ASPECTS OF PLACENTATION IN CERTAIN CERVIDAE

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INTRODUCTION

An account of the placentae and uteri at a relatively late stage of gestation of four specimens of Père David's deer (*Elaphurus davidianus*) has already been given by two of us (Harrison & Hamilton, 1952). The appearance and microscopical structure of the placentomes and the remarkable changes found in certain zones of the villi prompted us to look further into the whole question of placentation in other Cervidae which became available.

Brief and often incomplete accounts have been given of the placentae of a number of deer by earlier investigators. Weber, as early as 1832, and Bischoff (1854) described the form, and to some extent the structure, of the placenta of the roe deer (*Capreolus capreolus*). Swinhoe (1870) gave an account of reproduction of the Shanghai river deer (Chinese water deer) (*Hydropotes inermis*) and some details of the placenta of this deer were also given by Ewart (1878). Turner (1878*b*) described the gravid uterus and foetal membranes of the hog-deer (*Cervus porcinus*). The amniotic cavity had been opened before it came into Turner's hands and only one of the 'foetal caruncles' was attached to the 'maternal cotyledon'. In the same year Turner (1878*a*) gave an account of the foetal membranes after they had been shed by the reindeer (*Rangifer tarandus*). In 1879 Turner described the arrangement of the foetal membranes in the uterus of the Mexican deer (*Cervus mexicanus*).

Beauregard & Boulart (1885), in their note on the placentation of ruminants, referred to the number of 'cotyledons' among the following deer: *Panolia frontalis*, *Cervus porcinus*, *Cervus elaphus* and *Rangifer tarandus*. These authors were more concerned with the phylogenetic relationship of deer to other ruminants than with a description of the placenta. A brief note on the placenta of *Cervus sica* was given by the same authors in 1885. Boulart (1888) briefly referred to the placenta of the Mexican deer (*Cervus mexicanus*). Strahl (1906) and Andresen (1922) have described the placenta of several specimens of the red deer (*Cervus elaphus*) and Kolster (1909) has described that of *Rangifer tarandus* in some detail. Brief descriptions of placental details in other Cervidae are included in a valuable review by Andresen (1927). Harrison & Hyett (1954) have given an account of the growth, changes in form and the gross structure of the foetal and maternal elements of the placentome of the fallow deer (*Dama dama*) from the time of development of cotyledonary villi until the foetus reached a C.R. length of 40 cm.

MATERIALS AND METHODS

Specimens of intact pregnant uteri or portions of the uterus and placenta from eight species of Cervidae have been available for examination (see Table 1 for details). Pregnant uteri of Père David's deer (*Elaphurus davidianus*) were obtained from

Woburn Park, Bedford, through the kindness of the late Duke of Bedford and have already been described (Harrison & Hamilton, 1952). Through the kindness of the late Lord Leconfield, who had a large herd of fallow deer (*Dama dama*) at Petworth, we have been able to obtain stages of development from the unimplanted chorionic sac to late in gestation. Additional specimens of this deer have been obtained from Parham Park, Sussex, through the kindness of the Hon. Clive Pearson, from Knole Park, Kent, through the kindness of Lord Sackville, and from Quendon Park, Essex, through the kindness of Sir Arthur Ellis. Over two hundred uteri at different stages of pregnancy, and some twenty non-pregnant uteri form the basis of our collection. The details of the pregnant series are given in Table 1, but lack of space prevents a detailed tabulation of the dates; these and other details are available from the authors. Some aspects of development and growth of the placentomes from these animals have been described by Harrison & Hyett (1954).

The pregnant specimens from the other species form only a small and incomplete series, but are included here in order to provide a comparison of placentation in the Cervidae at least at certain stages of pregnancy. The red deer (*Cervus elaphus*) were obtained from Richmond Park, Surrey, through the kindness of the local authorities. The roe deer (*Capreolus capreolus*) were obtained from Petworth Park, Sussex, the Manchurian Sika (*Capreolus bedfordi*) and Indian spotted deer (*Cervus axis*) from Woburn Park, Bedfordshire, and the Japanese Sika (*Cervus nippon*) from Knole Park, Kent. The specimens of pregnant uteri from the reindeer (*Rangifer tarandus*) were flown by air from Norway either as fixed or frozen specimens. They were supplied through the kindness of Dr O. W. Tenjord, of the Norwegian Legation, London. The specimens were collected by District Veterinary Surgeons at Alta and Karasjok, Norway. The gross details are satisfactory but the microscopical appearances are poor.

The material was dealt with in three ways, depending on its freshness. The most recently killed specimens of pregnant uteri were opened and portions of the ovaries, uterus and placenta were removed and fixed in a variety of fixatives, including formaldehyde, formol-saline, Bouin's fluid, Zenker-formol, 80 % alcohol or acetone (chilled) and Rossman's fluid (chilled). Representative blocks of tissue were subsequently embedded in paraffin and sections were prepared at 3–10 μ. Small pieces of placentome (foetuses of 22 mm. and 200 mm. C.R. length) were fixed in Palade's or Dalton's fluid, embedded in methacrylate and ultra-thin sections were examined in a Siemens Elmiskop I electron microscope.

Sections were stained with Heidenhain's iron haematoxylin, Masson's trichrome (Wislocki's modification), Mallory's stain and Robb-Smith's modification of Foot's method for impregnation of reticulum. Frozen sections were stained with Sudan Black B. McManus' (1946) periodic acid-Schiff procedure was employed on the material fixed in Rossman's fluid. Phosphatases, alkaline and acid, were demonstrated in tissue fixed in chilled acetone or 80 % alcohol (Dempsey & Deane, 1946). Preparations were also made for examination by the phase contrast microscope.

The second method of examination involved the selection of suitable, undamaged pregnant uteri and their injection with coloured gelatin, Marco Resin or Latex. Warm 20 % gelatin containing Boston Chemical Company's Dispersion Colours (10 %) was the most convenient injection mass, because it entered the finest

capillaries and also allowed subsequent sectioning. Both maternal and foetal circulation were injected, or alternatively only one of them. These specimens were subsequently fixed in 10 % formalin.

Table 1. *Details of embryos and dates of collection*

Species	C.R. length in mm.	No. collected	Dates recovered
Fallow deer (*Dama dama*)	1·0	1	2 Dec.
	7·0–9·0	4	25 Nov.–2 Dec.
	12·0–17·0	8	25 Nov.–2 Dec. (1 on 12 Jan.)
	18·0–26·0	21	25 Nov.–18 Dec. (1 on 24 Feb.)
	27·0–40·0	8	8 Dec.–29 Jan.
	78·0–95·0	11	17 Dec.–29 Jan. (1 on 17 Feb.)
	99·0–110·0	31	7 Jan.–17 Feb. (1 on 2 Dec.; 1 on 23 Feb.)
	111·0–125·0	23	8–20 Jan.
	130·0–150·0	49	14 Jan.–10 Feb.
	152·0–170·0	24	25 Jan.–23 Feb.
	175·0–192·0	29	20 Jan.–26 Feb.
	195·0–220·0	21	10 Feb.–3 March (1 on 17 Jan.)
	222·0–290·0	27	17 Feb.–3 March
	375·0–399·0	5	12–16 April (1 on 1 March)
Red deer (*Cervus elaphus*)	150·0–170·0	4	8–15 Jan.
	190·0–215·0	9	6–20 Jan.
	300·0	1	14 Jan.
	730·0	1	1 June
Père David's deer (*Elaphurus davidianus*)	100·0	1	19 Feb.
	275·0	1	9 March
	330·0	1	10 Jan.
	360·0	1	10 Jan.
	380·0	1	13 Jan.
Manchurian Sika deer (*Capreolus bedfordi*)	241·0	1	10 March
Japanese Sika deer (*Cervus nippon*)	332·0	1	5 April
Indian spotted deer (*Cervus axis*)	420·0	1	12 April
Reindeer (*Rangifer tarandus*)	260–265	4	Not known
	270–280	4	Not known
	294	1	Not known
Roe deer (*Capreolus capreolus*)	12·0–20·0	3	16 Feb.–10 Mar.
	50·0–52·0	2	16 Feb.
	91·0–105·0	4	5–16 Feb.
	110·0–170·0	14	19 Feb.–10 Mar.
	216·0–235·0	3	10 March

The third group of specimens were those pregnant uteri which were fixed unopened in 10 % formalin, or in which the uterus was opened and the foetus or chorionic sac removed for subsequent fixation and preservation (see also details given by Harrison & Hyett, 1954). Sections were also prepared of the ovaries, uterus and vagina of the non-pregnant animals and stained with Heidenhain's haematoxylin and eosin. Samples of the amniotic and allantoic fluids were removed from pregnant uteri at different stages of pregnancy and were analysed for total reducing substances and fructose (Walker, 1954).

REPRODUCTION IN THE CERVIDAE

Observations on the reproductive patterns exhibited by the Cervidae have been made by Lydekker (1898), Heape (1901), and Seton (1909) and the review by Asdell (1946) summarizes much of the earlier information. Lydekker points out that 'many of the larger and more specialised deer are comparatively slow breeders, for although the hinds frequently, if not generally, breed annually, yet it is seldom that more than a single fawn is produced at birth, twins being rare, while triplets are practically unknown'.

The majority of deer are monoestrous and have a limited mating season from September to October. The young are born from April to June. There are, however, a number of exceptions to this generalization. Sambar deer (*Cervus unicolor*) and Mule deer (*Odocoileus hemionus*) do not mate until November or December. Roe deer (*Capreolus capreolus*) mate in July and August and parturition does not occur until the following May. A few species have no fixed breeding season. Indian spotted deer (*Cervus axis*) will breed at any period of the year and in captivity have dioestrous cycles throughout the year. The Red Brocket (*Mazama americana*) and the Muntjacs (*Muntiacus*) also have no fixed breeding season. In Thameng (*Cervus eldi*) the mating season lasts from the middle of March to the middle of May and parturition occurs in October and November.

The following observations give the general pattern of reproduction of the species which we have examined.

Mating in *Dama dama* in parks in southern England occurs throughout October, but may be extended into November. Recently ruptured follicles have been observed by us in December and once in early January. Parturition occurs in the period late May to July. The number of young is one; twins have only been found once in over 300 pregnant uteri examined. The gestation period is 230–240 days; there is no evidence of delayed implantation. Unfortunately we have no information whether the late mating was in animals born late in the previous season or in animals which were suckling their young for a longer period.

Mating in *Cervus elaphus* occurs in September and October, and parturition in May or June with a gestation period of 225–246 days: it is said to exhibit cycles in captivity. A continuous series of cycles of 3 weeks' duration is exhibited by *Cervus axis* in captivity (Heape, 1901) and the late Duke of Bedford stated that breeding occurred at any time of the year at Woburn (Bedford & Marshall, 1942). Gestation lasts 210–238 days.

Mating in *Elaphurus davidianus* occurs in captivity in England in June and July with parturition in mid-April to May; the latest date for parturition is September. *Capreolus bedfordi* is said to mate in July and parturition takes place in May or June. In *Cervus nippon* the gestation period is estimated to be 222–246 days and in *Rangifer tarandus* the period is 216–246 days.

Mating in *Capreolus capreolus* occurs in July and August, there is usually delayed implantation until December or January, but implantation can immediately follow ovulation (Prell, 1938). From the time of nidation pregnancy lasts 140–165 days, and young are usually born in May. Twinning (of like sex) appears to be the rule.

Twinning

Twinning has been found consistently in the European roe deer and once only in fallow deer in the specimens that we have examined. Of the twenty-six pregnant uteri of the roe deer examined, twenty-two of these contained twins; ten of the twin specimens had two corpora lutea in one ovary and ten had one corpus in each ovary, two had only one corpus luteum. All the twins were of similar sex. Lydekker (1898) reports that this species may produce one or two at birth and states that some of the older writers reported that the twins 'are always male and female'. In Manchurian roe there are usually twins.

In the genus *Odocoileus*, twins are generally found in the following species: Virginian roe (*Odocoileus virginianus*), Mule deer (*Odocoileus hemionus*) and Black-tailed deer (*Odocoileus columbianus*). In the Red Brocket (*Mazama americana*) a pair of twins are produced annually. In Himalayan Musks (*Moschus moschiferus*), Sambar deer (*Cervus unicolor*), American Wapiti (*Cervus canadensis typicus*), Indian Muntjac (*Cervus muntjac*) and in the Moose (*Alces alces*) one or two fawns may be produced. The Chinese water deer (*Hydropotes inermis*) is an exception to most deer in that it usually produces three or four young at birth.

PLACENTATION IN FALLOW DEER (*DAMA DAMA*)

Early stages in placentation

The largest number of early specimens available (see Table 1) are from *Dama dama*, but even in the youngest embryo (1 mm. overall length) an amniotic cavity has been formed and the allantois is large. No statement can therefore be made as to the method of formation of the amniotic cavity. The elongated chorionic sac of *Dama* fully occupies both horns of the uterus by the 7 mm. C.R. stage. That part of the sac in which the foetus is lying is much wider and longer, and the uterine horn containing it more swollen than that on the opposite side (Pl. 4, figs. 17, 18). The foetus usually lies nearer the cervix than the mid-point of the pregnant horn. The chorionic sac is transparent and elastic and tapers towards its extremities. The large allantois distends the chorion and is intimately apposed to it except where the amnion intervenes. The allantois increases steadily in size during gestation and from about the 20 mm. C.R. stage it ruptures through the tips of the chorionic sac to form an atrophic region, placed at the tubal extremity of each horn (Pl. 4, fig. 18).

Discrete circumscribed regions, or 'plaques', usually oval in outline, appear on the mesometrial aspect of the chorion from the 12 mm. C.R. stage (Pl. 4, fig. 17). They lie closely related to the elevated maternal caruncles and are the sites of development of the primitive villi of the cotyledons. Small folded elevations of chorion, the areolae, develop on the intercotyledonary regions from the 15 mm. C.R. stage. They are evenly distributed about 1 mm. apart, and appear to lie opposite the mouths of uterine glands. They measure 0·5–0·8 mm. in diameter at the 20 mm. C.R. stage and increase only slightly in size as pregnancy advances. No intercotyledonary villi have been observed.

The umbilical vessels are well developed by the 20 mm. C.R. stage and one artery and one vein extend from each side of the umbilical cord along the lesser curvature or

mesometrial aspect of the chorion towards its tips. The umbilical vessels run close to the developing cotyledonary regions and give off branches that pass circumferentially about the chorionic sac. These branches subdivide to supply the cotyledonary areas and continue to supply the intercotyledonary regions. A fine capillary plexus develops in the cotyledonary areas by the 22 mm. C.R. stage. The branches of the main veins run independently of those of the arteries.

Important changes occur in the chorion and the uterus during early pregnancy in all species of Cervidae. Villi develop on the circumscribed areas of the chorion to give rise to the primitive *cotyledons*. The villi are at first short and simple, later they elongate, branch and form tufts of long, filiform processes arising from a single main stem villus. They soon penetrate into *crypts* that develop in the uterine caruncles. These are flattened, sessile, ovoid structures lineally arranged along the mesometrial surface of the inside of the uterine horns. They are devoid of glands. Each cotyledon develops opposite a caruncle and when the villi have become established within the crypts a *placentome* is formed (Pl. 1, fig. 2). As pregnancy advances growth changes occur in the villi, the caruncles and later in the placentomes.

Villi appear first at about the 12 mm. C.R. stage and are arranged in a characteristic pattern. They arise as projections from small ridges, the majority of which lie transverse to the long axis of the cotyledon (Harrison & Hyett, 1954). These authors give details of the origin, number and arrangement of villi on a cotyledon. The growth and relationships of the villi and crypts are described later. No haematomata have been observed in relation to the chorion as Wimsatt (1950) found in sheep and Harrison & D'Silva (1956) in goats.

