). Like in other

members of the suborder therefore the gestation length in the cane rat is probably not due to delayed implantation but rather to the initial concentration in the proliferation and establishment of the definitive placenta and the extremely slow growth of the embryo. Illingworth et al. (1974) have found that in the guinea pig increased concentration of progesterone in the plasma did not delay pregnancy or inhibit parturition. They found that progesterone production rate and concentration in maternal plasma remained high and plasma clearance rate low. The slow rate of clearance was attributed to the presence, during pregnancy, of progesterone-binding globulin (PBG). However, Heap and Illingworth (1974) found that PBG was present in the plasma of several other S. American hystricomorphs. These two authors have speculated that PBG may play a role in the conservation of progesterone necessary for the maintainance of long gestations so characteristic of this suborder of rodents. The presence of PBG in the cane rat has not yet been demonstrated.

2. THE OVARIES

The unequal distribution of the embryos in the uterine horns coupled with the fact that there were always more corpora lutea in the ovaries

than the number of viable embryos strongly suggested that more eggs are shed and fertilised but many die and are resorbed early in gestation. Early embryonic mortality and resorption in the cane rat was reported by Asibey (1974) and in the plains viscacha and chinchilla by Roberts and Perry (1974).

A common feature of hystricomorph ovaries is the presence of numerous corpora lutea. With the exception of the guinea pig whose ovary has been described by Mossman (1966) as prosaic, the other hystricomorph rodents develop accessory corpora lutea during pregnancy. These accessory corpora lutea develop from atretic follicle (Mossman and Duke, 1973). In some, like the mountain viscacha, some follicles grow and become luteinised before rupturing (Pearson, 1949). In others, like the degu and the African porcupine the interstitial tissue become luteinised to give rise to an interstitial gland tissue which may be involved in steroid synthesis (Weir and Rowlands, 1974). Mossman and Duke (1973) have defined the interstitial gland tissue as any endocrine type of gland tissue found in the ovary or the mesovarium which is not thecal gland or luteal gland. In the coypu all the corpora lutea originated from ovulated

follicles at oestrus (Rowlands and Heap 1966). In the green acouchi, the true CL reaches a maximum size at the end of the second week p.c. and begins to regress. In the meantime the number of accessory CL increase as pregnancy advances. They contribute 25 - 100% of the total progesterone secreted by the ovary depending on the stage of gestation (Rowlands, Tam and Kleiman, 1970).

The cane rat ovary resembles that of the degu and other hystricomorphs with regard to the considerable amount of interstitial tissue. The interstitial cells and the theca interna undergo extensive luteinization during pregnancy. Luteinisation of the theca interna cells was reported in the guinea pig by Stafford et al. (1942), while the ability of these luteinized cells to synthesise progesterone and oestrogens was demonstrated in the chinchilla by Tam (1971, 1972). The increase in size of the theca interna and the concomitant decrease in size of the corpus luteum in the cane rat ovaries indicates an increasing role played by these cells in the production of progesterone and oestrogens in the late stages of pregnancy. This is supported by the fact that the luteinised cells are strongly eosinophilic and this may be attributed to the presence of abundant smooth endoplasmic reticulum, a cytoplasmic organelle

which is now believed to be closely associated with steroidogenic enzymes (Christensen and Gillim, 1969).

The development of accessory corpora lutea during pregnancy has also been reported in a number of mammalian species which are phylogenetically unrelated e.g. in the mare (Cole et al. 1931; Amoroso et al. 1948), in the nilgai, Boselaphus tragocamelus (Amoroso, 1958), in the African Elephant, Loxodonta africana (Perry, 1953) and in the pronghorn, Antilopa americana (O'gara, 1969). However, the regular occurance of these accessory ovarian structures in the rodent suborder, Hystricomorpha, whose members are spread in both New and Old Worlds must raise some interesting phylogenetic questions which will be discussed in a separate chapter.

Several authors have pointed out that one of the major requirements for viviparity in mammals is the production or conservation of adequate amounts of progesterone to enable the retention of the fertilized eggs within the genital tract for periods beyond the limits of the normal oestrous cycle (Amoroso, 1959; Heap, 1972; Heap and Illingworth, 1974; Illingworth et al. 1974). In some animals like the opossum,

the golden hamster, the mouse, the rabbit, the goat and the cow the ovary seems to be the only source of progesterone while in others like the monkey, the bitch, the mare, the cat, the sheep and woman the placenta seems to form an important source of progesterone especially after the first trimester of gestation (Amoroso, 1955; Linzell and Heap, 1968). A similar situation occurs among the hystricomorph rodents: in the guinea pig and cuis the placenta is an extra-ovarian source of progesterone (Illingworth & Challis 1973) while in the plains viscacha, the green acouchi and the chinchilla the ovary is the only source of progesterone during pregnancy (Tam 1970, 1974). The maintainance of the corpus luteum during pregnancy seems to be under the influence of an antiluteolytic factor produced by the blastocyst which overrides the luteolytic mechanism of prostaglanding F_2 produced by the uterus (Labbsetwar, 1970; Blatchley and Donovan 1969; Heap and Perry 1974; Anderson and Melampy, 1967; Perry, Heap and Amoroso 1973). The ultrastructure of the cane rat ovary reveals an abundance of agranular endoplasmic reticulum, numerous mitochondria and lipid droplets while that of the placenta (even in late gestation) reveals an abundance of the granular endoplasmic reticulum. It is now generally believed that cells with

abundant agranular ER are engaged in steroid biosynthesis while cells with abundant granular ER are concerned with protein biosynthesis and secretion (Bloom and Fawcett, 1975; Fawcett, 1966; Christensen and Gillim, 1969; Dellmann, 1971; Rhodin, 1974 etc.). It would appear from the ultrastructural appraisal of both the ovary and placenta that the cane rat, like the plains viscacha, the green acouchi and the chinchilla, is dependent on the ovary for the production of progesterone to maintain pregnancy. This, however, needs to be proved by performing a series of ovariectomies at different stages of gestation. Whatever its source, progesterone is the one hormone considered indispensable for the maintainance of pregnancy. Even in the hyrax, where plasma progesterone is very low during pregnancy (Heap, Gombe and Sale 1976) the removal of progesterone source (by ovariectomy) invariably results in abortion (Gombe, Oduor-Okelo & Amoroso 1977).

3. IMPLANTATION AND POST-IMPLANTATION DEVELOPMENT

Implantation can be defined as the first contact of the blastocyst with the uterine tissue for the purpose of establishing important physiological relationship. It involves the interaction of ovarian hormones namely oestrogen and

progesterone (Psychoyos, 1966; Mclaren, 1969; Finn 1967; Grant, 1973; Perry, Heap and Amoroso 1973; Heap, 1972; Heap and Perry 1974; Leiser 1975). That both the uterus and the blastocyst are equally essential for successful implantation, the subsequent differentiation of the blastocyst into the inner cell mass and trophoblast and the development of the decidual reaction is now well documented (Fawcett, 1950; Shelesnyak, 1952; Shelesnyak et al. 1967). When blastocyst are transplanted to ectopic sites like the kidney or testis, the trophoblast grows and invades the organ but the inner cell mass fails to differentiate into the embryo (Kirby, 1960; Finn, 1967; Porter, 1967; Yu-Chih-Hsu, 1971; Samuel, 1971). The complex series of interactions that take place between the blastocyst and the uterine epithelial before and during the process of implantation in mammals is beyond the scope of this thesis but reference should be made to some of the very interesting articles on this subject e.g. Boving (1959, 1965); Larsen (1961, 1962); Shelesnyak (1963) Finn and Mclaren (1967); Krishnan and Daniel (1967); Mclaren (1969) Bradbury et al (1970); Menke and Mclaren (1970); Steer (1970); Glasser (1972); Enders and Schlafke (1972); Clemetson et al. (1972); Holmes and Dickson (1973) and Enders and Schlafke (1974).