The structure of the chorion

The chorionic epithelium is composed of two main types of cell even in the youngest specimen (embryo 1 mm. overall length). They are (*a*) the columnar trophoblastic cells which form the majority and are arranged at first as a continuous sheet, 2–3 cell layers thick, and (*b*) the characteristic binucleate cells (or diplokaryocytes) which are distributed at intervals all over the young avillous chorionic sac and which have been extensively studied in ruminants (Wimsatt, 1951). This general arrangement is maintained throughout pregnancy except in certain regions of the villi (page 11).

The columnar trophoblast cells are 15–20 μ in height and 10–15 μ across: their spherical nuclei are 4–6 μ in diameter. In haematoxylin- and eosin-stained sections their granular cytoplasm is moderately eosinophilic and contains minute vacuoles. Nuclei are lightly stained, have a 'linum reticulum' and two or more nucleoli. Minute lipid droplets can be seen scattered throughout the cytoplasm in frozen sections. Alkaline phosphatase (Na-β-glycerophosphate substrate) is demonstrable as a band on the outer region of these cells. No PAS-positive material is present and no acidophilic proteinous crystals have been seen.

The fully differentiated binucleate cell is spherical, 15–20 μ in diameter, with two or more spherical nuclei up to 10 μ in diameter. They display similar cytological characteristics throughout pregnancy (Pl. 3, fig. 16). Chromatin in the nuclei is arranged in striking clumps and gives a marked Feulgen reaction. No distinct nucleoli have been observed. A 'Golgi zone' comparable to that described by Wimsatt (1951) can be demonstrated close to the nucleus.

The cytoplasm throughout most binucleate cells appears to exhibit even distribution of staining properties, but in a number it can be divided into predominantly basophilic supranuclear and acidophilic infranuclear portions (Wimsatt, 1951). A dome-like protuberance is frequently seen on the supranuclear uterine surface of binucleate cells lying superficially in the chorionic epithelium. This and the supranuclear cytoplasm has a distinct basophilic component, but there is often an acidophilic zone in the protuberance. It must be emphasized that this protuberance is only clearly seen when the trophoblast has separated from the crypt lining, and may well be an artefact. One or more large lipid droplets are frequently seen in the cytoplasm. Alkaline phosphatase (Na-β-glycerophosphate substrate) is distributed evenly throughout the cytoplasm, but is scanty or lacking in the nuclei. Some binucleate cells, however, possess only sparse amounts of the enzyme. No maternal erythrocytes, non-ferruginous pigments, or strongly acidophilic proteinous crystals as described by Wimsatt (1951) have been identified in binucleate cells of any deer placenta we have examined.

The binucleate cells give a strong and characteristic reaction with the PAS procedure (Pl. 4, fig. 21). Repetition of the analysis made by Wimsatt (1951) shows that the reacting material is a polysaccharide-protein complex. There is not the same infranuclear restriction of distribution of the material as in the sheep. It appears to be distributed evenly, as in the cow, throughout the cell except for a clearer area that may well correspond to the Golgi zone. Those cells at the tips of villi are always the most intensely stained.

Our material allows no statement on the time of their first appearance, but it is clear that an immense number of binucleate cells are formed during the duration of pregnancy. They may either reproduce themselves or arise from trophoblastic precursors: but we have found no evidence for the former hypothesis. Cells intermediate in form and characteristic between those of typical trophoblast cells and binucleate cells may be seen everywhere in the early chorion. In general the youngest and healthiest binucleate cells are seen in the inner part of the trophoblast (Wimsatt, 1951; Harrison & Hamilton, 1952). These cells contain only one large nucleus, but their abundant cytoplasm, PAS-positivity and other tinctorial, cytoplasmic and nuclear characteristics allow them to be readily distinguished. Nuclear division occurs mitotically without cytokinesis and two nuclei, more rarely three, are formed. At this stage the cells lie nearer the surface of the trophoblast and their plasma membrane makes contact with the crypt epithelium. The characteristic protuberance may now be seen and it is our contention that this may represent the start of an invasive process by which the binucleate cell enters the crypt epithelium (page 10).

The caruncle

The changes in the caruncle in the earliest stages of pregnancy have only been observed in detail by us in *Dama dama*. A few fairly young stages (12 mm. C.R. length) of *Capreolus capreolus* have been examined and also later stages of *Cervus elaphus* (Table 1). The appearance of these specimens, although at later stages, suggests that the caruncular changes and subsequent placentome formation would not be dissimilar from those in *Dama*. The quite different internal construction of

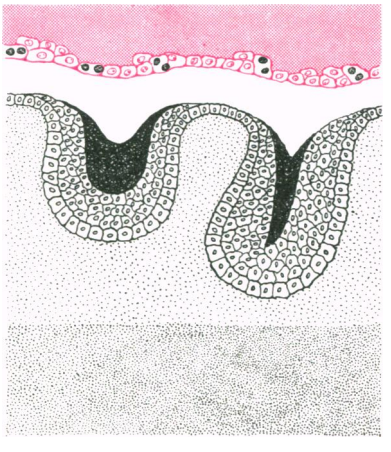
the older placentomes of *Rangifer tarandus* precludes any such assumptions for this species.

The appearances of the non-pregnant uterus of *Dama* have been described by Harrison & Hyett (1954). The caruncles are arranged in a row along the mesometrial aspect of the uterine lumen. They are elongated, sessile and leaf-like in immature animals; the central ones in each horn are the largest and up to 2 cm. in length and 8 mm. across. They become elongated ovoids supported by a short pedicle in older does. The average number is four in each horn (range 3–6). Each caruncle is covered by a low, single-layered cubical epithelium (Pl. 2, fig. 10). The cells are 6–10 μ in height and have dense eosinophilic cytoplasm with a densely stained nucleus situated centrally in each cell. No evidence of secretion is seen in the caruncular epithelium and none of its cells contains glycogen. The subjacent stromal tissue is devoid of glands and is densely cellular. It does not exhibit any strata of varying density. It is poorly vascularized, but many large, spiral vessels lie in the basal region. The caruncular tissue is not oedematous and contains few leucocytes. The closely packed stromal cells are surrounded by a dense feltwork of reticulin fibrils. Little glycogen or Schiff-positive material is discernible in caruncular stromal cells in the non-pregnant state. In those animals that had been pregnant the previous season the basal region of the caruncle exhibits the fibrous remnants of large obliterated blood vessels that were presumably those supplying the maternal septa.

Placentome formation does not commence until the embryo has surpassed a C.R. length of some 2 to 3 mm. Even at 7 mm. only the central caruncles of each horn exhibit any evidence of crypt formation. Changes occur first in the central region of each caruncle. The caruncular epithelium becomes thicker, the cells enlarge to some 20 μ in height, become two-layered in places, and their nuclei become enlarged and vesicular. Rows of solid epithelial ingrowths develop from the caruncular epithelium. They are arranged lineally, at intervals of about 1 mm. transverse to the long axis of the caruncle (Harrison & Hyett, 1954). The ingrowths are at first conical or spherical, only some 100 μ deep and 50 μ in diameter, and are composed of hypertrophied epithelial cells (Pl. 2, figs, 7, 8; Pl. 4, figs. 18, 20). The ingrowths increase in size until some 0·2 mm. in greatest diameter, when the central cells exhibit degenerative changes. Their nuclei are fragmented and their cytoplasm is dense and aggregated. The central zone is occupied by strongly Schiff-positive material which is partly glycogen but mostly of a glycoproteinous nature. The central region next obtains communication with the uterine lumen and the Schiff-positive material is lost, either by absorption *in situ* or by drainage to the exterior, where it is possibly absorbed by the trophoblast. The latter lies, at this stage, in loose contact with the surface of the caruncle and is presumably held against it by pressure exerted by the ever-increasing size of the allantoic sac. We can find no evidence that it is the development of the chorionic villi that initiates crypt formation. Distinct villi appear to develop only after crypt formation has advanced to some extent. There is no doubt, however, that folds occur in the chorion very easily after death, and it is impossible to be sure that small folds or protuberances of the fixed chorion do not represent primitive villi, although they do not look like them.

Each crypt becomes elongated as it grows, but remains between 0·1 and 0·25 mm. in diameter throughout its length. Those crypts in the centre of the central caruncle

in each horn enlarge first. Even by the 20 mm. stage the smaller caruncles at each end of a horn only show early stages of crypt formation. The primitive crypts are invaded by definitive villi as soon as the central cellular debris has started to disappear. The tips of the villi extend into the remnants of this material at the bases of some crypts, even where they have reached a depth of 0·6 mm. The crypts are at first lined by a single layer of cubical epithelial cells, 12–15 μ in height, and with distinct cell boundaries. The layer is continuous with the covering of the surface of the caruncle. The lining at the bases of the crypts is often several cells thick; this may indicate further growth in depth (Pl. 2, fig. 9; Pl. 4, fig. 22). The majority of these



Text-fig. 1

Text-fig. 2

Text-fig. 1. Diagram to show the method of formation of the primitive crypts in *Dama*. Binucleate cells are present in the trophoblast, but not in the epithelium of the caruncle which is intact and shows downward invaginations with necrotic centres.

Text-fig. 2. Diagram to show primitive villi within developing crypts in *Dama*. Note the hyperplasia at the base of each crypt.

cells appear healthy, with a vacuolated cytoplasm and with vesicular nuclei 6–10 μ in diameter. Others have dense, pyknotic, shrunken nuclei. Interspersed amidst these cells are some binucleate cells which can be recognized by their larger size (15–20 μ in diameter), less heavily stained cytoplasm, dense nuclei and polyhedral or oval shape. They also contain Schiff-positive material whereas the lining epithelium has little or none. Alkaline phosphatase activity can be demonstrated in the cytoplasm and these cells are in every way identical to the binucleate cells of the trophoblast (page 7): the significance of their presence in the crypt lining is discussed on page 29.

The stromal tissue surrounding the crypts shows little change during early crypt formation. When the crypt has reached a depth of 0·5 mm. there is some evidence of increase in density of that tissue about the tip of the crypt. Intercellular material is faintly Schiff-positive, and there is a slight decidual reaction. The caruncle now

begins to exhibit three zones. A superficial zone, nearest the surface epithelium, is 1–2 mm. thick and contains the deepening crypts. It is oedematous and capillaries lie in it parallel to the long axis of the crypt and extend towards the surface. A narrow intermediate zone of young, cellular stromal tissue is only 0·25 mm. thick. Its appearance and the presence of occasional mitoses suggest that it is a zone of active growth. It is perforated by small arteries and veins that branch into capillaries of the superficial zone. A basal zone, varying in thickness from 1 to 5 mm., blends with the loose connective tissue lining the muscle of the uterine horn. It contains fibroblasts, collagenous fibres and many large spiral vessels. The general arrangement of the developing placentome is shown in Text-figs. 1–4 and illustrated in Plates 2 and 4.

Later changes in the crypts

The crypts are up to 2 mm. in depth and 0·25–0·5 mm. in diameter in the largest caruncles by the 27 mm. C.R. stage. They are then lined by cuboidal cells two to three layers thick on the sides of the crypt; there is a denser accumulation of cells at the bases. Binucleate cells invade the crypt lining in considerable numbers at this stage. There is at first no evidence of lining destruction; the binucleate cells appear to probe the lining by insinuation of their cytoplasm between the cuboidal lining cells. Eventually they become intercalated in the lining where many can be distinguished by their intense PAS-positivity; they are supported by a strongly PAS-positive basement membrane. Capillary loops are now arranged close to this membrane. The stromal cells of the caruncular tissue surrounding the crypts are enlarged and vacuolated. Tissue oedema is present and droplets of glycogen are scattered throughout the superficial areas of the caruncle.

Many binucleate cells present in the crypt lining have little or no PAS-positive material. Others show varying degrees of nuclear degeneration, some possess more than two nuclei; occasional mitotic figures can be observed in these cells. Some cuboidal cells also show evidence of nuclear degeneration (pyknosis) and some are collapsed or distorted in shape. Areas of extracellular eosinophilic amorphous material (PAS-positive, but not removed by diastase) are present at the junction of the trophoblast and the cuboidal cells.

From the 30 mm. to the 70 mm. C.R. stage the crypt lining appears to contain: (*a*) cuboidal cells that are considered to be derived from those maternal cells that originally lined the primitive crypt; (*b*) cuboidal cells undergoing degeneration; (*c*) invading binucleate cells, possessing abundant glycoprotein, that are foetal in origin; (*d*) binucleate cells, with little or no glycoprotein; some are degenerating and are intercalated amongst the cuboidal cells. No leucocytes are present and there is no evidence of haemorrhage from maternal vessels.

The fully differentiated villus

The final form and characteristics of a fully differentiated villus in *Dama dama* are shown in Text-fig. 4. The overall shape is filiform and a villus measures up to 25 mm. in total length. The main stem of the villus is 5–6 mm. in length and 1–2 mm. in diameter, and divides into two to six secondary villi, 10–15 mm. in length. These taper gradually from 0·4–0·5 mm. at their origin to 0·2–0·3 mm. at their point of

division into short tertiary villi 3–5 mm. in length. Three zones, each with special trophoblastic characteristics, are recognizable and it is our conviction that they each have different functions. They are: (*a*) a storage zone; (*b*) a zone of attrition; (*c*) a zone of physiological exchange (Text-fig. 4).

The storage zone

This is represented by the trophoblast of the great part of the main-stem villi and the arcades between them (Pl. 3, fig. 11). The trophoblast forms a single layer of tall, narrow columnar cells up to 60 μ in height. Their nuclei lie at the base or in the central third of the cells. The apices of these cells are frequently distorted by protuberances or knobs of cytoplasm projecting from the surface (Pl. 3, fig. 14). There is a PAS-positive basement membrane, deep to which is a row of mesenchymal cells; only a few capillaries are present. Binucleate cells are absent in this zone. Numerous droplets of PAS-positive material are present within the apical cytoplasm and in the cytoplasmic protuberances; many of the droplets lie outside the cells (Pl. 3, fig. 12). Diastase treatment and other tests indicate that the greater part of this material is glycogen. The amount of glycogen present increases as pregnancy advances. This zone of the villus may or may not be in contact with the necrotic tissue of the maternal crypt (page 14).

There is an abundant distribution of alkaline phosphatase (Na-β-glycerophosphate substrate) as a band along the outer surface of the columnar trophoblast. It is particularly marked in the distal part of this zone and here there is some intracellular distribution within individual cells. The surface band is less marked, or absent, in the arcades between villi, but there is a narrow distribution along contiguous cell membranes. In places degenerated debris of giant cells may be seen lying against the edges of the trophoblast.

The zone of attrition

This is a narrow zone extending for only 0·5–1·0 mm.; it frequently includes the point of division of the main stem villus and extends a short way down the secondary villi. It is the region of the definitive villus that obtains intimate contact with the crypt wall. The single layer of tall columnar cells with abundant glycoprotein content is abruptly replaced by a double- or treble-layered trophoblast (Pl. 3, fig. 15; Text-figs. 3, 4). This consists of columnar trophoblast cells with many binucleate cells amongst them. The former contain no PAS-positive material, the latter have fine PAS-positive granules distributed throughout their cytoplasm. A fine PAS-positive basement membrane supports the trophoblast and in places it sends slender folds between its cells. The trophoblast separates easily from the crypt lining but where it has not separated it appears to be in contact by processes of varying thickness between which lies granular eosinophilic material (page 14).

The zone of physiological exchange

This zone comprises the remainder of the villus and it is throughout in contact with the crypt lining. The exact nature of this contact is obscure, but under the light microscope it appears to be an edge-to-edge contact by cytoplasmic processes from the trophoblast cells separated by vacuoles and small lakes of amorphous

eosinophilic material (page 17). When the trophoblast separates from the crypt lining, as it does only too readily after death, its outer margin has an irregular appearance (Harrison & Hamilton, 1952, pl. 4, fig. 13). The trophoblast has characteristics similar to the zone of attrition, except that (*a*) the binucleate cells are fewer and progressively contain more PAS-positive material towards the tip of the villus; (*b*) intra-epithelial capillaries are present and (*c*) there is an interlocking of microvilli (page 15).

Throughout the zone binucleate cells may be seen deep in the trophoblast, at its surface and in an intermediate position (Pl. 3, fig. 16). Some of them can also be seen invading the crypt lining and lying deep in it in contact with the maternal connective tissue. Many of these cells appear healthy, but many show degenerative changes in one or both nuclei. Alkaline phosphatase (Na-β-glycerophosphate substrate) is distributed in a broad band along the region of contact between the trophoblast and the crypt lining (Pl. 3, fig. 13). The edge of the trophoblast appears to possess the greatest quantity, but there is a marked intracellular distribution in many but not all of the binucleate cells. This continuous phosphatase barrier is not found in the placentomes of the sheep and cow (Wimsatt, 1951).

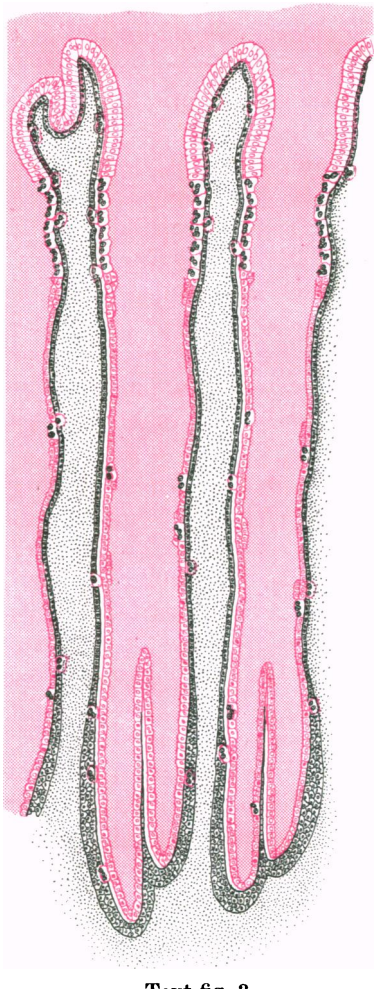
The crypt lining in relation to the differentiated villus

The crypt lining starts to become differentiated into three zones at the 70 mm. C.R. stage. These zones correspond spatially to those already described for the villus (page 11); they are clearly present by the 90 mm. C.R. stage and persist until term. The deepest part of each crypt is subdivided into short basal recesses that receive the tertiary villi (Pl. 3, fig. 16). These regions of the crypt, and the much longer region above it which encloses each secondary villus, maintain the same characteristics throughout the rest of pregnancy. The crypt lining is from one to two cell layers thick except at the bottom of the subsidiary crypts, where it is often thicker. In any specimen several types of cell may be seen in the crypt lining. In younger specimens the lower cuboidal type predominates, but as pregnancy advances more and more of the crypt lining becomes syncytial in character. Numbers of degenerating cuboidal cells can be seen in the fundi of the crypts, particularly in stages from 100 to 500 mm. C.R. length. Some of these cells retain their cuboidal shape and exhibit nuclear degeneration; others are distorted and both cytoplasm and nucleus are fusiform. Mitotic figures are present in some binucleate cells and may occasionally be seen in the cuboidal cells. The binucleate cells contain abundant PAS-positive glycoprotein and cytoplasmic alkaline phosphatase before they enter the crypt lining. Some of those binucleate cells incorporated in the lining retain these characteristics, but the majority possess only small amounts or no glycoprotein. Alkaline phosphatase is found only at the junction between the lining binucleate cell and the trophoblast.

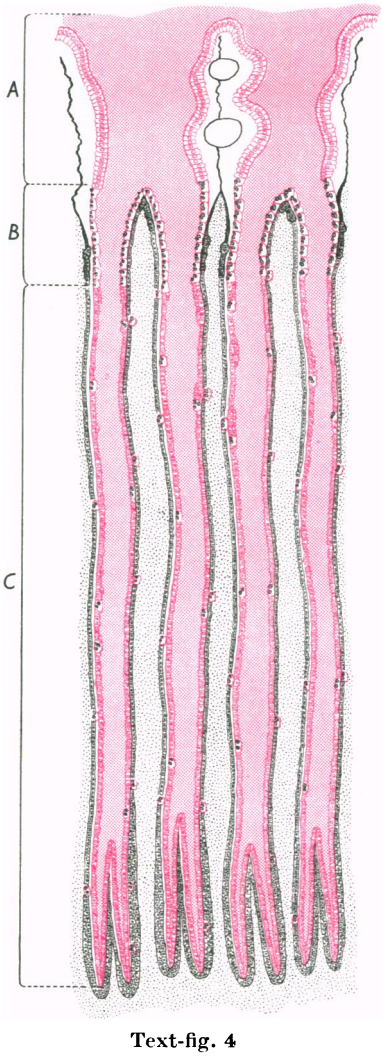
The tissue of the septa consists of a PAS-positive basement membrane supporting the crypt lining, stromal cells and a few fibroblasts. Little collagen is present; a reticular network enmeshes the stromal cells. A network of capillaries surrounds each crypt and lies immediately subjacent to the basement membrane. The endothelial cells are thin and contain alkaline phosphatase; a PAS-positive membrane surrounds the endothelial cells (page 17).

The lining of the crypts changes markedly in its characteristics in the regions

opposite the 'attritional' intermediate zone of the villus (page 11). This region of the crypt really represents its true mouth, as the maternal tissue beyond it exhibits degenerative changes. It is a restricted zone and extends only for 1 mm. at the most.



Text-fig. 3



Text-fig. 4

Text-fig. 3. Diagram to show the branching of villi and development of crypts in the early placentome of *Dama dama*.

Text-fig. 4. Diagram to show the branching of villi and destruction of maternal septa in a definitive placentome of *Dama dama*. Three zones are shown in the villus: (*A*) zone of storage, (*B*) zone of attrition, (*C*) zone of physiological exchange.

All evidence of cuboidal cells is lost in this zone and the only cells observable are giant cells of various types (Pl. 3, fig. 15). Healthy binucleate cells with high PAS-positivity are present in the deeper parts of the zone. There are also many areas occupied by large masses of syncytium or plasmodial masses, the characteristics of

which differ markedly from syncytial areas deeper in the crypt. These masses are often up to 80 μ in length and 40 μ across and may contain as many as 30 nuclei in one section. They are usually separated from each other by distinct boundaries or indentations, but in places two or more masses appear to be continuous through slender bridges of cytoplasm. The cytoplasm is conspicuously eosinophilic and densely granular; it does not contain PAS-positive material. Alkaline phosphatase in abundant quantities is distributed throughout the cytoplasm and is most concentrated at the maternal border. The nuclei are characterized by their dense basophilia, and not infrequently more than one shows pyknotic changes. Mitotic figures have not been observed despite careful searching of many sections.

Binucleate cells of characteristic appearance are abundantly present in the trophoblast and in the region between the trophoblast and syncytial masses. The syncytial masses nearer the surface of the placentome frequently exhibit degenerative changes. Karyorrhexis and pyknosis occur and the whole mass often breaks away from the crypt wall. The crypt wall becomes altogether denuded of syncytium, binucleate and cuboidal cells. Characteristic changes are present in the tissue of the maternal septa in this region: the endothelial cells of the capillaries are shrunken, the nuclei slender and pyknotic; and no blood is present in the much compressed lumen. Stromal cells and fibroblasts have pyknotic nuclei and, higher in the septa, lose their nuclei completely.

The superficial parts of the maternal septa exhibit striking changes, particularly at the tips (Pl. 5, fig. 24). The septa are devoid of an epithelial covering and show varying degrees of cellular degeneration. The tips of the septa are necrotic, nuclei are absent and the tissue consists of amorphous, eosinophilic material with some reticular and collagenous fibres embedded in it. The material displays less and less PAS-positivity towards the tip; some glycogen is present as droplets lying against the wall. Alkaline phosphatase is abundant in the deeper parts of the septa but is altogether absent in the necrotic tip region.

The terminal parts of the maternal septa at the 78 mm. C.R. stage are still covered by epithelium and show no evidence of degeneration. They are devoid of epithelium for a terminal length of 3–4 mm. and have necrotic tips extending for 1·5 mm. at the 100 mm. C.R. stage. At the 175 mm. C.R. stage the area devoid of epithelium is 4 mm. in length and the necrotic region is 2 mm. in length and is reduced to a fine filamentous thread. Near term, at the 290 mm. C.R. stage, the terminal region has increased to 5 mm. in length and the greater part of it is necrotic and finely filamentous.

This terminal region, particularly the necrotic part, fits loosely between the bases of the main-stem villi and it is frequently plicated. If the chorion and as much of the villi as possible be stripped from a fresh placentome and the exposed surface of the caruncle be placed under water the necrotic filamentous, terminal portions wave freely about the more solid mass of the caruncle.

Details of placentomes in a series of Dama

An analysis was made of the characteristics of the placentomes in the uterine horns of a series of 81 fallow deer pregnant with foetuses varying in size from 85 to 350 mm. The corpus luteum was in the ovary on the same side as the uterine

horn containing the foetus in 72 % of the specimens; transmigration of the ovum had therefore occurred in 28 %. Migration occurred from the left to the right horn four times as often as from right to left. The commonest number of placentomes was eight and the commonest arrangement was four in each horn. The greatest number was fifteen (nine in one horn) and in two does the least was four, but the horn without the foetus contained no placentomes. There was no significant difference between the number of placentomes in either horn whether one horn contained the foetus or not. The largest placentomes were found in the pregnant horn in 54 % and the total gross volume of the placentomes was greater in the pregnant horn in 60 % of the specimens.

There was no increase in the average number of placentomes as pregnancy advanced. There were small accessory placentomes only a few millimetres in size in a few specimens both early and late in pregnancy and there was no evidence of neo-formation of small placentomes during pregnancy. The general tendency is for the larger placentomes to become nearly equal in size towards the end of pregnancy (Pl. 1, fig. 3). Placentomes in specimens near term may vary in length and breadth by several centimetres, but they do not exceed 4·5 cm. in height.

Fine structure of the trophoblast and crypt lining in Dama

The structure of the trophoblast, of the cells lining the crypts and the junctional region between the two at the 200 mm. C.R. stage have been examined with the electron microscope. The trophoblast, in every region it has been examined, possesses microvilli on its external surface (Pl. 6, figs. 27, 28). In the depths of the crypts they intimately interlock with microvilli on the surface of the cells lining the crypts. Those on the columnar cells of the main-stem villus lie free in what appears to be cellular debris. The microvilli of the trophoblast cells measure 1·4–3·5 μ in length and 1400–2100 Å in width. They occasionally branch and have tapering tips. The microvilli of the cryptal cells have similar characteristics but are slightly smaller (Pl. 8, fig. 39).

The trophoblast in the zone of physiological exchange contains cubical cells with simple cell membranes and giant cells. The apical region of the *cubical cells* between the microvilli and the densely packed mitochondria is about 2 μ thick and contains numerous profiles of what appear to be vesicles often arranged in rows directed towards the microvilli (Pl. 6, fig. 28; Pl. 7, fig. 29). Some vesicles have a dense lining 350 Å thick. The majority of the mitochondria are arranged in a row on the outer aspect of the nucleus (Pl. 6, fig. 28). They tend to be elongated, 2000 Å in width and 20000 Å in length, and are densely osmiophilic. A few mitochondria are sparsely distributed in the perinuclear region. Endoplasmic reticulum is scanty and few vesicles are present other than in the superficial zone. The trophoblast possesses a basement membrane about 2000 Å thick. Foetal capillaries abut against the bases of the cubical cells and also lie within the trophoblast; a cubical cell may almost completely encircle a part of the capillary. The foetal capillaries have a basement membrane about 350 Å thick which is separated from that of the trophoblast cells by a clear space 700 Å thick. The intervening space does not appear to be markedly lamellated as described by Björkman and Bloom (1957) in the bovine placenta. No deficiencies or pores have been seen in the foetal endothelium; small processes or protuberances project into the lumen (Pl. 8, figs. 36, 37).

Binucleate cells have also been recognized lying within the maternal crypt lining (Pl. 8, fig. 30). They possess a few short microvilli (300–2800 Å in length) on the aspect in contact with the villus (Pl. 8, fig. 31). Short clefts are occasionally present between them and neighbouring crypt cells. There is an inclusion-free zone in the cytoplasm nearest the villus. It contains only a few small vesicles. Most of the mitochondria lie in a row between the nuclei and the cell surface nearest the villus. The mitochondria are small, spherical and frequently contain one or more small vesicles. The endoplasmic reticulum is perinuclear and is well developed in some binucleate cells: a Golgi apparatus is present. Vacuoles from 0·3 to 1·5 μ in diameter are present in the inner cytoplasm. They have clear centres, but occasionally a peripheral zone of finely granular material is present. Some binucleate cells have collections of osmiophilic granules which are related to the endoplasmic reticulum. The nuclei show localized aggregations of electron-dense material and also nucleolar-like structures with a reticular pattern.

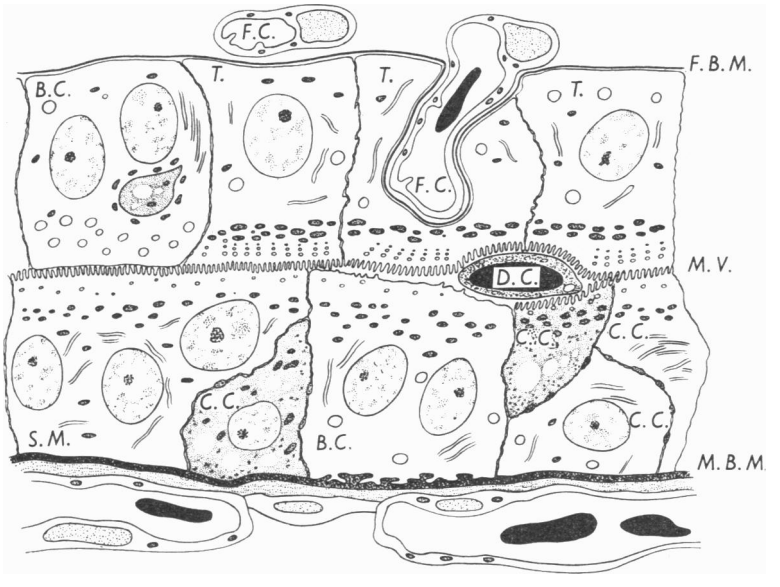
The *columnar* trophoblast cells of the main stem of the villi have a much clearer cytoplasm than that of other trophoblast cells. The adjoining plasma membranes of contiguous cells are simple, but separate near the cell surfaces to provide an intercellular cleft containing microvilli. Mitochondria tend to be aggregated in the superficial regions of the cell; they resemble those of the cubical cells. Small vesicles 350–14000 Å in diameter are present in some number in the superficial zone and more sparsely throughout the rest of the cell (Pl. 8, fig. 38). Endoplasmic reticulum is scanty and little detail is discernible in the cytoplasm apart from sparsely distributed osmiophilic granules.

The crypt lining shows much complexity of structure, is often several cells thick and contains various cell types. In general it appears more electron-dense than the trophoblast. There are simple cubical cells of varying electron-density and exhibiting different types of vacuolation. Binucleate cells are present (page 12) and also regions occupied by a limited syncytium which may or may not be accompanied by other types of cell (Pl. 6, fig. 28).