It is generally believed that the prerequisite for implantation is the shedding of the zona pellucida and this is especially so among the rodents - mouse, rat, hamster rabbit - in which extensive studies have been carried out (Boyd and Hamilton 1952; Amoroso 1952; Orsini and Donovan 1971). In the guinea pig however, it is not lost prior to attachment. As early as 1883, von Spee had observed that in this species the blastocyst was already fixed by processes which extended from the implanting pole through the zona pellucida and came into direct metabolic relationship with the uterine epithelium. This observation has since been confirmed by Blandau (1949); Orsini and Donovan (1971); Parr (1973). Although similar trophoblastic processes have not been observed in other hystricomorph rodents, it may be due to lack of material at the critical stage and the cane rat may well exhibit the same phenomenon as the guinea pig. However, the cane rat blastocyst does send trophoblastic outgrowth from the implantation pole which invade the uterine tissue. Whether these trophoblastic outgrowths penetrate through the zona pellucida was not observed.

The timing of implantation in various hystricomorph rodents has been carefully recorded by Roberts and Perry (1974) and ranges from between day 5 post coitus in the chinchilla to day 7 post coitus in the guinea pig and degu with the exception of the plains viscacha - day 18 post coitus. In the absence of any evidence of delayed implantation, it may be tentatively concluded that the time of implantation in the cane rat falls between day 5 - 7 post coitus. No predetermined implantation sites were observed in the cane rat but Table I and Fig. 10 b shows the uneven distribution of conceptuses in the uterine horns. However, it can be concluded with certain that like other hystricomorph rodents both ovaries and uterine horns of the cane rat display equal competence throughout life. A notable exception among these rodents is the mountain viscacha in which ovulation and implantation are restricted to the right ovary and right uterine horn respectively and only one ovum is released at each oestrum (Pearson 1949; Winsatt 1975). It is however, interesting to note that restricted right horn competence has also been recorded in a number of antelopes like the Uganda kob (Buechner, 1961); the common duiker, Sylvicapra grimmis (Child and Mossman, 1965); and the impala Aepyceros melampus (Mossman and Mossman, 1962;

Kayanja 1969, 1972). In these antelopes, however, both ovaries are functional and therefore uterine migration of the ovum is a common phenomenon.

Although early implantation stages of development were not available, the materials at my disposal were fairly suggestive that implantation in the cane rat is mesometrial and secondarily interstitial - an originally eccentrically implanated blastocyst becomes excluded from the uterine cavity by the overgrowth of the endometrial tissue. The epithelium of the implantation cavity undergoes disintegration probably providing an important source of histiotroph for the blastocyst.

The blastocyst lies free in the implantation cavity except for the attachment pegs at the abembryonic pole (Fig. 11 a, c). It has elongated into a cylindrical tube typical of the guinea pig and other hystricomorph rodents (Sansom and Hill, 1931; Amoroso 1952; Roberts and Perry, 1974). Implantation among the rodents ranges from central and superficial in the rabbit to completely interstitial and antimesometrial in the guinea pig (Boyd and Hamilton 1952; Mossman 1937). That the implantation in the guinea pig is completely interstitial and antimesometrial has now been

accepted mainly through the works of Duval (1892); Von Spee (1901); Wilson (1928); Sansom and Hill (1931); Mossman (1937) etc. It is important, however, to point out that Mclaren (1926) did offer an alternative interpretation to the above. He contended that the implantation in the guinea pig was eccentric and not interstitial. This view was strongly denied by Sansom and Hill (1931). The implantation has been described as completely interstitial and antimesometrial also in the cuis, casiragua, degu, chinchilla and the plains viscacha (Roberts; 1973; Tibbitts and Hillemann 1959; Roberts and Perry, 1974). Perrotta (1959) suggested that in the Canadian porcupine the implantation is incompletely interstitial due to the relatively large size of the blastocyst, while Wimsatt (1975) stated that in the mountain viscacha implantation is always mesometrial. It is interesting to note that while antimesometrial implantation is now accepted for the guinea pig and other hystricomorph rodents, there is yet no explanation as to how the definitive chorio-allantoic placenta develops mesometrially.

The interpretation that the implantation in the cane rat is initially eccentric but later becomes secondarily interstitial is based on the following two observations:-

a) Figs. 11a, c, d show the blastocyst lying free in a large cavity except for the attachment at the abembryonal pole. The orientation of inner cell mass is antimesometrial. The cavity occupied by embryo seems to be too large to have been created by the blastocyst itself. The situation contrasts sharply with the interstitial implantation in the guinea pig, plains viscacha and chinchilla where the blastocyst wall is in very close contact with the maternal connective tissue (see Sansom and Hill, 1931; Figs. 9, 10, 11, 12, 17, 18 and 19 and also Roberts and Perry, 1974; Figs. 4 and 5). The large cavity in the cane rat specimen is not an artefact as the tissue is well fixed.

b) Figs. 15, 16 & 18 show the formation of the chorionic placenta in an enlarged pocket of the uterine cavity which is lined by an epithelium and is continuous, through a narrow, epithelially lined channel, with the main uterine cavity. The amnio-embryonic mass, which has differentiated into the embryonic disc and amnion, is suspended by an extensive bilaminar chorion in the uterine lumen. It can be said that the blastocyst dropped into a uterine crypt situated mesometrially and after implanting itself, the amnio-embryonic mass pushed itself through the patent neck of the crypt back into the main uterine lumen.

What, however, remains to be elucidated is how the eroded epithelium of the crypt is later reestablished as evidenced in Fig. 15, 16 & 17. The existence of the trophoblastic layer of the guinea pig blastocyst has been the subject of considerable controversy. Selenka (1882) and Duval (1889) have described its existence as a trophoblastic ectoderm while Graf Spee (1901) strongly rejects its existence in the guinea pig, transient or otherwise.

This view is also shared by Mclaren (1926) who expressed serious doubts as to whether such an outer trophoblastic ectodermal wall has ever been observed. Mclaren makes an interesting statement " .... while the polar thickening undoubtedly gives origin to the trophoblast of the Trager, the layer of cells bounding the cavity in the rest of its extent becomes directly converted into the lamella continuous with the definitive endoderm, and represents the endoderm of the inverted yolk sac wall in mouse and rat." In the next breath he makes a rather contradicting statement: "that the wall of the cavity of the blastocyst (which strictly does not deserve this name) consist of the yolk sac endoderm."

In a critical review of previous literature and a re-examination of the pictures of Selenka, Duval, Graf Spee, Maclaren (1926), and the Heaps collections at Cambridge, Wilson (1928) has concluded that the trophoblastic layer of the guinea pig blastocyst does exist. Sansom and Hill (1931), after an extensive study of the guinea pig blastocysts concluded that the trophoblastic wall of the blastocyst has a temporary existence in that it soon undergoes degeneration and disappears and the visceral yolk sac endoderm comes into contact with the uterine epithelium. This view is supported by Roberts and Perry (1974) who say that in most hystricomorph rodents there is no evidence of a parietal yolk sac wall.