The cubical cells have a distinct plasma membrane and microvilli on their foetal aspect (page 15). Their cytoplasm may be relatively clear or may be markedly electron-dense. A few small vesicles are present in a narrow superficial zone (up to 1·0 μ thick) below the microvilli, but they are not as plentiful as on the foetal side. The majority of the mitochondria are arranged in a thick row below the inclusion-free superficial zone. The mitochondria appear less dense and somewhat smaller than those in the trophoblast. Endoplasmic reticulum is well developed in some cubical cells (Pl. 6, fig. 27). Many cubical cells contain heavily vacuolated areas; the clear-centred vacuoles appear to be included in the plasma membranes of two contiguous cells. Other cells have plicated plasma membranes where they are in contact.

The appearances of the binucleate cells are described above: those seen in the crypt lining cannot be differentiated from those in the trophoblast. The syncytial regions possess surface microvilli of moderate length (up to 1·0 μ). The clear superficial zone is not marked and contains few vesicles. Mitochondria are small and are arranged in a broad row near the foetal surface, but some are present throughout the cytoplasm. Endoplasmic reticulum is present in all regions of the syncytial masses. Vacuoles are small and few in number.

Maternal capillaries lie deep to the crypt lining and are separated from it by a space up to 5000 Å thick, and a basement membrane to the crypt lining about 1800 Å thick. Small projections from the endothelial cell may enter the intervening space, which contains irregular heterogeneous material (Pl. 8, figs. 34, 35). In places binucleate cells extend as far as a maternal capillary and the plasma membrane of the binucleate cell may show small infoldings along this margin.



Text-fig. 5. Diagram to show the structure of the trophoblast (above) and the crypt lining in *Dama* as seen with the electron microscope. *B.C.* = binucleate cells, one of which is in the crypt lining. *C.C.* = cuboidal crypt cells of varying electron density. *D.C.* = degenerating cell in junctional zone. *F.B.M.* = basement membrane to trophoblast *T.* *F.C.* = foetal capillaries, one intra-epithelial. *M.B.M.* = basement membrane of crypt lining with maternal capillaries below it. *M.V.* = microvilli of columnar trophoblast interdigitating with those of the crypt lining cells, but absent from the binucleate cells. *S.M.* = syncytium in the crypt lining.

At intervals the microvillous junction between the trophoblast and crypt lining contains what appear to be degenerating cells. They are markedly electron-dense, with granular and often heavily vacuolated cytoplasm. Their nuclei are particularly dense. Where such degenerating cells are present the adjacent trophoblast is devoid of the characteristic rows of small vesicles (Pl. 6, fig. 28). Remnants, or portions, of such cells may also be seen enclosed by, or included within, the trophoblast (Pl. 6, fig. 28) and the binucleate cells. The relationships of the cell types seen in the trophoblast and crypt lining are illustrated diagrammatically in Text-fig. 5.

PLACENTATION IN OTHER DEER

Japanese Sika deer (Cervus nippon)

A single pregnant uterus of a Japanese Sika deer (*Cervus nippon*) was obtained on 5 April 1951. The almost spherical uterus was 35 cm. in length, 27·5 cm. in breadth and 108 cm. in circumference. The right uterine horn contained a male

foetus 332 mm. in C.R. length. Six large placentomes were present, three in each horn. They were approximately spheroidal (Pl. 1, fig. 4) and were mounted on a short pedicle of maternal tissue. Their greatest diameters were:

Horn with foetus (cm.)	Other horn (cm.)
7·0, 6·0, 5·0	9·0, 7·0, 5·0
8·5, 7·0, 6·0	7·0, 5·0, 5·0
5·0, 3·5, 4·0	7·0, 7·0, 6·0

The secondary villi arise from a single squat stem 3–5 mm. in length in the large placentomes. The filiform secondary villi are up to 3 mm. in length and 5 mm. in diameter. Some three to five arise from each main stem villus. They bifurcate into tertiary villi 0·5–3·0 mm. long. The characteristics of the trophoblast covering are essentially similar to those of *Dama* at an equivalent stage of development. The most marked differences are seen in the covering of the main-stem villi and the arcades between them. The trophoblast is tall columnar as in *Dama*, but there is less glycogen in the cells or lying outside them. There is also a markedly increased number of degenerating masses, with many pyknotic nuclei, lying against the surface of the trophoblast.

The lining of the maternal crypts resembles that of *Dama*. The septa can be divided into three zones similar to those described on page 12. The deeper parts of the crypts are lined with varying proportions of simple cuboidal cells with distinct cell boundaries and moderately densely stained nuclei, binucleate cells, and elongated stretches of what appears to be syncytium with densely stained nuclei. The septa lose their epithelial covering in a manner comparable to that in *Dama*. The region of the mouth of each crypt exhibits dense syncytial masses and binucleate cells which send projections of cytoplasm into the septum. These projections appear to penetrate the crypt wall and extend between the stromal cells. All the cells in the septa show degenerative changes in this region. There are necrotic filamentous tips to the septa.

Indian spotted deer (Cervus axis)

A single specimen of pregnant uterus of the Indian spotted deer (*Cervus axis*), obtained on 12 April 1951, contained a female foetus 420 mm. in C.R. length. There were four large placentomes in the pregnant right horn and two large placentomes and one small in the other. The largest placentome was an elongated ovoid, the others were flattened spheres. Their dimensions were:

Horn with foetus (cm.)	Other horn (cm.)
7·0, 6·0, 4·0	6·0, 5·0, 4·0
4·0, 4·5, 3·5	9·0, 5·0, 3·0
5·0, 5·0, 3·0	2·5, 2·5, 2·5
6·0, 4·0, 3·5	

The villi have a general arrangement similar to that of *Dama* in that several filiform villi arise from a stout main-stem villus. The main-stem villi in a large placentome are 1·5–2 mm. long and 0·6–0·9 mm. in diameter. The secondary villi are 8–10 mm. in length and divide into two to four fine tertiary villi 1·5–2 mm. in length. Three zones are discernible in the crypts and villi similar to those described for *Dama* (page 11). The crypts are lined by a single layer of low epithelial cells 8–10 μ

in height which does not appear to be a syncytium. Only occasional binucleate cells are present in the lining of the deeper parts of the crypts. The lining cells at the crypt's mouth show evidence of pyknosis and degeneration and are not present in the most superficial part of the crypt wall. This part shows some evidence of attrition, but it is not marked as in equivalent stages of *Dama*.

The structure and histological characteristics of the villi are generally similar to those in *Dama*. The main-stem villi and arcades between them are lined by cells that are not as large or as tall as the typical columnar cells in this region in *Dama*. There is only a small amount of glycogen in or outside these cells.

There is a compact block of tissue several centimetres in extent in one region of one placentome. The compact arrangement appears to be due to the absence of any mucoid connective tissue in the villi. These are slender, often only 30–40 μ in diameter, and are encased in thick-walled crypts.

Manchurian Sika deer (Capreolus bedfordi)

A single pregnant uterus of the Manchurian Sika deer (*Capreolus bedfordi*) was obtained on 10 March 1951. The uterus was spherical and about 73 cm. in circumference. The right horn contained a foetus 241 mm. in C.R. length. Six large placentomes of approximately similar size were present. They were ellipsoidal in shape, but were markedly flattened against the uterine wall. Each was attached by a short pedicle. Their greatest diameters were:

Horn with foetus (cm.)	Other horn (cm.)
8·0, 6·5, 2·5	6·0, 5·5, 2·0
6·0, 5·0, 2·0	6·5, 5·0, 2·0
5·5, 4·0, 2·0	5·0, 4·5, 2·0

The structure of the placentome is generally similar to that of *Dama* in the late stages of pregnancy. Each placentome is, however, more flattened and the villi are shorter. The plump main-stem villi are 4–5 mm. in length and 0·25–0·5 mm. in diameter. Several (2–4) filiform secondary villi, 10–12 mm. long and tapering from 0·4 to 0·15 mm. in diameter, arise from them. Some secondary villi bifurcate into short tertiary villi 1–1·5 mm. long and 0·15–0·10 mm. in diameter.

The basic construction of the villi resembles that in *Dama* and three zones are clearly discernible in both villi and crypt lining. The main-stem villi and arcades between them possess a covering of high columnar trophoblast rich in glycogen. The superficial region of the crypt wall is necrotic for 3–3·5 mm. and is devoid of a lining. The trophoblast contains many binucleate cells and is several layers thick at the true mouth of the crypt. The crypt lining shows extensive degenerative changes in this region, many degenerating giant cells being included. The remainder of the crypt lining is somewhat thicker than in *Dama*. It is up to 30 μ in thickness in some areas and consists of layers of discrete cuboidal cells 9–10 μ in height with eosinophilic cytoplasm. Binucleate cells are rarely seen in the crypt lining, and when present appear degenerate.

Red deer (Cervus elaphus)

Fifteen specimens of pregnant uteri of the red deer have been examined. All contained single foetuses which varied in C.R. length (Table 1) from 150 to 730 mm. (near term). The number of placentomes varied from six to ten; the commonest number was eight, four in each horn. There were only two placentomes in one horn, four or more in the other, in two animals. Where there were only two or three placentomes in one horn, they were consistently larger than those in the other horn. The placentomes in the other specimens were of almost equal size and were elongated ovoids raised on a short pedicle (Pl. 1, fig. 1). At the 150 mm. C.R. stage they measured about 40 × 35 × 30 mm. in overall dimensions; the largest placentome was not always in the horn containing the foetus. Near term the overall dimensions of the largest placentome were 110 × 70 × 55 mm.

The arrangement of villi within a placentome and the form of the villi exactly resembles that in *Dama*, as do the details of the cellular construction of the trophoblast and the crypt lining. Our findings confirm those of Strahl (1906).

Reindeer (Rangifer tarandus)

Only nine uteri from pregnant reindeer were in a reasonably preserved condition. The foetuses were all singletons, varying in C.R. length from 260 to 294 mm. The placentomes were ovoid or ellipsoid and mounted on short pedicles; a few displayed a kidney-like shape. The usual number of placentomes was six, three in each horn. The greatest number in one horn was five, the smallest three; where the number was greater than three the additional placentomes were much smaller. The corpus luteum was present in the ovary on the side of the uterine horn that contained the foetus. The dimensions of the placentomes in the uterus of a reindeer pregnant with a foetus 265 mm. in C.R. length were:

Horn with foetus (cm.)	Other horn (cm.)
3·0, 2·5, 2·5	7·5, 3·5, 3·5
3·25, 2·5, 2·5	6·5, 3·5, 3·0
0·75, 0·7, 0·3	8·5, 3·25, 3·5
3·0, 2·0, 2·5	
2·5, 2·0, 1·5	

If a large placentome is sliced longitudinally it can be seen to be sub-divided by septa of maternal connective tissue into a series of lobules (Pl. 5, fig. 25). These are pyramidal in form with their apices directed towards the depths of the caruncle (see also Turner, 1878 *a*, *b*; Kolster, 1909; and Andresen, 1927). A broad, short, main-stem villus, up to 2 cm. in diameter, enters the base of each lobule. A large number of branches arise from each main-stem villus. Those nearest the surface of the placentome are straight and directed laterally; those deeper in the placentome are more obliquely arranged and branch repeatedly to form a complicated mass of secondary and tertiary villi (Pl. 5, fig. 25). The laterally directed villi possess an obvious mesodermal core (Pl. 5, fig. 26), whereas those deeper in the placentome possess only a sparse amount of mesoderm. The total overall length of one tuft of villi in a lobule of the largest placentomes at the stages described is between 1·5 and 2 cm. The villi fit into maternal crypts and thus the latter are arranged in a more complicated manner

than in *Dama*. The deepest divisions of the crypts are, however, arranged nearly vertically to the base of the caruncle.

Unfortunately the preservation of the specimen is adequate only for a brief description of the cellular structure of the trophoblast and crypt lining. The main-stem villi and the arcades between them are covered by a single layer of trophoblast consisting mainly of tall columnar cells. In places, and particularly on the lateral branches, the columnar cells are replaced by a layer of cuboidal or squamous cells two or three cells thick. Where the more complex branching of the villus begins the trophoblast contains numerous binucleate cells and some cuboidal cells. Towards the extremities of the subsidiary branches the number of binucleate cells is scanty and cuboidal cells predominate.

The main maternal septa that divide the placentome into lobules are relatively stout and are up to 1 mm. thick. Complex folded subsidiary septa extend inwards into the lobule. The superficial ones are horizontal and form crypts for the laterally directed villi. Those septa nearest the chorion are necrotic and devoid of any lining; others slightly deeper in the lobule are lined by syncytial masses and individual binucleate cells and do not show degenerative changes. Masses of degenerating cellular debris are present in the more superficial crypts. The lining of the remainder of the crypts is uniform and appears to consist of a single or double layer of cuboidal cells with distinct cell membranes. In places the lining has disintegrated post-mortem into individual cuboidal cells. Binucleate cells are present within the crypt lining.

Roe deer (Capreolus capreolus)

A total of thirty-two adult roe deer were killed at Petworth Park between 5 February and 10 March 1952 and 1953. Twenty-six does were pregnant (Table 1); one specimen, not included in the Table, contained two macerated foetuses, one at the tubal end of each horn. There were no caruncles in the uterus and it was presumed to be abnormal. Twenty-two of the pregnant does contained two foetuses (one in each horn), four contained singletons. Ten of the twin pregnancies had two corpora in one ovary, ten had one corpus in each ovary and two had only a single corpus luteum. Therefore 84 % of this small series displayed twinning and at least 7·7 % showed either monozygotic twinning or ovulation of two ova from one follicle. The fact that all the twins were of like sex strongly suggests that monozygotic twinning does occur, and that only one egg is in fact fertilized. It was noticed that fusion of the chorion in the twin pregnancies always occurred, but the two circulations could not be shown to anastomose after injection of coloured material into the umbilical vessels of one twin. The two amniotic cavities did not communicate, nor could it be shown that the allantoic sacs were in continuity. It would appear that the fertilized ovum separates at the two-cell stage, and that one of the separated blastomeres migrates to the opposite horn. The chorionic sacs become apposed and the chorions fuse at a later date, but probably before the 20 mm. C.R. stage.

The corpora lutea in those does that were carrying twins and had two corpora lutea show little histological difference and could well have ovulated at the same time. There was no evidence of multiple follicle formation in the ovaries that were examined. Further discussion of twinning in deer will be found on page 5.

The placentomes develop from flattened, sessile caruncles that are nearly circular in outline; later they become spheres or ovoids raised on a pedicle. The villi are only a few millimetres long by the 20 mm. C.R. stage, but are already lying in shallow crypts. The crypts are 4–5 mm. deep by the 80 mm. C.R. stage, but the villi separate easily from them. The usual number of placentomes is eight, with four in each horn. The smallest number in one horn was two, the greatest six. The average diameters of the placentomes at the 230 mm. stage were:

Horn with foetus (cm.)	Other horn (cm.)
5·7, 3·7, 3·3	7·2, 4·0, 3·8
5·1, 3·7, 3·6	6·7, 4·2, 3·0
7·2, 5·0, 4·0	3·6, 3·0, 2·5
5·6, 3·9, 3·5	5·6, 4·0, 3·5
	4·2, 3·3, 3·1

The arrangement of the villi resembles superficially that in *Dama* or *Cervus* rather than that in *Rangifer*. The main-stem villus gives off a number of short branches, however, which all branch again to form tufts of short terminal villi. Some of these terminal branches project laterally into short horizontal crypts, a feature which distinguishes the placentomes of *Capreolus* from those of the Cervinae. The trophoblast displays the same regional differentiation as in *Dama* except that the zone of storage with its tall columnar cells is reduced in extent due to the short main-stem villi. Some columnar cells contain degenerated cellular remnants. The caruncle is subdivided by a few main and many subsidiary septa so that the crypts exhibit a more complex arrangement than in *Dama*. The crypts are lined predominantly by a single layer of cubical cells; occasional binucleate cells are present, and in some regions there is evidence of a syncytium. There does not appear to be the same type of necrotic filament to the tips of the maternal septa as in *Dama* and *Cervus*, although our material does not extend beyond the 235 mm. stage. The apices of the maternal septa may end as rounded knobs (see Andresen, 1927) devoid of epithelium and showing evidence of degeneration (Pl. 5, fig. 23) or as narrow strands still with some cellular organization but with degenerating epithelium. Hyperplasia of the septal epithelium also occurs about the mouths of the crypts but few binucleate cells are present in the crypt lining. Many binucleate cells on the surface of the trophoblast and in contact with these hyperplastic regions have intracytoplasmic inclusions and pyknotic nuclei.

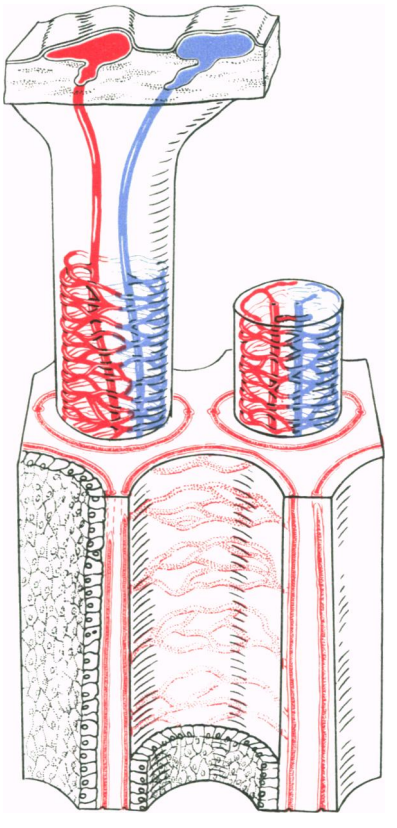
BLOOD SUPPLY OF VILLUS AND CRYPT

The arrangement of the blood vessels in the placentome of *Elaphurus* has been described by Harrison and Hamilton (1952). Similar arrangements were found to exist in the placentomes of *Dama* and *Cervus* which had been injected with coloured gelatin. The structure of the foetal and maternal capillaries as seen under the electron microscope is described on page 15. The following additional observations are emphasized and some are illustrated in Text-fig. 6 and Plate 8, figs. 34–37.

Capillaries are present in the villi of the largest cotyledonary plaques of *Dama* at the 12 mm. C.R. stage and probably earlier. Intra-epithelial capillaries have not been observed until about the 100 mm. C.R. stage. They are present in all Cervidae

we have examined and are always covered by narrow strips of trophoblast however closely they extend to the surface.

The foetal endothelium is closely applied to the trophoblast and the intervening space seen in electron micrographs is narrower than that seen between the maternal endothelium and the crypt lining (page 17). The pattern displayed by the capillary network in a villus in *Dama* and *Cervus* is similar to that in *Elaphurus*. There are no intra-epithelial capillaries in the main-stem villi or the arcades between them.



Text-fig. 6. Diagram to show the arrangement of the central artery and vein in a villus of *Dama dama*: note the circumferential anastomosing capillary plexus. The crypt lining has been partially removed from one crypt to show the trellis-like arrangement of the maternal capillaries.

The capillaries are distributed relatively sparsely deep to the columnar cells as opposed to the closely arranged circumferential plexus that is present in the rest of the villus.

Each placentome is supplied by branches of the uterine artery and vein which enter the base of the caruncle. Many, if not all, of the vessels penetrating the basal zone of the caruncle are spiral. Subsidiary arterial branches, which are not spiral, enter the septa vertically and give off short laterally directed arterioles. These divide into leashes of capillaries that extend round a segment of the crypt wall, always

deep to the crypt lining and generally in a horizontal plane, i.e. at right angles to the long axis of the villus.

The leash of capillaries join to form a horizontal venule which drains into the vertically placed septal vein (Text-fig. 6). The vertical vessels of the septa are arranged at intervals in the wall of each crypt so that a villus is surrounded by what may be termed a series of trellis-works of maternal vessels.

DISCUSSION

The literature on placentation in ruminants is voluminous (Andresen, 1927; Wimsatt, 1950, 1951; Amoroso, 1952, for references) and no attempt is made here to review or consider all the problems. Certain aspects only are discussed, especially where our material appears to have provided relevant information.

Implantation

The process of implantation, that is to say the mode of attachment of foetal cotyledon to maternal caruncle, has not until now been observed in the Cervidae, although Strahl (1906) examined one specimen of *Cervus elaphus* with an early embryo. In the ewe Assheton (1906) found that the caruncular epithelium was distinctly degenerated in the regions where the chorion made contact and formed a cotyledonary burr. He maintained that the epithelium was not subsequently regenerated but was replaced by binucleate cells. Hammond (1927) reported that in the cow the caruncle was completely denuded of epithelium prior to implantation. Björkman (1954) examined bovine material after fixation or freezing of the chorion *in situ* and found that 'the caruncular epithelium is intact to a large extent during implantation'. We have used similar methods to preserve our material and have found no evidence of denudation of the caruncular epithelium during implantation in *Dama* or of an early invasion or replacement of the superficial caruncular epithelium by any foetal cells.

Björkman (1954) reviews the problem as to how the crypts are formed by downgrowth and subsequent canalization of strands of uterine epithelium, with the villi penetrating them later. In *Dama* the crypts develop before the primitive villi are formed. The caruncular epithelium exhibits localized areas of hyperplasia, the central regions of which become necrotic. The epithelial invagination becomes canalized as a result of disappearance of the necrotic material. The primitive crypts are arranged in rows transverse to the long axis of the caruncle (Strahl, 1906; Turner, 1879; Harrison & Hyett, 1954). The primitive villi enter the crypts after the disappearance of the central necrotic core and only after their establishment within the crypts are any binucleate cells seen within the crypt lining. The subsequent growth changes in the caruncle and villi are described by Harrison & Hyett (1954).

The placentomes

The placentomes in ruminants vary in number, size and shape; there may be as few as five in Père David's deer (oligocotyledonary), or as many as 160–180 in goats (polycotyledonary). The usual number of placentomes in the deer examined by us is six to eight. The largest placentomes have been found in Père David's deer and the smallest in cows. Andresen (1927) has described the different shapes of placentome

encountered in ruminants. In deer they all eventually become pedunculated in contrast to the convex, concave or sessile shapes in other ruminants. Andresen considered that the Cervidae have placentomes with a shape specific for each form, but he had not available specimens from stages throughout pregnancy. Our material, in particular that from *Dama* (see also Harrison & Hyett, 1954), shows that the form of the placentome changes markedly as pregnancy advances, and that the shape of neighbouring placentomes in one uterine horn may also vary. There is no correlation between size of placentome and whether its horn contains the foetus as Strahl (1906) suggested. When there are only two placentomes in a horn they are always large.

The construction of the placentomes and the arrangement of the villi in those genera in the subfamily Cervinae (*Dama, Cervus, Elaphurus*) is remarkably similar. The placentomes of *Rangifer* are differently constructed in that the villi branch in more complex fashion and the arrangement appears to resemble that described for some other members of the subfamily Odocoileinae (*Hydropotes*, Ewart, 1878; *Mazama*, Andresen, 1927). The placentomes of *Capreolus capreolus* display a somewhat intermediate condition (page 21), but as Andresen (1927) observed, may be differentiated from those of *Cervus* by the tufts of short, much-branched villi and numerous lateral branches.

No new placentomes are formed during pregnancy. Small accessory placentomes are formed at the same time as, or shortly after, the main ones, and presumably arise as the result of the existence of small regions of caruncular tissue outside the main caruncles (Kolster, 1903).

We find three distinct zones of cellular differentiation and activity in the definitive villus of *Dama*; they are comparable with similar zones described by Strahl (1906), Kolster (1908) and Andresen (1927) in the placentome. These authors did not, however, possess histochemical and other methods of analysing the functions of the components of each zone. We recognize, from the tip to the base of the villus, (*a*) a zone of physiological exchange (Wachstumzone of other authors); (*b*) a zone of attrition (Umwandlungszone); (*c*) zone of storage (Rückbildungszone). We feel that the division of the placentome into zones is better replaced by an analysis of those exhibited by the villus. We prefer calling the main part of a villus a zone of physiological exchange as that appears to be its main function; growth changes occur everywhere in the placentome as Björkman (1954) also maintains. Electron micrographs show that the trophoblast possesses well-developed microvilli in this zone. Large numbers of small intracytoplasmic vesicles lie in the apical region of the trophoblast cells between the microvilli and the lineally arranged mitochondria. These appearances strongly suggest the presence of a transfer mechanism; enzyme systems are also situated in this region. The zone of attrition is one where the activities of the binucleate cells, and perhaps other factors, cause hyperplasia of the crypt lining, its subsequent destruction and also that of maternal connective tissue with the formation of atrophic filamentous threads on the tips of the septa. The zone of storage is characterized by the tall columnar trophoblast cells of the main stem villi and the arcades between them. These cells have apical microvilli and contain quantities of glycogen and glycoprotein.

The definitive placentome also exhibits three zones in the maternal septa corresponding in position to those analysed in the villus. The greater part of each crypt

has a complex lining containing several cell types (see later) most of which take part in physiological exchange. At the mouths of the crypts is a second zone characterized by the presence of large syncytial masses (plasmodial masses of Björkman, 1954) with from three to thirty dense nuclei. Many show evidence of pyknosis and advanced degeneration. This zone lies opposite the zone of attrition of the villus. It has been suggested (Assheton, 1906; Wimsatt, 1950; Amoroso, 1952) that these masses may arise from binucleate cells that have left the trophoblast to enter the crypt lining. Björkman (1954), however, points out that the staining characteristics of the plasmodial masses of the cow resemble those of the cellular crypt lining, and the same is true in *Dama*. We consider these masses to be formed from the crypt lining, and they are probably maternal in origin. The third septal zone is one of complete attrition of maternal tissue. The crypt lining is completely absent and the septal connective tissue is reduced to an atrophic strand. The term diaphthoro-epithelio-chorial was introduced to describe this relationship by Harrison and Hamilton (1952) and has been discussed by Harrison & Hyett (1954) in that it illustrates an adaptation of the placentome in deer for maximal physiological efficiency.

The lining of the crypts

There has been disagreement as to the nature and origin of the crypt lining in ruminants. Andresen (1927) maintained that the lining is often syncytial and that where a syncytium is found it is derived from the trophoblast. This generalization was considered to be premature, and probably erroneous, by Wimsatt (1950). He quotes the opinions of Sedlaczek (1912), Wislocki (1941) and Wislocki & Fawcett (1949) who had examined specimens of the antelopes *Hippotragus bakeri*, *Rhyncotragus kirkii* and *Antilocapra americana*. All had considered that the syncytium lining the crypts was maternal in origin. In the cow Kolster (1902), Jenkinson (1906), Chiodi (1927) and others considered that the lining was derived from modified uterine epithelium. Hammond (1927) maintained the lining was not epithelial in nature but consisted of connective tissue, plasma or lamellar cells. Strahl (1906, 1912) considered the syncytium to be maternal in origin in *Cervus elaphus*. Kolster (1909) disagreed with Strahl and maintained, as did Andresen (1922), that the syncytium also arose from trophoblast in *Cervus elaphus* and in *Rangifer tarandus*. Assheton (1906), on the other hand, considered the syncytium in the crypts of the sheep to be formed from binucleate trophoblastic cells. Kolster (1909, 1910) supported Assheton's contention, as did Andresen (1927) and others. Wimsatt (1949) at first took the view that maternal connective tissue gave origin to the syncytium, but later (1950, 1951), and as the result of careful histochemical investigations, revised his view and supported Assheton's interpretation. Björkman (1954) is convinced that if bovine material is well fixed, distinct cell boundaries may be seen in the crypt lining. He also observed mitoses and states dogmatically that the crypt lining is cellular and of maternal origin. Later he confirmed this statement by using an electron microscope to study the foetal-maternal junction (Björkman & Bloom, 1957).

Harrison & Hamilton (1952) considered the crypt lining in *Elaphurus* to consist of distinct cuboidal cells with definite cell membranes and used phase contrast microscopy to illustrate the lining. The crypt lining varies in the different deer

described in this paper from a single row of cuboidal cells to a complex layer containing several cell types. The lining also changes in complexity and construction as the placentome grows. It does not remain static, but is everywhere changing in its composition in that it exhibits growth, suffers invasion and destruction and may even undergo metaplasia.

Electron micrographs confirm our observations with the light microscope that the crypt lining, at least in *Dama*, contains several types of cell. There are numerous *cuboidal cells* which we believe from our study of successive stages in placentome development to be maternal in origin. They undergo mitosis, but also show degenerative changes. They possess microvilli which interdigitate with those of the trophoblast. They are supported at their bases on a continuous basement membrane. *Binucleate cells* are present, and we are convinced that they have migrated there from the trophoblast (see later). Their histochemical characteristics are similar whatever their position, but they appear to lose their PAS-positivity after they have become incorporated in the crypt lining. They also show evidence of nuclear pyknosis and cytoplasmic degeneration when within the crypt lining or in contact with it. They do not possess pronounced microvilli when any part of their surface is included in the region of the foetal–maternal junction. Where their plasma membrane comes in contact with the crypt basement membrane it shows much irregular folding. Certain regions of the crypt lining are definitely syncytial. These regions extend for distances of at least a millimetre and are interspersed amongst cuboidal and binucleate cells. Microvilli are present and both in their staining characteristics and ultrastructural features they resemble the cuboidal cells. For these, admittedly mainly morphological, reasons we consider the limited regions of syncytial lining to be derived from the maternal cuboidal cells. If, on the other hand, they are derived from binucleate cells it means that the latter, after invasion of the crypt lining, lose certain characteristic cytological features and take on those of the cuboidal cells of the lining proper. Neither Björkman & Bloom (1957) nor we have found any evidence for such a transformation.

Binucleate and placental giant cells

The origin, classification and time of appearance of giant cells, Riesenzellen, megalokaryocytes, diplokaryocytes or binucleate cells in the placenta and related endometrium have occupied the attention of investigators for many years. It has been postulated that they may arise from the trophoblast, the decidua, or from the maternal endothelium. Hubrecht (1889), Duval (1895), Sobotta (1903) and Jenkinson (1906) were of the opinion that the giant cells were of trophoblastic origin. Disse (1906) in field mice, and Sansom (1922) in water voles, believed that they were of maternal origin. Sansom (1927) stated that in the rabbit two kinds of giant cells were present, larger cells derived from the trophoblast and mesometrial cells formed from the endothelial lining of maternal vessels.

Orsini (1954), following at least two other investigators (Snell, 1941 and Alden, 1946), has classified giant cells in rodents into three types. Primary giant cells arise by hypertrophy of the abembryonic and lateral areas of the blastocyst wall and play an active role in the penetration of the uterine epithelium. Later in development, the cells become restricted to an area bounded internally by Reichert's membrane

and externally by the degenerating decidua; some of the cells may actually penetrate the decidua. Secondary giant cells arise as a result of the hypertrophy of the superficial cells of the träger and with the subsequent development of the placenta they form an integral portion of its superficial surface. Tertiary giant cells are found in the maternal tissue, appearing first in relationship to blood vessels and the intramyometrial connective tissue. They are most numerous in the antimesometrial region at the time of the greatest degeneration of the decidua capsularis: they degenerate slowly after parturition in the hamster. Orsini states that these tertiary giant cells are not likely to be confused with decidual cells because of their intravascular location, large size, time of appearance, and typical staining reactions. A further point stressed by Orsini in favour of the trophoblastic origin of these cells is the fact that when there is a large interval between adjacent gestation sites 'the invasive cell population declines as one passes from the periphery of a site to the interembryonic area'.