The findings in this study, as regards the cane rat, differ fundamentally from those of Graf Spee (1901), Mclaren (1926) and Roberts and Perry (1974) regarding the guinea pig and other hystricomorph rodents. In the cane rat blastocyst the trophoblastic outer wall exists and does not degenerate. Figs. 11c, d & 13 a clearly show the

differentiation of the endoderm from the inner cell mass. They also show the continuity of the trophoblastic wall with the ectoplacental trophoblast at the implantation site. This continuity can be traced in later stages of development as shown in Figs. 15 & 16. A critical examination Fig. 5 of Roberts and Perry (1974) shows a solid inner mass at the embryonic pole and a smaller cell mass at the opposite pole with a cavity in between. All the three structures are contained within a cellular wall and one would presume that this structure constitutes the "blastocyst," with a well defined trophoblastic wall. In the text, they talk of the parietal trophoblast undergoing disintegration yet what they call the parietal trophoblast, is probably maternal connective tissue and possibly disintegrating uterine epithelium. The interpretation offered here is that after implantation, that portion of the trophoblastic wall which does not participate in the formation of chorio-allantoic placenta differentiates into an epithelial lining which surrounds the embryo and is continuous with the ectoplacental trophoblast, an observation which has been made in the guinea pig by Harman and Pricket (1932). It is concluded here the cane rat blastocyst, unlike that of the other hystricomorph

rodents displays the conditions of a normal Eutherian blastocyst and it is suggested that a more critical reexamination of the so called inverted yolk sac and its formation in the hystricomorph rodents will be required to streamline what Wilson has aptly called "a retrograde change unparalleled in any other known Eutherian group, not excepting the closely related types of rodents exhibiting inversion or entype". It may be said that the pictures provided by various authorities on hystricomorph placentation are very similar to those in this study (cane rat). The only difference is the question of interpretation. There was no maternal blood in the cavity of the cane blastocyst. Roberts and Perry (1974) have claimed that in the chinchilla, coypu and Viscacha the "ectoplacental cavity" is frequently filled with extravasated maternal blood. Such an observation is interesting because since the parietal trphoblast is disintegrating it means that maternal blood comes into direct contact with the inverted endoderm. But to date only the trophoblast is known to possess immunosuppressive mechanism to enable the conceptus to survive maternal lymphocytic attacks (Amoroso, & Perry, 1975). Endodermal cells are not known yet to possess this quality and it would be interesting to examine how the embryo survives in these rodents. It is concluded

here that Roberts and Perry's interpretation is erroneous and incompatible with the known facts to date.

4. THE DEFINITIVE PLACENTA OF THE CANE RAT

The chorio-allantoic placenta of the cane rat portrays essentially the same features as those which have been described for the guinea pig (Amoroso, 1952; 1961), the Canadian porcupine (Perrotta; 1959), the Chinchilla (Tibbitts and Hillemann, 1959), the mountain viscacha (Pearson, 1959), the nutria (Hillemann and Gaynor, 1961), the cuis, plains viscacha coypu and chinchilla (Roberts and Perry, 1974). Amoroso (1952) has distinguished four major regions of the guinea pig chorio-allantoic placenta:

a) The trophoblastic area consisting of labyrinthine and spongy zones;
b) the accessory or subplacenta;
c) the junctional zone;
d) the decidua basalis.

These characteristics are well developed in the cane rat. Like in other hystricomorph rodents the chorio-allantoic placenta of the cane rat is lobulated and the complex labyrinth consists of two sets of closely parallel tubes:

a) the trophoblastic tubules consisting of syncytial trophoblast which enclosed maternal blood and

b) the allantois capillaries embedded in the foetal mesenchyme.

The spongy zone consists of coarse syncytium enclosing spaces filled with maternal blood only. The subplacenta, which is so well developed in the cane rat, has so far not been described in any mammalian species outside the hystricomorph suborder. First described by Duval (1892), it develops from the cytotrophoblastic lamellae that form the floor of the central excavation (of Duval). Like in the guinea pig the cane rat subplacenta consists of cells that are closely packed in ovoid groups. The intercellular spaces within the subplacenta are filled with an amorphous, PAS-positive, diastase resistant precipitate (Fig. 35 & 36). Davies, Dempsey and Amoroso (1961 a, b) suggested that the subplacenta might be involved either in gonadotrophin synthesis or in the absorption of materials from the decidua. Similar results have also been obtained in the cuis, casiragua, the chinchilla and the plains viscacha by Roberts (1973). Bambrah (personal communication) has demonstrated high concentration of gonadotrophin-like material in

the cane rat sub-placenta by immunofluorescence.

Roberts and Perry (1974) have suggested that the sub-placenta may be involved in the transfer of nutrients of high molecular weights such as polysaccharides and proteins from the decidua to the foetal tissues. This is unlikely. It would be more logical to assume that these cells are involved in secretion rather than absorption. Recent studies have shown a high affinity binding of progesterone and other hormones in guinea pigs during various reproductive stages (Diamond, Rust and Westphal, 1969; Feder, Resko and Goy, 1968; Heap and Deanesly 1967). Heap and Illingworth (1974) have in fact shown the existance of a progesterone-binding globulin (PBG) among the hystricomorph rodents during pregnancy. They have postulated that the subplacenta may well be the site of PBG synthesis. Recently, however, Bambra (personal communication) has demonstrated the presence of a gonadotrophin in the subplacenta of the cane rat using the peroxidase method. This result indicates that the subplacenta of the hystricomorph rodents may be a specialised endocrine organ. Bambra and Gombe (1978) have also shown that the chorionic cells of the rat placenta produce a human chorionic gonadotrophin/ pregnant mare serum gonadotrophin -

like substance which plays an immunosuppressive role during pregnancy. The gonadotrophin synthesised by the cane rat placenta may play a similar role in immunosuppression.

The fine structural studies reveal that the placenta of the cane rat is haemodichorial in some places and haemotrichorial in others according to the classification of Enders (1965). The paucity of ergastoplasm, mitochondria and secretory granules indicates that the cellular trophoblast is more concerned with growth and differentiation giving rise to syncytial trophoblast than with a specialised function. That the syncytial trophoblast derives from the cytotrophoblast has been stated by several authors including Hamilton and Boyd (1960); Wynn and Davies (1964); Pierce and Midgley (1963). The syncytial trophoblast portrays features which are generally associated with protein biosynthesis namely a well developed and dilated cisternae of granular endoplasmic reticulum, numerous mitochondria and many secretory granules (Fawcett 1966; Dellmann, 1971; Rhodin, 1974; Bloom and Fawcett, 1975). Enders (1965) and King and Tibbitts (1976) have also reported the abundance of granular endoplasmic reticulum, numerous mitochondria and secretory droplets in the syncytial trophoblast of the guinea pig and chinchilla

placentae, respectively. Syncytial trophoblast has been implicated in the synthesis of human chorionic gonadotrophin-HCG- (Midgley and Pierce, 1962; Pierce et al. 1962; Pierce and Midgley, 1963; Wynn and Davies, 1964; Macnaughton, Coutts and Browning, 1970; Dempsey & Luse, 1971) and pregnant mares serum gonadotrophin, PMSG, (Hamilton, Allen and Moor, 1973; Allen, Hamilton and Moor, 1973). The profuse development of microvilli on the syncytial trophoblast was remarkable. This is suggestive of cells with considerable absorptive activity. Such a profuse feature has not been received in any of the other hystricomorph rodents.