The binucleate cells in ruminants have a less complex cycle than those found in rodents; this is undoubtedly related to the type of implantation and placentation. They have been found in a large number of species (Assheton, 1906; Kolster, 1909; Andresen, 1927; Wimsatt, 1951; Björkman, 1954 are key references) including all the deer examined by us. Wimsatt (1950, 1951) and Björkman (1954) have provided a detailed history of these cells in the placenta of the sheep and cow. We would agree with Wimsatt (1951) who states 'the binucleate cells in different ruminants resemble one another morphologically, but there are indications that they are not completely equivalent functionally in all species'. He goes further and says that 'in many of their chemical and physiological attributes they resemble the syncytial trophoblast of the deciduate placenta and may with considerable justification be interpreted as the homologue of this tissue in the non-deciduate placentae of ruminants'. While not as invasive as those found in the human placenta (Boyd & Hamilton, unpublished), the binucleate cells of deer may well perform similar functions. Björkman points out that the terms 'binucleate' and 'diplokaryocyte' are not quite adequate, as the cells can sometimes be 'mono-' or 'polynucleate'. Irrespective of the number of nuclei, these cells are large with a high content of easily stainable granular cytoplasm. Although there have been other views (Kolster, 1909), modern investigators all agree that Assheton, Strahl and Andresen were correct in considering the binucleate cells to be trophoblastic in origin. Wimsatt (1951) finds that they do not make their appearance until about the 17th day of gestation. He states that they are formed by direct transformation of the columnar trophoblast through a gradation of stages into the typical definitive binucleate cells. Greenstein, Murray & Foley (1958) state that in the cow both binucleate cells and columnar trophoblast cells arise independently 'from a primitive polygonal "stem cell" which is characteristic of the early blastocyst'. We have insufficient early material to comment on this statement. Once the cell has matured into a typical binucleate cell, it apparently loses the power to reproduce itself. Increase in the number is probably brought about by transformation of primitive cells into further binucleate cells. The binucleate cells in sheep are especially numerous at the tips of the villi, less numerous along the sides and moderately plentiful between the bases of the arcades and in the membranous chorion; they remain in the same positions throughout pregnancy.

In the cow, Wimsatt found that binucleate cells are no more numerous at the tips of the villi than elsewhere, whereas Kolster (1909) finds them more numerous at the tips. We find no binucleate cells in the main-stem villi nor in the arcades between them in deer.

Many investigators have observed binucleate cells outside the chorion in several types of ruminants (Wimsatt, 1951). Assheton (1906) and Wimsatt (1950) found that in sheep the binucleate cells attach themselves to the denuded connective tissue of the crypt and form a primitive trophoblastic syncytium. In the cow Björkman (1954) states that binucleate cell formation is a property of both maternal and foetal tissues, but that interchange of binucleate cells from foetal and maternal epithelia may occur. Kolster (1909) observed binucleate cells passing from the trophoblast into the lining of the crypts in reindeer. The binucleate cells did not injure the lining and ultimately degenerated there. In *Dama*, binucleate cells pass from the trophoblast into the crypt lining from the time the villus has occupied a crypt. From then until term typical binucleate cells can be seen at intervals along the crypt lining. We have no convincing evidence that they give rise to the syncytial regions found in the crypt lining.

The function of the binucleate cells is still a matter for speculation. In sheep and in deer there is little doubt that on occasions the binucleate cells are phagocytic; some pigment granules derived from destroyed pigment cells are frequently found within them. It has been further suggested (Assheton, 1906, Kolster, 1909 and Andresen, 1927) that the binucleate cells produce a secretion which plays a part in destruction of uterine tissue. Wimsatt (1951, p. 259) comments that this is purely inferential since the cells do not possess pre-secretory granules. Björkman (1954) considers that they are responsible for the production of chorionic gonadotrophin and Greenstein *et al.* (1958) suggest their secretions may help to maintain the corpus luteum. Our electron micrographs show that giant cells lack well-developed microvilli and subjacent minute intracytoplasmic vesicles. This suggests that they are not primarily concerned with physiological exchange between mother and foetus.

SUMMARY

1. Details of patterns of reproduction are given for several species of Cervidae.

2. A description is given of implantation and of the development of the placentome in *Dama dama*, and of the appearances of the placentomes at certain stages of pregnancy in several other species of deer.

3. The morphological characteristics of the pedunculated placentomes including the arrangement of the villi are given; all species are oligocotyledonary.

4. Three zones are recognized in the villi and maternal septa. They are called zones of physiological exchange, attrition and storage.

5. The cytological and electron-microscopic characteristics of the trophoblast and crypt lining are described for the first time in Cervidae.

6. Trophoblastic and cryptal microvilli are well developed in *Dama* and small intracytoplasmic vesicles are pronounced in the apical regions of the trophoblast cells.

7. Binucleate giant cells are a constant feature of the trophoblast of the Cervidae. They contain a characteristic PAS-positive carbohydrate-protein complex. They are phagocytic; they lack microvilli.

8. The crypt lining in *Dama* consists of maternal cuboidal cells with apical microvilli, and in limited regions the lining is a syncytium also possessing microvilli. Binucleate cells, devoid of microvilli, are also included in the crypt lining. Remnants of dead cells are found in the foetal-maternal junction separating the microvilli of the two layers.

9. The uterine epithelium remains intact in the intercotyledonary region.

10. In most deer the tips of the maternal septa eventually undergo attrition to become atrophic filaments and the diaphthoro-epithelio-chorial condition prevails.

11. The foetal capillaries are arranged as an anastomosing circumferential plexus about the central artery and vein of the villus; many are intra-epithelial. The maternal capillaries form a trellis-work on the septal wall and are not intra-epithelial.

12. The origin and fate of the binucleate giant cells and the components of the crypt lining are discussed.

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REFERENCES

ALDEN, R. H. (1948). Implantation of the rat egg. III. Origin and development of primary trophoblast giant cells. *Amer. J. Anat.* **83**, 143–181.

AMOROSO, E. C. (1952). Placentation. In *Physiology of Reproduction* (ed. Marshall), pp. 127–311. London: Longmans, Green.

ANDRESEN, A. (1922). Uber die Semiplazenta multiplex der *Cervus rufus* Cuv. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **65**, 544–569.

ANDRESEN, A. (1927). Die Plazentome der Wiederkäuer. *Morph. Jb.* **57**, 410–485.

ASDELL, S. A. (1946). *Patterns of Mammalian Reproduction.* Ithaca, New York: Comstock Publ. Co.

ASSHETON, R. (1906). The morphology of the ungulate placenta, particularly the development of that organ in the sheep, and notes upon the placenta of the elephant and Hyrax. *Phil. Trans.* B, **198**, 143–220.

BEAUREGARD, H. & BOULART, M. R. (1885). Note sur la placentation des ruminants. *J. Anat., Paris*, **21**, 93–99.

BEDFORD, Duke of, & MARSHALL, F. H. A. (1942). On the incidence of the breeding season in mammals after transference to a new latitude. *Proc. roy. Soc.* B, **130**, 396–399.

BISCHOFF, T. L. W. (1854). *Entwicklungsgeschichte des Rehes.* Giessen.

BJÖRKMAN, N. (1954). Morphological and histochemical studies on the bovine placenta. *Acta Anat.* **22**, Suppl. 22/2, 1–97.

BJÖRKMAN, N. & BLOOM, G. (1957). On the fine structure of the foetal-maternal junction in the bovine placentome. *Z. Zellforsch.* **45**, 649–659.

BOULART, M. R. (1888). Note sur la placenta du *Cervus mexicanus. C.R. Soc. Biol., Paris*, 8th Series, **5**, 422.

CHIODI, D. V. (1927). Sulla fine struttura della placenta di *Bos taurus. Arch. ital. Anat. Embriol.* **24**, 150–188.

DEMPSEY, E. W. & DEANE, H. W. (1946). The cytological localization, substrate specificity and pH optima of phosphatases in the duodenum of the mouse. *J. cell. comp. Physiol.* **27**, 159–179.

DISSE, J. (1906). Die Vergrosserung der Eikammer bei der Feldmaus (*arvicola arvalis*). *Arch. mikr. Anat.* **68**, 215–251.

DUVAL, M. (1895). Le placenta des carnassiers. *J. Anat., Paris*, **31**, 38–80.

EWART, J. C. (1878). The fecundity and placentation of the Shanghai River Deer. *J. Anat., Lond.*, **12**, 225–228.

GREENSTEIN, J. S., MURRAY, R. W. & FOLEY, R. C. (1958). Observations on the morphogenesis and histochemistry of the bovine preattachment placenta between 16 and 33 days of gestation. *Anat. Rec.* **132**, 321–342.

HAMMOND, J. (1927). *The Physiology of Reproduction in the Cow.* Cambridge University Press.

HARRISON, R. J. & D'SILVA, J. L. (1956). Uptake of radio-potassium (^{42}K) by the uterus and placenta during the advancement of pregnancy in the rat and the goat. Ciba Foundation: *Colloquium on Ageing*, **2**, *Ageing in Transient Tissues*, pp. 148–160. London: Churchill.

HARRISON, R. J. & HAMILTON, W. J. (1952). The reproductive tract and the placenta and membranes of Père David's Deer (*Elaphurus davidianus* Milne Edwards). *J. Anat., Lond.*, **86**, 203–225.

HARRISON, R. J. & HYETT, A. R. (1954). The development and growth of the placentomes in the Fallow Deer (*Dama dama* L.). *J. Anat., Lond.*, **88**, 338–355.

HEAPE, W. (1901). 'Sexual seasons' of mammals and the relation of the 'pro-oestrum' to menstruation. *Quart. J. micr. Sci.* **44**, 1–70.

HUBRECHT, A. A. W. (1889). Studies in mammalian embryology. 1. The placentation of *Erinaceus europaeus* with remarks on the phylogeny of the placenta. *Quart. J. micr. Sci.* **30**, 283–404.

JENKINSON, J. W. (1906). Notes on the histology and physiology of the placenta in Ungulata. *Proc. Zool. Soc. Lond.* **1**, 73–96.

KOLSTER, R. (1902). Die Embryotrophe placentarer Saüger, mit besonderer Berücksichtigung der Stüte. *Anat. Hefte*, 1 *Abt.* **18**, 455–505.

KOLSTER, R. (1903). Weitere Beiträge zur Kenntnis der Embryotrophe bei Indeciduaten. *Anat. Hefte*, 1 *Abt.* **20**, 231–321.

KOLSTER, R. (1909). Weitere Beiträge zur Kenntnis der Embryotrophe. III. Über den Uterus gravidus von *Rangifer Tarandus*, H. Sm. *Anat. Hefte*, 1 *Abt.* **38**, 101–192.

KOLSTER, R. (1910). Weitere Beiträge zur Kenntnis der Embryotrophe. IV. Zur Kenntnis der Chorionepithels. *Anat. Hefte*, 1 *Abt.* **40**, 149–178.

LYDEKKER, R. (1898). *The Deer of All Lands.* London: Rowland Ward.

MCMANUS, J. F. A. (1946). Histological demonstration of mucin after periodic acid. *Nature, Lond.*, **158**, 202.

ORSINI, M. W. (1954). The trophoblastic giant cells and endovascular cells associated with pregnancy in the hamster, *Cricetus auratus*. *Amer. J. Anat.* **94**, 273–331.

PRELL, H. (1938). Die Tragzeit des Rehes. *Zuchtungskunde*, **13**, 325–345.

SANSOM, G. S. (1922). Early development and placentation in *Arvicola* (*Microtus*) *amphibius*, with special reference to the origin of placental giant cells. *J. Anat., Lond.*, **56**, 333–365.

SANSOM, G. S. (1927). The giant cells in the placenta of the rabbit. *Proc. roy. Soc. Lond.* B, **101**, 354–368.

SEDLACZEK, S. (1912). Über Placentarbildung bei Antilopen. *Anat. Hefte*, 1 *Abt.* **46**, 573–597.

SETON, E. T. (1909). *Life Histories of Northern Animals. An Account of the Mammals of Manitoba.* London: Constable.

SNELL, G. D. (1941). The Early Embryology of the Mouse. In *Biology of the Laboratory Mouse.* Philadelphia: Blakiston Co.

SOBOTTA, J. (1903). Die Entwicklung der Eies der Maus vom Schlusse der Furchungs-periode bis zum Auftreten der Amniosfalten. *Arch. mikr. Anat.* **61**, 274–330.

STRAHL, H. (1906). Über die Semiplacenta—multiplex von *Cervus elaphus* L. *Anat. Hefte*, 1 *Abt.* **31**, 199–218.

STRAHL, H. (1912). Zur Kenntnis der Wiederkäverplacentome. *Anat. Anz.* **40**, 257–264.

SWINHOE, R. (1870). On a new deer from China. *Proc. Zool. Soc. Lond.* 89–92.

TURNER, W. (1878*a*). Note on the foetal membranes of the Rain-deer (*Rangifer tarandus*). *J. Anat., Lond.*, **12**, 601–603.

TURNER, W. (1878*b*). On the placenta of the hog-deer (*Cervus porcinus*). *J. Anat., Lond.*, **13**, 94–98.

TURNER, W. (1789). On the cotyledonary and diffused placenta of the Mexican deer (*Cervus mexicanus*). *J. Anat., Lond.*, **13**, 195–200.

WALKER, D. G. (1954). Fructose in the foetal fluids of deer. *Nature, Lond.*, **173**, 309–310.

WEBER, E. H. (1832). In *Handbuch der Anatomie des Menschen* (ed. Hildebrandt), 4th ed. Braunschweig.

WIMSATT, W. A. (1949). Some important cytological and cytochemical features of the binucleate giant cells characteristic of the placenta of ruminants and their interpretation. *Anat. Rec.* **103**, 522.

WIMSATT, W. A. (1950). New histological observations on the placenta of the sheep. *Amer. J. Anat.* **87**, 391–458.

WIMSATT, W. A. (1951). Observations on the morphogenesis, cytochemistry, and significance of the binucleate giant cells of the placenta of ruminants. *Amer. J. Anat.* **89**, 233–281.

WISLOCKI, G. B. (1941). The placentation of an antelope (*Rhynchotragus kirkii nyikae* Heller). *Anat. Rec.* **81**, 221–241.

WISLOCKI, G. B. & FAWCETT, D. W. (1949). The placentation of the pronghorned antelope (*Antilocapra americana*). *Bull. Mus. comp. Zool. Harv.* **101**, 545–559.

EXPLANATION OF PLATES

PLATE 1

Photographs of the placentomes of various Cervidae. The umbilical vessels have been injected with coloured gelatin and the allantois has been opened in each specimen.

Fig. 1. Placentomes of a red deer (foetus 200 mm. C.R. length). $\times \frac{1}{4}$.

Fig. 2. Placentomes of a fallow deer (foetus 110 cm. C.R. length). $\times \frac{1}{6}$.

Fig. 3. Placentomes of a fallow deer (foetus 290 mm. C.R. length). Note the well-vascularized membranous chorion. $\times \frac{1}{8}$.

Fig. 4. Placentomes of a Japanese Sika deer (foetus 332 mm. C.R. length). $\times \frac{1}{8}$.

Fig. 5. Placentome of a Père David's deer (foetus 275 mm. C.R. length). $\times \frac{1}{3}$.

PLATE 2

Fig. 6. Photograph of placentomes of a red deer pregnant with a 150 mm. C.R.-length foetus. The uterine vessels have been injected with yellow gelatin. About natural size.

Fig. 7. Photomicrograph of a caruncle of a fallow deer pregnant at 22 mm. showing early crypt formation. PAS and Light Green. $\times c.$ 3.