If the cane rat placenta produces a mucoprotein compounds, as is suggestive from the ultrastructural observation of the syncytial trophoblast, what is the nature of this compound? Is it a gonadotrophin and if so what is its role in the maintainance of pregnancy? It has been stated by many authors that the foetus and its membranes is under normal circumstances a successful "allograft". Several hypotheses have been put forward to account for the priviledged immunologic status of the foetus. These hypotheses include, among others:-

a) the antigenic immaturity of the foetal tissues;
b) the immunological indolence or innertness of the mother;
c) the uterus is a priviledged site;
d) inhibition of the cellular immune mechanism by invasive trophoblast;
e) the immune tolerance and
f) the existance of an anatomical barrier between the mother and conceptus (Parks and Zimmer, 1972; Amoroso and Perry, 1975).

All these hypotheses have been discarded except the last one listed - the existance of an anatomical barrier between the foetal and maternal tissues. Two factors have been suspected to act as an immune barrier; the trophoblastic cell and the fibrinoid extracellular layer of Nitabuch. In a series of experiments Kirby et al. (1964) and Bradbury et al. (1970) have demonstrated the presence of a thick amorphous sulphated glycoprotein (mucoprotein) coating the surface of trophoblastic cells seperating the trophoblastic cells from the maternal tissue. They have therefore concluded that the amorphous fibrinous layer, rather than the trophoblastic cell acts as the immunologic barrier during pregnancy. Wynn (1967) with the aid of

electron microscopy, has demonstrated the presence of a fibrinoid layer in haemochorial placentae but not in the epitheliochorial types as in the mare and the cow. Kirby et al. (1964) stated that the fibrinoid substance was derived from the trophoblast - at least in the mouse, while Bulmer and Dickson (1960) demonstrated the widespread distribution of a PAS-positive diastase-fast material of a glycoprotein nature. Hulka, Hsu and Beiser (1961) have stated that the trophoblastic tissue in the placenta was capable of absorbing circulating maternal antibodies. All these findings are in agreement with the conclusion in this thesis that the cane rat syncytial trophoblast secrete a glycoprotein. Whether the glycoprotein is a gonadotrophic hormone remains to be proven but that it acts as an immunologic barrier is increasingly becoming obvious although the existance and nature of such a barrier in epitheliochorial types of placentae is yet to be determined. It may be speculated that the glycoprotein produced by the trophoblastic cells, on attachment to specific receptors on the trophoblast cells themselves, renders the latter safe from immunologic attack.

5. THE AMNION

The formation of the foetal membrane in the cane rat is essentially the same as that described in the guinea pig (Mossman, 1937) and other hystricomorph rodents (Roberts and Perry, 1974). Although amniogenesis was not traced from its earliest stages, it is deduced from Fig. 14 and 19 that amnion formation is by cavitation. Fig. 18, 19 shows the amnio-embryonic mass in the shape of a sigmoid ring with the amniotic cavity already formed. Mossman (1937) has shown a correlation between the mode of implantation and the type of amniogenesis especially so in the order Rodentia. He points out that in those species like the rabbit and the squirrels where implantation is superficial, amniogenesis is by folding while in the Dipodomys and Muridae in which implanation is eccentric "the folds as such, have practically disappeared and in Cavia no obvious relation to the folding method can be detected." Implantation in the cane rat has been stated earlier to be secondarily interstitial and therefore would fit in well with a cavitation type of amniogenesis.

The cane rat amnion is avascular. This is in agreement with the findings of Roberts and Perry (1974) although Tibbitts and Hillemann (1959)

reported the presence of primordial blcod cells in the mesoderm of the chinchilla amnion. Blood formation by first intention has been reported in the amniotic mesoderm of baboon, Papio papio by Noback (1946). Mossman (1937) reported that secondary vascularisation of the amnion occurs after its fusion with allantois in artiodactyls, perissodactyls and carnivores. However, in the cane rat, even after fusion with allantois, no blood vessels were traced.

In the early stages of development the amnion remains fairly avascular, but in the later stages of gestation it comes very close to and in many areas fuses with the so called 'chorion' but no trace of vascularisation was observed. The cane rat amnion is also studded with white rounded pustules from mid gestation onwards. As already mentioned, histologically they consist of a series of coiled layers of loose fibrous tissue with small deeply staining nuclei. PAS reaction does not reveal any glycogen granules. Among the hystricomorph rodents, similar pustules have so far been described only in the plains viscacha (Roberts and Perry, 1974). Pustules have been described among several unrelated mammalian species. They have been observed in the mare and cow (Amoroso, 1952), in

the African elephant (Amoroso and Perry, 1964). They have also been observed on the amniotic surface and even recovered from the foetal stomach in the hyrax, oryx, eland (Oduor-Okelo, unpublished observation), and hippopotomus (Amoroso, personal communication). Histologically, these amniotic and stomach hexagonal cells are ladden with glycogen granules as revealed by PAS-stain (Fig. 69, 70) and they are ectodermal in origin. The cane rat pustules are mesodermal derivatives and their precise function is unknown. They may merely represent regressing mesodermal outgrowths of incomplete organogenesis.

6. PARIETAL TROPHOBLAST AND THE CHORION

It has been stated already that the existence of the parietal trophoblast in the guinea pig and other hystricomorph rodents has been denied by many authorities (Maclaren, 1926; Sansom and Hill, 1931; Mossman 1937; Roberts and Perry 1974). It is quite clear from the excellent review of the literature by Wilson (1928) that the controversy concerning the existence of the parietal trophoblast has been largely due to different interpretation of the earlier works by Selenka (1883, 1884), Duval, (1882) and Spee (1901).

From what was available it is clear that the cane rat has a true yolk sac although it appears to be a short-lived structure. It has been observed in this study that the umbilical cord of the cane rat foetus contains five large blood vessels namely, two umbilical arteries, one umbilical vein and two vitelline vessels. In the human embryo, after the umbilical cord is formed, it consists of an external covering of amniotic ectoderm, a core of mesenchyme in which lie two arteries, a vein, vitelline vessels and remnants of vitello-intestinal duct (Harrison, 1964). It would appear, therefore, in the cane rat the presence of the vitelline vessels represent the true yolk sac, whose cavity has been obliterated and the remnants of its structure incorporated into the umbilical cord. It is interesting to note here that in animals like the elephant shrews, which have large, prominent yolk sacs, (See Figs. 47 a, 56a, b & c) there are only three blood vessels in the umbilical cord namely two umbilical arteries, and one umbilical vein. The structure, which has hitherto been referred to as the inverted yolk sac is none other than the parietal trophoblast which in later stages becomes vascularised by allantoic mesoderm to become the chorio-allantoic

membrane. When this membrane becomes closely apposed to the uterine epithelium an extensive epitheliochorial placenta is formed whose functions may not be secondary to those of the haemo-chorial placenta. Hemmingway and Brambell (1961) stated that in the rabbit, guinea pig and the monkey the young were born with a fair equipment of antibodies and that the route of transmission was via the uterine cavity and the yolk sac. In the human, they stated that the yolk sac was vestigial and the chorion covered the whole surface of the conceptus and hence the route of transmission was likely to be via the chorio-allantoic placenta. Recently King (1974) has described the presence of a surface coat or glycocalyx on the yolk sac endoderm of the guinea pig and suggested that the glycocalyx provided receptors for antibodies prior to being absorbed by pinocytosis. The structure which King and others refer to as the yolk sac is the same as "chorio-allantoic membrane" of this study. Hence this membrane in the cane rat may well serve as an important route of antibody transmission from the mother to the foetus.