Fig. 8. Photomicrograph of another caruncle from the same specimen as above showing early crypt formation. H. and E. $\times 400$.

Fig. 9. Photomicrograph of the tip of a developing villus within a shallow crypt in a caruncle of a fallow deer pregnant with a 27 mm. foetus. H. and E. $\times 600$.

Fig. 10. Photomicrograph of the edge of another caruncle of the same specimen as above showing the continuity of the duct lining of an uterine gland with the caruncular epithelium. H. and E. $\times 400$.

PLATE 3

Fig. 11. Photomicrograph of the zone of attrition (page 11) of the placentome of a fallow deer (foetus 280 mm. C.R. length). H. and E. $\times 150$.

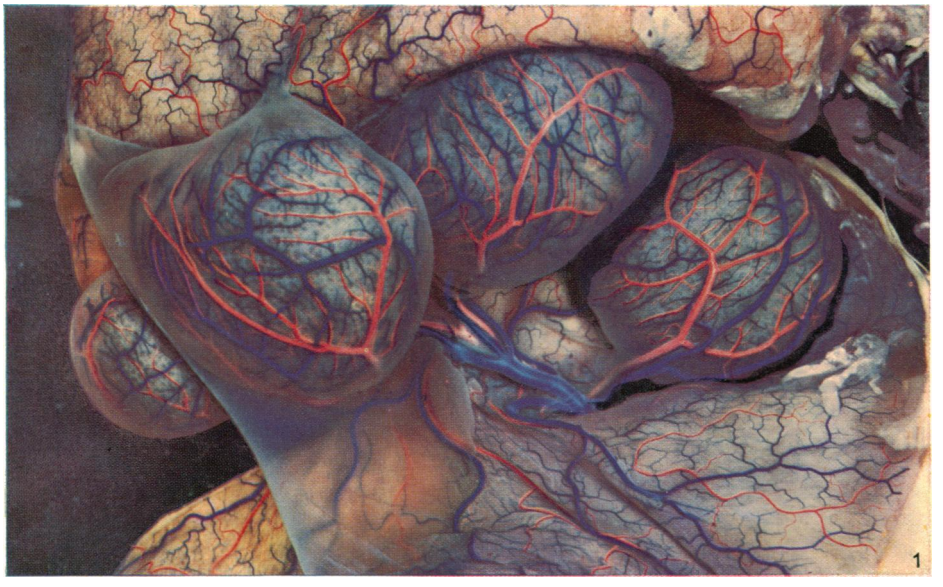
Fig. 12. Photomicrograph of a similar region of a placentome of the same animal as above showing glycogen in the columnar trophoblast cells of the main stem villi and the arcades. PAS and Light Green. $\times 180$.

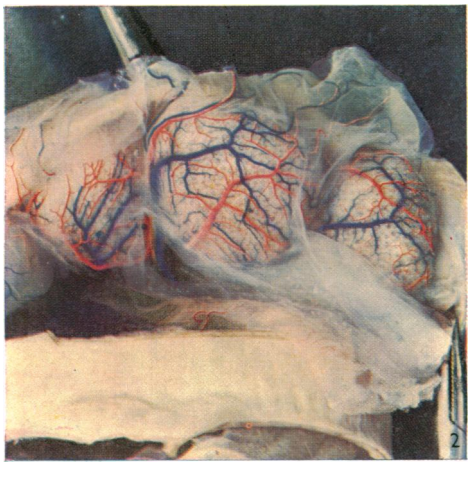
Fig. 13. Photomicrograph to show distribution of alkaline phosphatase (Na-β-glycerophosphate substrate) along the edge of the trophoblast and in maternal endothelial cells of the placentome of a fallow deer (foetus 190 mm. C.R. length). $\times 180$.

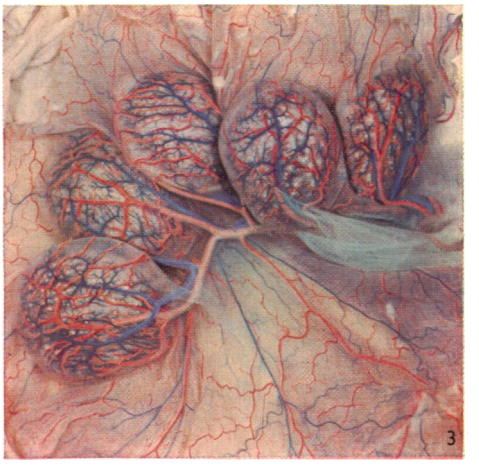
Fig. 14. Photomicrograph of the columnar trophoblast cells of the main-stem villus (zone of storage, page 11) of the placentome of a fallow deer (foetus 220 mm. C.R. length). Compare with fig. 12. H. and E. $\times 500$.

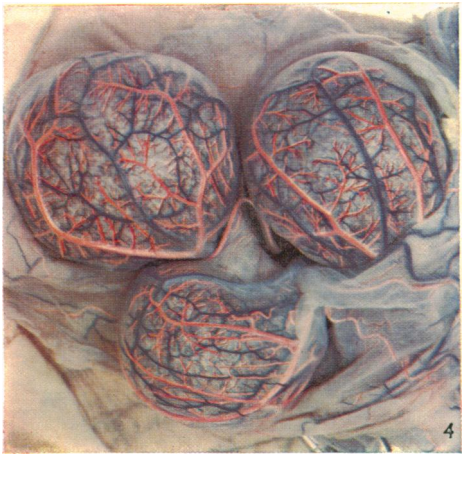
Fig. 15. Photomicrograph of the lower part of the zone of attrition (page 11) of the placentome of a fallow deer (foetus 180 mm. C.R. length). Binucleate cells are present in the trophoblast and in the crypt lining; syncytial masses are present on the right of the central maternal septum. H. and E. $\times 180$.

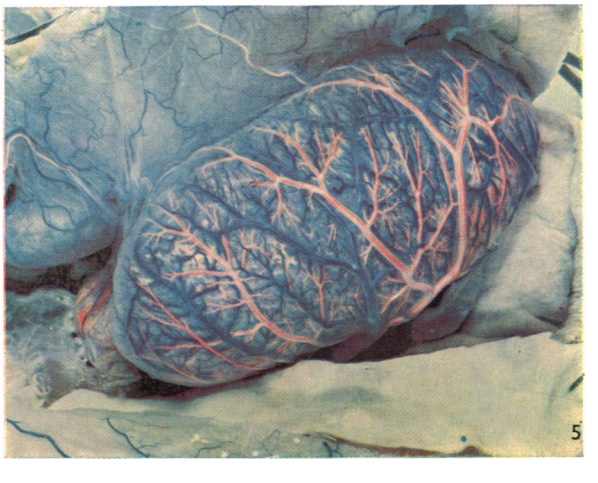
Fig. 16. Photomicrograph of the tip of a villus in the placentome of a fallow deer (foetus 180 mm. C.R. length). H. and E. $\times 90$.











HAMILTON, HARRISON AND YOUNG—ASPECTS OF PLACENTATION IN CERTAIN CERVIDAE

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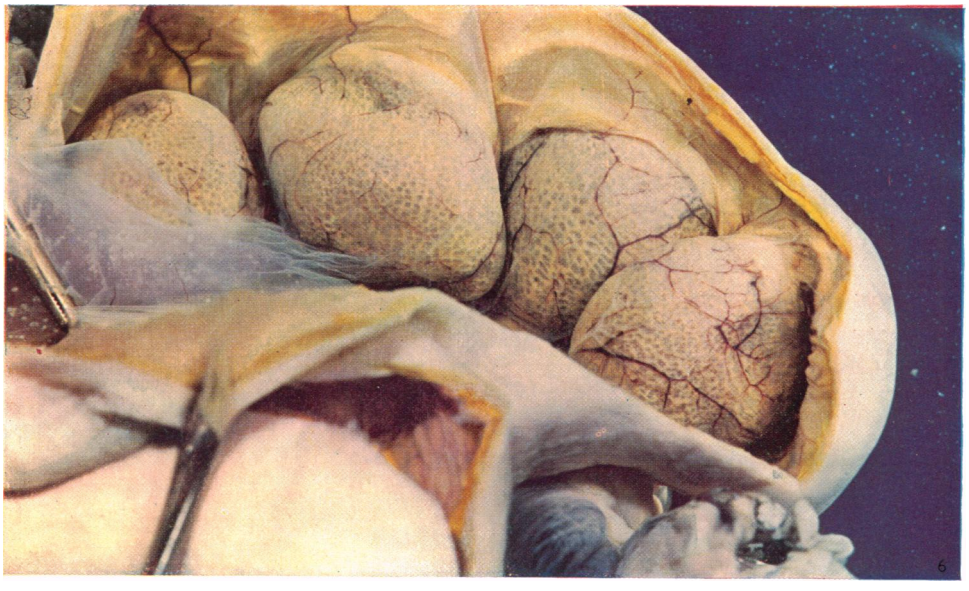
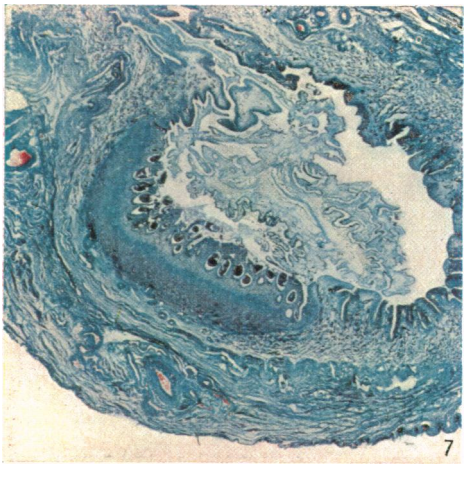
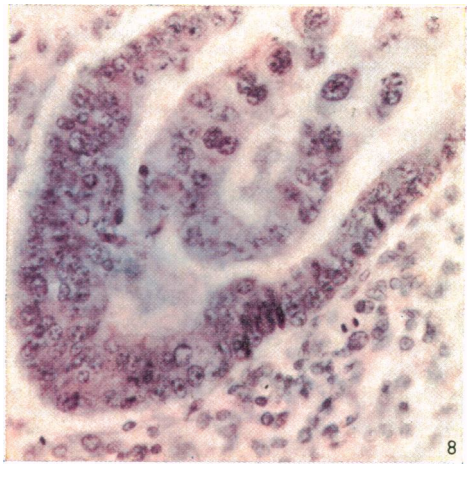
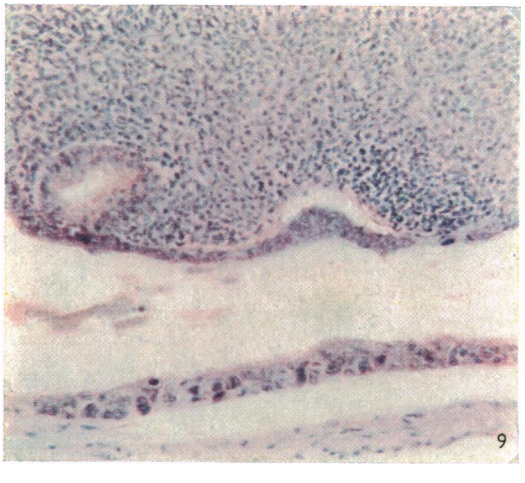
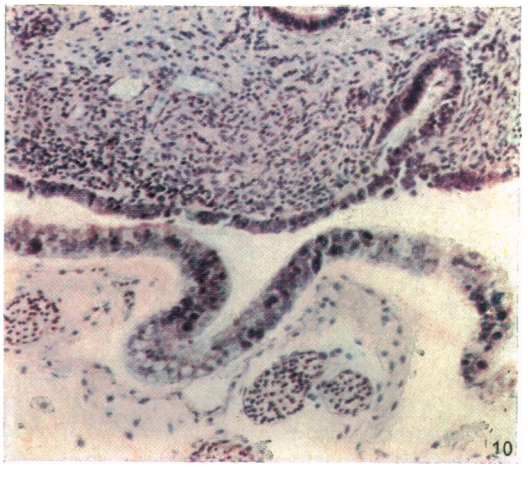