7. THE ALLANTOIS

The development of the allantois in the cane rat was not studied but in all probability, resembles that in other rodents in which there is no allantoic vesicle but otherwise a well developed vascular allantoic mesoderm is present and takes part in the formation of the placenta (Mossman, 1937; Amoroso 1952; Roberts and Perry, 1974).

8. THE DECIDUA BASALIS

Unlike the guinea pig and other hystricomorph rodents a decidua capsularis does not develop in the cane rat. The remarkable reaction of the decidua basalis as seen in the cane rat (Fig. 42). has not been described in any other hystricomorph rodent except the African mole rat, Bathyergus janetta (Mossman and Luckett, 1968). They stated that the early gestation sacs in the mole rat bulges mesometrially rather than antimesometrially due to the hypertrophy of the basal decidua and the giant cells derived from it. They also stated that the giant cells become so numerous by mid-pregnancy that they obscure the gestation sac myometrium. A similar reaction was observed in the African cane rat. The giant cells are very

large, are multinucleated and have very vacuolated cytoplasms; some of these cells lying adjacent to the perimetrium (Fig. 42b). These giant cells also contained fibrous structures in their cytoplasm. In near term pregnancy these cells diminish in size and lie intermingled with muscle fibres. It is concluded that these cells may be mesenchymal in nature. They are probably myomerial smooth muscle cells which have reverted to the mesenchymal status during pregnancy and which again differentiate into smooth muscles towards the end of pregnancy. It would be interesting, however to examine their presence after artificially induced decidual reaction.

The mesometrial bulging of the gestation sac myometrium as reported in the grey mole rat by Mossman and Luckett (1968) may indicate that implantation in this rodent is mesometrial. If this be so, then four important differences emerge between the New World hystricomorphs on the one hand and the African cane rat and mole rat on the other. These differences are:-

a) an eccentric implantation which becomes secondarily interstitial (at least in in the cane rat);

b) a mesometrial implantation as suggested by the mesometrial bulging of the gestation sac myometrium;

c) an unusually rapid and great hypertrophy of the decidua basalis and its giant cells which are also present in the mesometrium and

d) the presence of the parietal trophoblast (in the cane rat blastocyst) as opposed to its total absence or early disintegration in other hystricomorph rodents.

The pattern of circulation is very similar to that in the guinea pig and other hystricomorph rodents.

PART II

B. THE ELEPHANT SHREWS

The elephant shrews are strictly confined to the African Continent. The three species of elephant shrews under study display many features which are similar to those described in Elephantulus myurus by Van der Horst and Van der Horst and Gillman.

1. The ovaries:

The ovaries of the three species are lobulated and are completely surrounded by an ovarian capsule with a simple cuboidal ciliated epithelium very similar to that of E. myurus and other species of elephant shrews (Van de Horst 1943; Van de Horst and Gillman, 1945; Tripp 1970). Where the corpus luteum bulges above the surface of the ovary, the overlying capsular epithelium is stretched to become a simple squamous epithelium. R. chrysopygus and P. tetradactylus are strictly monovular and give birth to a single young per litter while E. rufescens is a biovulator with probable intrauterine transfer of eggs as evidenced by specimen 550 in which there were two conceptuses, one in each horn; but the two corpora lutea in the right ovary only (Fig. 46). Egg transfer to the contralateral horn has been reported also in E. rozeti, P. tetradactylus and R. petersi by Tripp (1970). The strict oligo-ovulation in R. chrysopygus, P. tetradactylus and E. rufescence contrasts strongly with the wasteful ovulation in E. myurus as reported by Von de Horst and Gillman (1941) and confirmed by Tripp (1970). These authors have reported an ovulation rate of up to 60 eggs per ovary. Included also among the superovulators are E. edwardi, M. proboscideus, E. capensis and E. brachyrhynchus (Van de Horst

1944; Tripp, 1970). On the basis of their ovulation rates, Van de Horst (1944) has divided the Macroscelididae into two genera namely:-

a) wasteful ovulators

b) conservative ovulators

However, this disparate ovulation is not peculiar to the Macroscelididae. It is also displayed by the rodent Suborder Hystricomorpha in which as many as 200 to 800 eggs can be shed by the plains viscacha whilst the mountain viscacha releases only one egg. It would, therefore, be hard to justify a division of Macroscelididae into two genera on the basis of their ovulation rates alone. Although Tripp (1970) confirmed that the large number of corpora lutea present in the ovaries of E. myurus were of primary origin, the possibility that the corpora lutea were formed from unovulated follicles during the early stages of pregnancy cannot be discounted. This is what happens in the case of the mountain viscacha (Pearson, 1949). It may also be possible that some corpora lutea are formed at intervals during pregnancy. Such a phenomenon has been reported in the rat where small corpora lutea are formed at intervals of 4 to 5 days during pregnancy without any evidence of ovulation (Swezy and Evans 1930).

2. The Pregnant Uterus

Implantation in the three species under study takes place in a special cavity, the embryo chamber, situated at the caudal end of each uterine horn. The cavity is lined by the uterine epithelium except at the site of placental formation. It is continuous with the main uterine lumen (Fig. 47 and 48). This finding is in agreement with those of Van der Horst (1947; 1950) for E. myurus. Implantation in the elephant shrews can therefore be termed eccentric and mesometrial. Van der Horst (1947) has, however, described an interstitial implantation in E. myurus but this was an abnormal situation.

Although the development of the embryo from the time of fertilization to the time of implantation was not studied in this thesis, it is worth recalling that Van der Horst (1942) claimed that the eggs of E. myurus are completely naked, having lost their zona pellucida and zona radiata shortly after ovulation. This claim has since been disclaimed by Tripp (1970) as an artefact due to fixation in Bouin's fluid. It is now well established that in mammals the zona pellucida persists until shortly before implantation or in some instances it disintegrates

during the process of implantation and apparently the elephant shrews are no exception. Another interesting phenomenon which has been described in E. myurus by Van der Horst (1942) and Van der Horst and Gillman (1942) is the formation of the four cell stage blastocoel. These authors further stated that in E. myurus the embryo cannot develop beyond the four cell stage unless it is implanted. The consequence of this development is that the morulla stage is by-passed and a typical unilaminar vesicle or blastula is formed. What is however, more interesting is Van der Horst's (1945) assertion that in E. myurus there is no polarisation of the early blastocyst into an inner cell mass and an outer trophoblast layer. First the blastocoel appears in the four cell stage and this is then followed by the differentiation of the inner cell mass by aggregation of amoeboid like cells from the peripheral wall. Such a development is closer to what is found in Amphioxus than what has so far been described in Placentalia. In the Placentalia, the morula first differentiates into an inner cell mass and an outer trophoblastic layer which is then followed by the appearance of the blastocoel or segmentation cavity. It is unfortunate that no further work on elephant shrews has been done to

verify or disapprove Van der Horst contention: a contention which must undoubtedly raise some interesting questions of phylogenetic importance.

3. The Placenta of Elephant shrews

The structure of the definitive placenta in the three species of Macroscelididae studied resemble the definitive placenta of E. myurus (Van der Horst 1950) and M. proboscideus (Starck, 1949) in all the essential components. In the three species P. tetradactylus, R. chrysopygus and E. rufescens two types of placenta exist simultaneously. There is, in the first place a well developed and circumscribed discoidal placenta of the haemo-chorial type according to Grosser's (1927) classification. Secondly the chorion, which is richly vascularised by allantoic vessels, is in intimate apposition with the uterine epithelium to constitute an epithelio-chorial placenta (Fig. 47a and 47b). Van der Horst (1950) stated that the large yolk sac, functioning as a placenta may be regarded as an epithelio-chorial placentation. This terminology brings unnecessary confusion as it is quite clear that after the establishment of the exocoelom the yolk sac is completely separated from the somatopleure and therefore it is only the somatopleure, with its outer covering

of trophoblast that can make contact with the uterine epithelium, first establishing a chorionic placenta and later an epithelio-chorial placenta. The existance simultaneously of two placentas of totally different types is not peculiar to the elephant shrews. Gerard (1929) has described the presence of an epithelio-chorial and a syndesmo-chorial placentae simultaneously in Galago demidovii while Hill (1938) found a diffuse placenta and a small disc of haemo-chorial placenta in the water shrew, Potamogale velox. Van der Horst (1950) stated that the epithelio-chorial placenta of E. myurus absorbs food supplied by the uterine glands. This may well be the case in the other species of elephant shrews especially when it is noted that there are extensively dilated uterine glands in the submucosa of the uterine wall (Fig. 47 a, b). PAS and Alcian blue stains demonstrated the presence of an acidic mucopoly-saccharide surface coat of both the chorionic trophoblast and the uterine epithelium. It has been quoted above that in the rabbit and guinea pig the route of transmission of antibodies from the mother to the foetus is via the uterine cavity and the well vascularised yolk sac (Hemmingway and Brambell, 1961; King, 1974).

In the human, however, the yolk sac is vestigial and the chorion covers the whole surface of the conceptus hence the route of antibody transfer is across the chorion-allantoic placenta. Since the chorion in the elephant shrews is richly vascularised and is in intimate apposition with the uterine epithelium it may be reasonable to postulate that the well established epithelio-chorial placenta in these species is involved in some way with the transmission of molecules (e.g. antibodies) from the uterine cavity to the foetus. However, such a postulation needs to be tested -- it requires among other things, the elimination of the role of colostrum in antibody transfer from mother to the new born (as occurs in the horse and cow) and also the direct transfer of antibodies across the haemochorial placenta.

The haemochorial placenta in the three species consisted of the columnar zone, the zone of proliferation and the spongy zone. This arrangement resembled exactly what has been described in E. myurus (Van der Horst 1950) and M. proboscideus (Starck 1949). P. tetradactylus and E. rufescens differed from R. chrysopygus in the structure of their basal region. In Petrodromus and Elephantulus the spongy zone is followed by a degenerative zone,

very much similar to the situation depicted in Macroscelides by Starck (1949). The foeto-maternal junction is represented by a necrotic zone as has been described in the results. Like in Macroscelides, the 'chorionic laeve' ends at the junction of the spongy trophoblast and the zone of degeneration (Fig. 57). In Rhynchocyon the spongy zone was followed by a distinct layer of basal trophoblastic cells which was continuous with the chorion at the placental periphery. This basal layer of trophoblast rested on a well defined wall of fibrous connective tissue. This fibrous layer is reminiscent of the granulation tissue reaction observed in chronic inflammatory conditions. In Rhynchocyon this layer is the equivalent of the junctional necrotic zone of other mammals and appears to act as the maternal limitation to foetal trophoblastic invasion. There were also many lymphocytes in the region between the basal columnar trophoblasts and the fibrous layer. Since these lymphocytes appear to be unable to attack the trophoblast this reinforces the hypothesis that these trophoblastic cells have a coating of an immuno suppressive substance.

A similar basal layer of trophoblastic cell has not been described in the other members of the family Macroscelididae but it has been

described in the placenta of the hyrax by Wislocki and Westhuysen (1940). The Macroscelididae and the Hyracoidae ware however, not phylogenetically related and whereas the hyrax placenta can also be divided into columnar and spongy zones, the similarity of placental morphology of the two families is a result of parallel and independent evolution. The similarity may be a result of adaptation but this will require an indepth study.

4. The Foetal Membranes

a) The Amnion

Early stages of amniogenesis were not available but probably amnion formation is by cavitation as in E. myurus (Van der Horst 1944; 1950;) rather than by folding. The amnion in the three species is extremely thin and in many places it is closely applied to the allantois.

b) The Yolk sac

The yolk sac is a distinct structure, and is relatively large and vascularised. Its intimate association with the uterine epithelium was not observed in this study. It is enclosed by the extensive allantoic membrane with which it blends at its lower border forming a distinct circle 47a; 56a, b. The yolk sac blood vessels are

characterised by their tall endodermal cells which form the wall. Whether the yolk sac persists to the end of gestation or regresses gradually was not determined but its presence in some fairly advanced conceptuses was suggestive that it probably does persist to the end of gestation.

c) The Allantois

Van der Horst (1944) has described the development of the Allantois in E. myurus in great detail. He says that the allantois appears as a little sac with a thick wall that hangs down in the extracoelomic cavity like a short tail. On reaching the placental surface, it begins to spread out over the surface. A similar situation was observed in R. chrysopygus; Fig. 48 b shows the allantois coming from the posterior end of the embryo and spreading over the surface of the chorion. At this stage the allantois is still not vascularised. In the later stages of development, the allantois in Macroscelididae portrays a very interesting pattern of development as shown in Fig. 56a, c (E. rufescens). The allantois sends several "arms" across the exocoelomic cavity to vascularise the chorion and together with the amnion, completely surround the yolk sac. This pattern of allantoic development is also observed

in Rhynchocyon and Petrodromus (Fig. 47a, b) but has not been described in other mammalian species to the best of my knowledge. The significance of such an arrangement is yet unclear. The presence of allantoic vesicle is observed in all the three species and this characteristic is considered important in determining phylogenetic relationship within an order (Mossman, 1937).

5. The Pattern of Circulation

Van der Horst (1950) stated that as a rule three decidual arteries contribute towards the formation of the placental arteries. He further stated that two sets of arterial capillaries enter the placenta, one set having been formed by the fusion of the terminal portions of two decidual arteries while the other came from the terminal portion of the third. The decidual vein always remained single. In this study it was observed that only one decidual artery actually penetrated the placenta and on reaching the foetal side it bifurcated into a left and right branch. Two veins, one on each side of the placental artery, left the placenta at the mesometrial side. These two veins soon joined, in the degenerative or junctional region to form one vein that entered the decidua. Similar findings were described and depicted in M. proboscideus

by Starck (1949). The counter current blood flow, therefore, involves the seeping through the trophoblastic lacunae of maternal blood from the foetal side of the placental disc towards maternal side while the foetal blood runs through the allantoic vessels from the junction of the spongy and columnar zones upwards towards the foetal side.

An interesting feature of the placental circulation in the elephant shrews (particularly well shown in Rhynchocyon and Petrodromus), was the presence of large giant cells in the lumen of placental and decidual vessels. Similar giant cells were also described in the decidual vessels of E. myurus by Van der Horst (1950) and in M. proboscideus by Starck (1949). Both authors called them decidual giant cells. These cells invade the vascular wall and in some instance they replace the vascular endothelium. In this study, however, two types of giant cells were observed:-

a) the giant cells present in the lumen of the terminal portions of the placental artery which were multinucleated with abundant cytoplasm and resembled the trophoblastic giant cells (Fig. 67 a, b).

b) the other type of giant cell was confined to the myometrial vessels (Fig. 61, 62).

As already described they could be single or binucleated and could represent decidual cells or hypertrophied endothelial cells (Fig. 61, 62). The presence of these giant cells in the lumen of maternal blood vessels raises the possibility that they may (and especially the trophoblastic ones) be transported to other organs in the body. The transportation of trophoblastic cells to other parts of the body especially to the uterus and lungs of the mother has received considerable attention in humans because of the extensively reported neoplastic nature of these cells. (Jacobson and Enzer, 1959; Lepow, 1959; Parks, 1958; Douglas et al. 1959). Salvaggio et al., (1960)have also reported the presence of syncytiotrophoblast in the umbilical cord blood, in the heart and liver of new borns. Trophoblastic emboli have recently been reported in the lung of chinchilla killed two months postpartum by Billington and Weir (1967). They also found the trophoblastic cells in the myometrium during the course of normal pregnancy and postulated that the haemochorial type of placenta is a prerequisite for trophoblast deportation. Although this line

was not pursued in this study, the possibilities of trophoblast deportation cannot be ruled out especially since giant cells, were found in the maternal myometrial blood vessels.

Another interesting finding in the decidua of the three species of elephant shrews was the transformation of the smooth muscles in the walls of the coiled arteries. The walls of these blood vessels consisted of large giant cells many of which were binucleate. The cytoplasm contained large bifringent PAS-positive granules that were diastase-resistant. The whole structure resembled a "subplacental gland" with a well defined outer epithelium that was continuous with the uterine epithelium. Similar glandular structures were described and depicted by Starck (1949) in M. proboscideus. He called the structure the "mesoplacentarium". There is no reference to such a structure in E. myurus by Van der Horst (1950). In the three species studied these large granular cells were even found in the walls of the mesometrial arteries. The precise role of these granular cells is not known. It is, however, now well known that the smooth muscle cells of the efferent arteriole in the mammalian kidney are modified into cells which contain

large granules. These cells - juxtaglomerular cells - are known to be the site of production of renin or its precursor (Rhodin, 1974; Bloom and Fawcett 1975). While the nature of the granules in the arterial wall of "mesoplacentarium" are not yet known, their presence in the pregnant uterus of the three species and in M. proboseideus indicates yet another characteristic that binds these species towards a common origin and their possible endocrine function cannot be overlooked. Starck (1949) described three types of cells in Macroscelides uterus during pregnancy:

a) Multinucleate large periarterial decidual cells which invaded the intima of the arteries;

b) multinucleate large syncytial elements which clad the lumen of the coiling uterine arteries and had pale nuclei and vacuoles;

c) small decidual cells with two nuclei and homogenous inclusions in their cytoplasm:

these being found in the vessel walls and in the endometrium. Similar cells were found in this study. Some of these may be endothelial in origin while

others trophoblastic or decidual. The hypertrophy of some of the endothelial cells or their secretion may act in the regulation of blood flow into the placenta.

The close association between the veins and arteries in the mesometrium poised some interesting questions. In recent years considerable attention has been focused on the role of Prostaglanding F_2 in the regression of the corpus luteum. It is believed that PGF_2 is produced locally in the uterus, diffuses directly from the veins into the arteries to be carried to the ovary (McCracken et al. 1972; Ginther, 1974; Del Campo and Ginther, 1974). Ginther (1974) and Del Campo and Ginther (1974) have described the very close contact betweeen the arterial and venous walls in the uterus of sheep, cattle, guinea pig, rats, hamsters and swine and concluded that such a relationship may favour the direct passage of uterine luteolytic substance between the utero-ovarian vein and artery through the intercellular spaces in the intervening tissue. A close relationship between arteries and vein has also been observed in the pampiniform plexus of the bovine (Amann and Ganjam, 1976) and the rhesus monkey testes (Einer-Jensen and Waites, 1977). These authors have suggested that such a close

relationship could facilitate the transfer of androgens from the testicular vein to the testicular artery for recirculation in the testis and epididymis. Einer-Jensen and Waites (1977) have further suggested that such a relationship is favourable for heat exchange (testicular temperature in the rhesus monkey was 32.9± 0.2°C while the deep body temperature was 37.2± 0.2°C). It also helps to attenuate the arterial pulse rate to the testis. The fusion of the tunica adventitia of the veins and arteries was observed in the mesometrium of the Macroscelididae. Such a relationship may reduce the arterial pulse pressure to the placenta. The presence of PAS-positive granules in the cells of the mesometrial arterial wall and the coiled arteries is suggestive that a substance is being synthesised. What this substance is and whether it can be transfered from the arterial wall to the veins are questions which will need further studies.

TABLE 3

SOME COMMON REPRODUCTIVE CHARACTERISTICS OF HYSTRICOMORPHA

	NAME	Range of gestation in days	Litter Size	Initial Attachment	Implantation	Amniogenesis	Chorioallantoic placenta	Sub Placenta	Decidual reaction
1.	Guinea pig (*Cavia porcelli*)	59 - 72	1 - 13	antimesometrial	Interstitial	Cavitation	haemochorial	present	
2.	Chinchilla (*chinchilla lanifer*	105 - 115	1 - 6	antimesometrial	Interstitial	Cavitation	haemochorial	"	
3.	Mountain Viscacha (*Lagidium peruanum*)	90	1	mesometrial	Interstitial	Cavitation	Haemochorial	"	
4.	Plains viscacha (*Lagostomus maximus*)	145 - 166	1 - 4	antimesometrial	Interstitial	Cavitation	haemochorial	"	
5.	Canadian porcupine (*Erethizon dorsatum*)	230	1 - 2	?	Incompletely	?	haemochorial	"	

TABLE 4

SOME REPRODUCTIVE CHARACTERISTICS OF THE TWO SUBFAMILIES OF ELEPHANT SHREWS

RHYNCHOCYONINAE & MACROSCELIDINAE

	CHARACTER	RHYNCHOCYONINAE	MACROSCELIDINAE	SOURCE OF INFORMATION
*1.	Ovulation rates	Strictly monovular	Range from monovular in Petrodromus to polyovular in E. myurus	Van der Horst (1944): Personal observation
2.	Length of gestation	40 - 60 days	40 - 60 days	Rathbun (1976)
*3.	No. young born	Strictly one	Range from one to maximum of two	Van der Horst (1950) Personal observation
4.	Implantation	In bursa embryonica and mesometrial	In bursa embryonica and mesometrial	Van der Horst (1950) Personal observation
5.	Placental type	Haemochorial	haemochorial	Van der Horst (1950) Personal observation
6.	Placental structure	3 distinct layers columnar, proliferative and spongy	3 distinct layers columnar, proliferative and spongy	Vauder Horst (1950) Starck (1949); Personal observation

	CHARACTER	RHYNCHOCYONINAE
*7.	Junctional zone	Basal columnar trophoblast resting on a layer of hyeline connective tissue
8.	Yolk sac	Large and persistent
9.	Inversion of germ layers	No
10.	Allantoic vesicle	Present
11.	Mesoplacentarium	Absent
12.	Granules in mesometrial arterial wall	Present
13.	Epitheliochorial type of placenta secondary to haemochorial	Present

MACROSCELIDINAE	SOURCE OF INFORMATION
No basal columnar trophoblast: Instead necrotic zone	Personal observation
Large and persistent	Personal observation
No	
Present	Personal observation
Present	Starck (1949) Personal observation
Present	Personal observation
Present	Van der Horst (1950)
	Personal observation

VIII PHYLOGENY

1. Phylogenetic relationships between the New World and the Old World Hystricomorph Rodents

The Rodent suborder Hystricomorphs contains about 13 known species and of these the guinea pig, Cavia porcelli, is widely used as a laboratory animal, hence it is the most extensively studied. Lovocat (1974) has stated that only those rodents which are simultaneously hystricognathous and hystricomorphous should be included in the suborder. He further defined the term "hystricomorph" as a particular condition of the infraorbital region in which the infraorbital foramen is large and accomodates the passage of an important portion of the masseter muscle as is found in the Hystrix.

On the basis of the foetal membrane and placental structure several important similarities emerged in this study which clearly indicate the close relationship between the Old and New World hystricomorph rodents. Mossman (1937) described the foetal membranes as among the most conservative characters having been least influenced by environmental factors or selective pressures. He listed four characters that he considered to be most consistent within a mammalian order namely:-

a) the orientation of the embryonic disc to the uterus;

b) the condition of the vascular splanchnopleure of the yolk sac;

c) the type of placentation - whether villous or labyrinthine, epithelio-. syndesmo-, endothelio-, or haemochorial,

d) the nature of the allantois i.e. the presence or absence of the allantoic vesicle.

Although Luckett (1969) has pointed out that it is the development relationship of the conservative foetal membrane characteristics that are phylogenetically significant and not their appearance in late stages of pregnancy, there is a justification in emphasising the later stage appearance of the foetal membranes when dealing with members of the same order or suborder. It has been mentioned already that the cane rat shares a number of important reproductive features with the New World hystricomorphs and these are shown in Table III. However, two of these are worth mentioning again:-

a) the early and complete entypy and

b) the presence of a well developed subplacenta

The possession of the subplacenta has not been described in any other mammalian order or suborder outside the hystricomorpha. Although the phenomenon of complete entypy is questioned in this thesis, the similarities in the pictures provided by various authors and those provided here for the cane rat show that fundamentally the development of the foetal membranes in the hystricomorph rodents is the same: The differences are questions of interpretations.

Wood (1972, 1974) and Wood and Patterson (1970) have stated that the many similarities between Old World (Caviomorph) and the New World (Phiomorph) rodents are due to parallelism rather than to retention of common ancestral characters or to a trans Atlantic migration by one or the other. Wood (1972) supported his argument against the theory of trans Atlantic migration by pointing out the inability of all other terrestial animals to use such a route. Lovocat (1974) on the other hand, pointed out that the probability of finding so many coincident characteristics in two unrelated infra-orders is unlikely. He therefore concluded that these similarities must be due to close genetic

affinities from a common ancestor. He has also provided fossil record evidence (Lavocat, 1967) to support the theory that the African phiomorphs and the South American caviomorphs have evolved from a common ancestral stock.

From the evidence presented in this thesis on the ovaries and the foetal membranes of the cane rat, Thryonomys swinderianus and those provided for Bathyergus janetta by Mossman and Luckett (1968) and for Hystrix cristata by Luckett (1971) it is concluded that the similarities are such that parallelism as advocated by Wood (1974) and Wood and Patterson (1970) is an unsatisfactory explanation for the relationship between the New and Old World hystricomorphs. A sounder explanation lies in very close genetic affinities among these groups of rodents resulting from a common ancestry from the African continent.

2. Phylogenetic relationships between the Macroscelididae and the Tupaiidae

The phylogenetic position of the elephant shrews is more complicated than that of the hystricomorph rodents. Their taxonomic position is yet to be determined. They have been associated and dissociated at one time or another with the primates

or with the Ungulates. But more commonly they have been classified with the insectivores (Young 1962). They have also been placed in the family Menotyphla together with Tupaiidae (Tree shrews) thus falling within one of the two families of Insectivores (Peters, 1864). But, as has been pointed out by Simpson (1945), the Insectivores is "a scrap basket for small animals of generally primitive characters that are not clearly referable to some more distinctive orders", the confussion about the taxonomic position of the Macroscelididae becomes quite apparent. Van der Horst (1950) concluded after an extensive literature review that the elephant shrews were very primitive mammals closely related to Erinaceus. However, Portmann (1935) listed the following characters as primitive in mammals:-

a) great number of offspring;

b) short periods of gestation;

c) birth of premature young;

All the elephant shrews that have been studied so far give birth to a maximum of two young per litter, the gestation periods are relatively long for their body size (ranging from 40 to 60 days) and the young are nidifugous at birth. Rathbun, (1976), has recorded that the young are capable of

rapid flight within hours after birth. Hence the elephant shrews are not primitive.

On the basis of their foetal membrane and placental structure the elephant shrews resemble each other as has already been described. The placenta is of the haemochorial type with a columnar, proliferative, spongy and basal zones. A similar placental architecture has been observed in the hyrax (Wislocki and Westhuysen, 1940; Oduor-Okelo, unpublished observation) but this shows more of parallel and independent evolution than divergence from a common ancestor. The placental affinities of the Macroscelididae differ greatly from those of Tupaiidae. The placentation in Tupaiidae (Tree shrews) is endothelio-chorial and the foetus is provided with a double or bidiscoidal placenta (Hill, 1965; Luckett, 1969). Several other lines of investigation have also revealed the absence of any relationship between the Tupaiidae and Macroscelididae. Carlsson (1922) for instance, found no relationship between the two families after comparing their skeletons, skins, musculature, dentition, gastro-intestinal tract, central nervous system and the reproductive tract. Buettner-Janusch and Hill (1965) compared the structure of haemoglobin in various mammals and found that the finger print pattern of Tupaia glis

Hb differed from the haemoglobin peptide patterns of Rhynchocyon and Petrodromus. Their keen senses, mode of locomotion, paired association, litter size and territoriality, which Rathbun (1976) considered to be phylogenetic within the family, together with their placental affinities as described by Van der Horst (1950) and Starck (1949) and in this thesis strongly suggest that the Macroscelididae are a distinct group of mammals which are certainly not primitive and deserve an order of their own separate from the Insectivora on the one hand and Tupaiidae on the other and as suggested by Buttler (1956) and Patterson (1965) the family Macroscelididae should be placed as the sole member in the order Macroscelidea.

The difference in the structure of basal trophoblast between Rhynchocyon on the one hand and Petrodromus and Elephantulus on the other would support the division of the family Macroscelididae into the two subfamilies of Rhynchocyoninae and Macroscelidinae. The similarities and differences between the two subfamilies are listed in table 4.

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