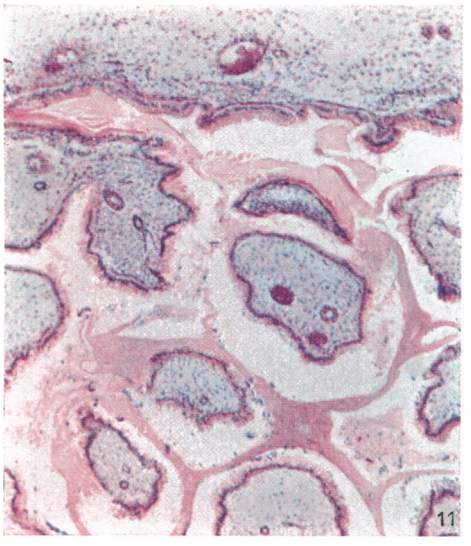








HAMILTON, HARRISON AND YOUNG—ASPECTS OF PLACENTATION IN CERTAIN CERVIDAE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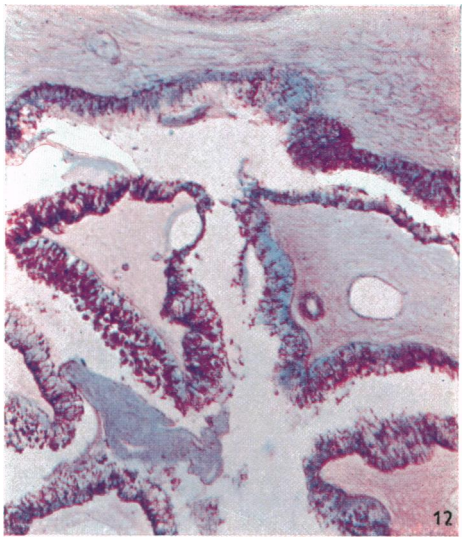
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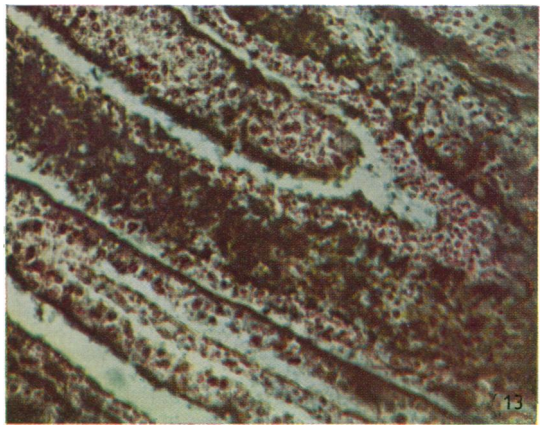
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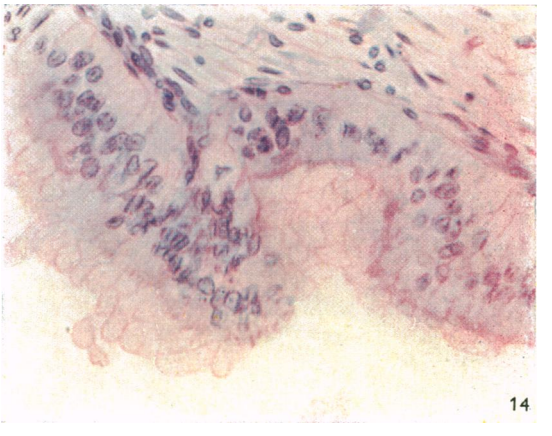
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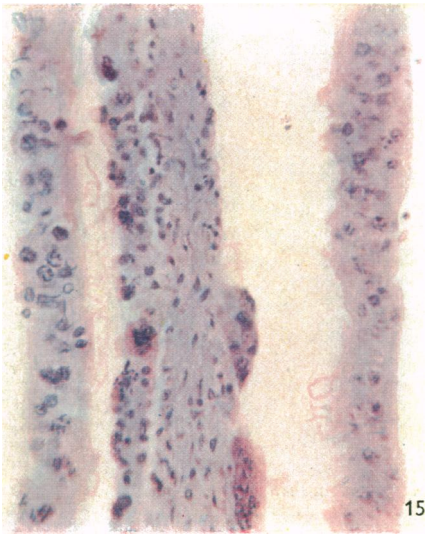
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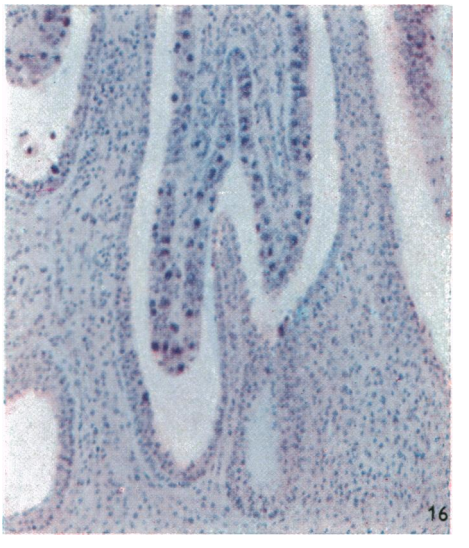
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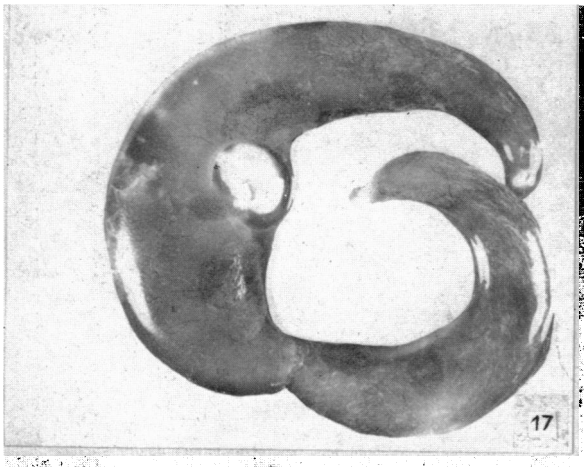
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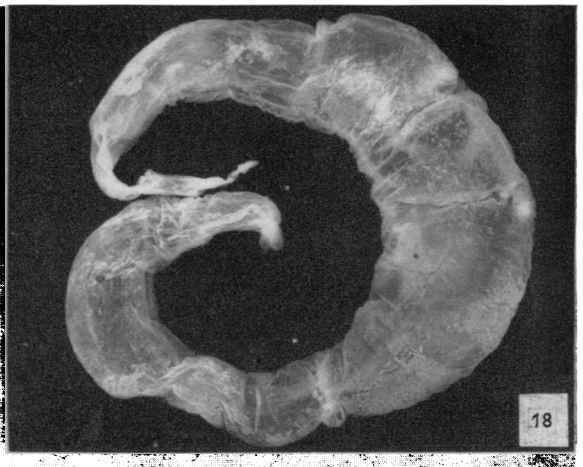
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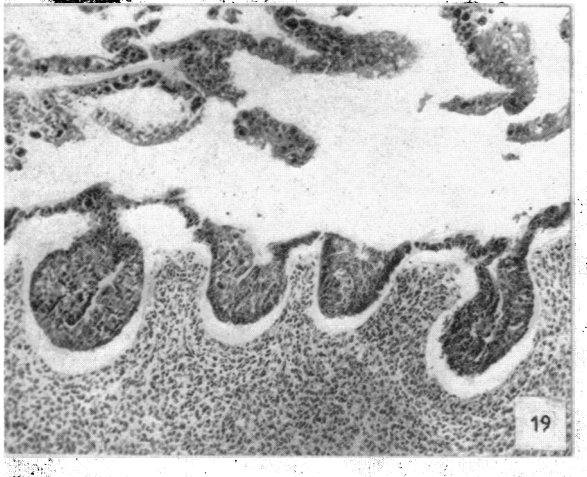
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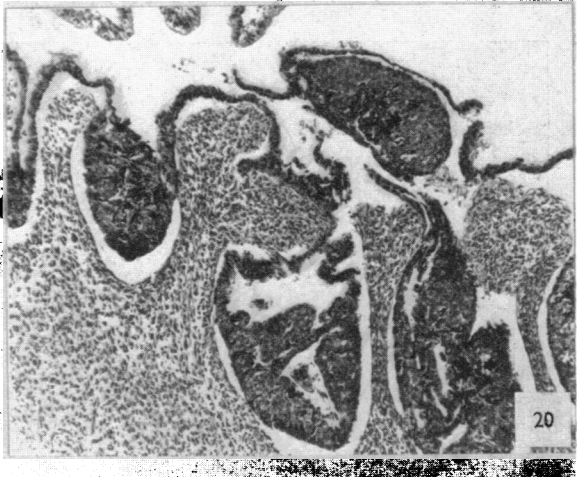
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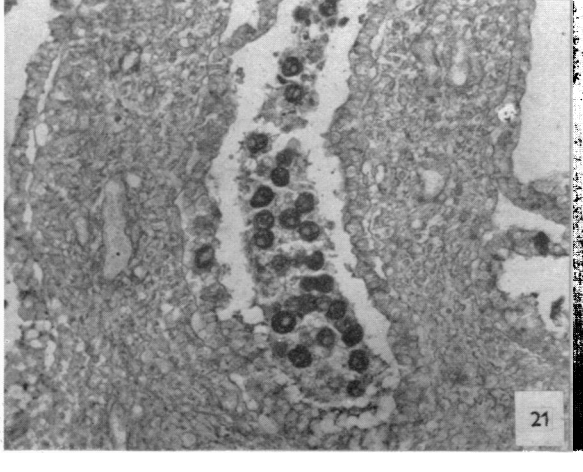
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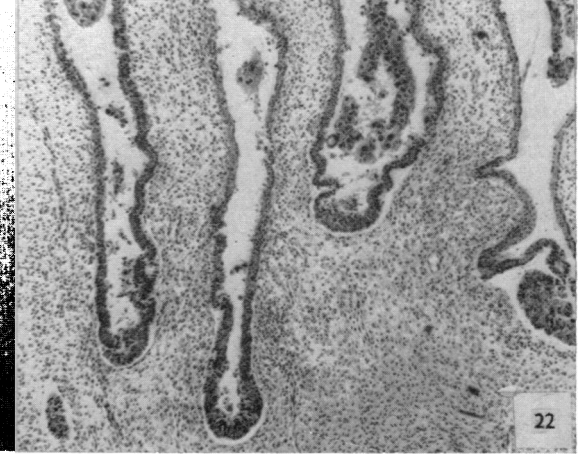
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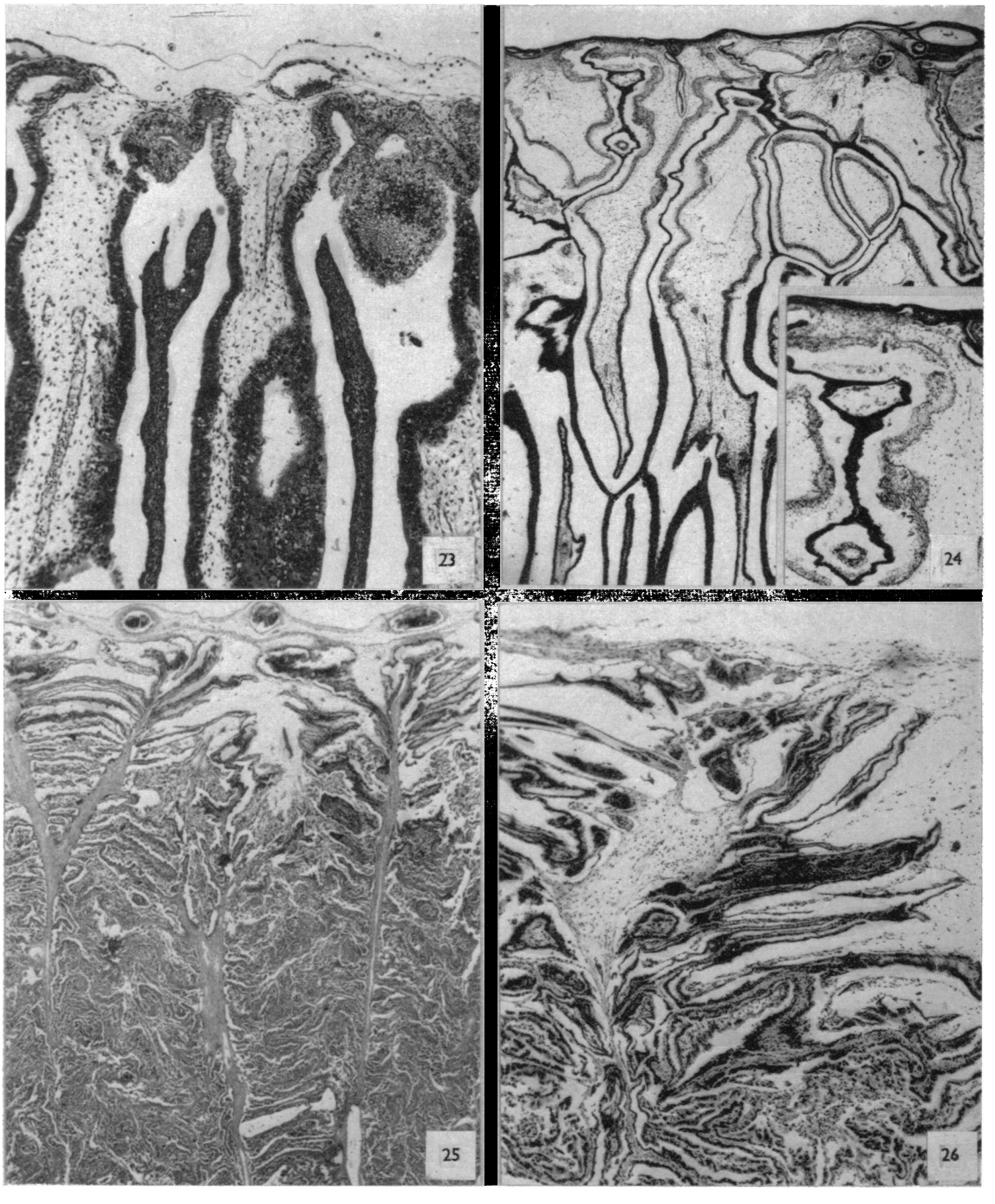
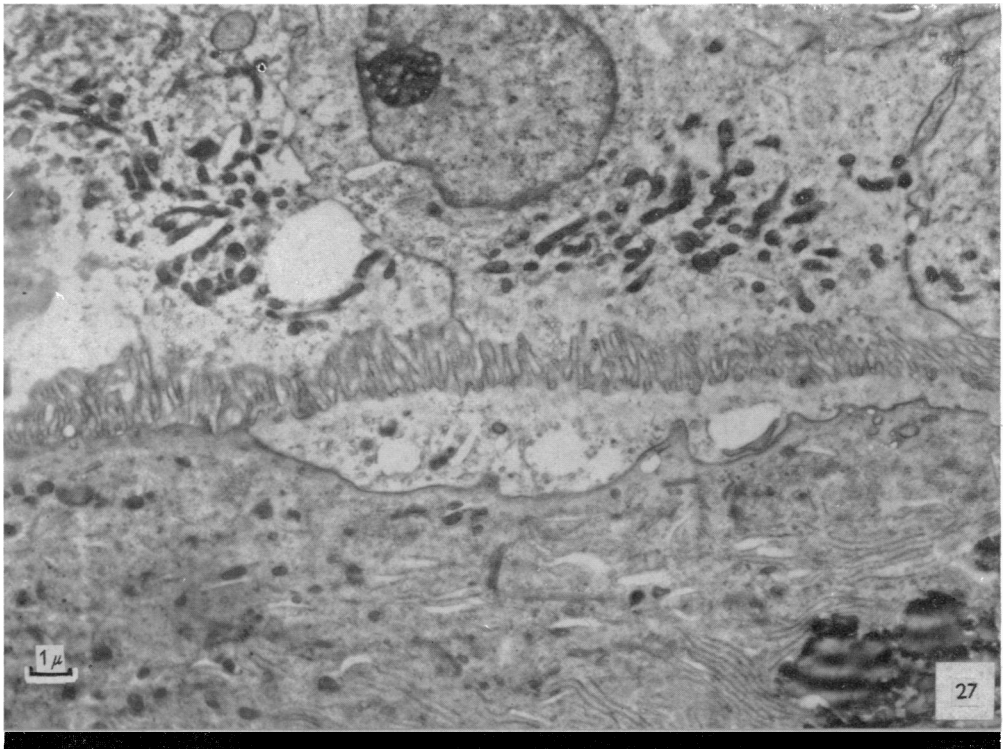


HAMILTON, HARRISON AND YOUNG—ASPECTS OF PLACENTATION IN CERTAIN CERVIDAE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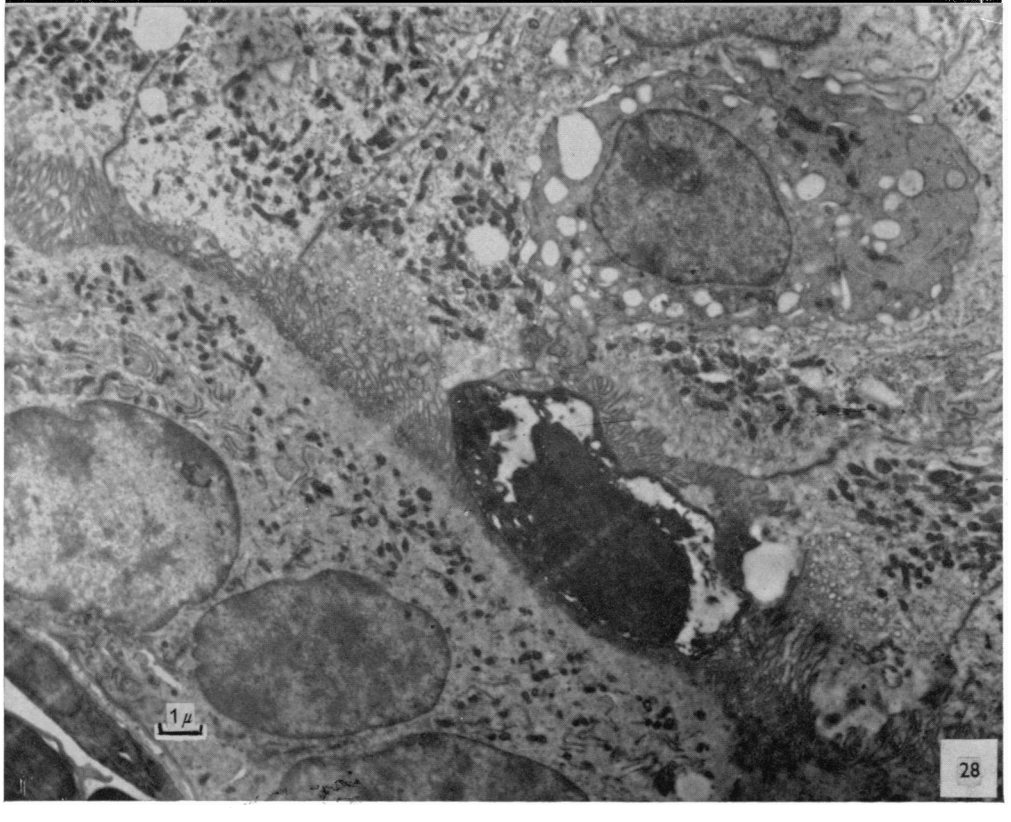
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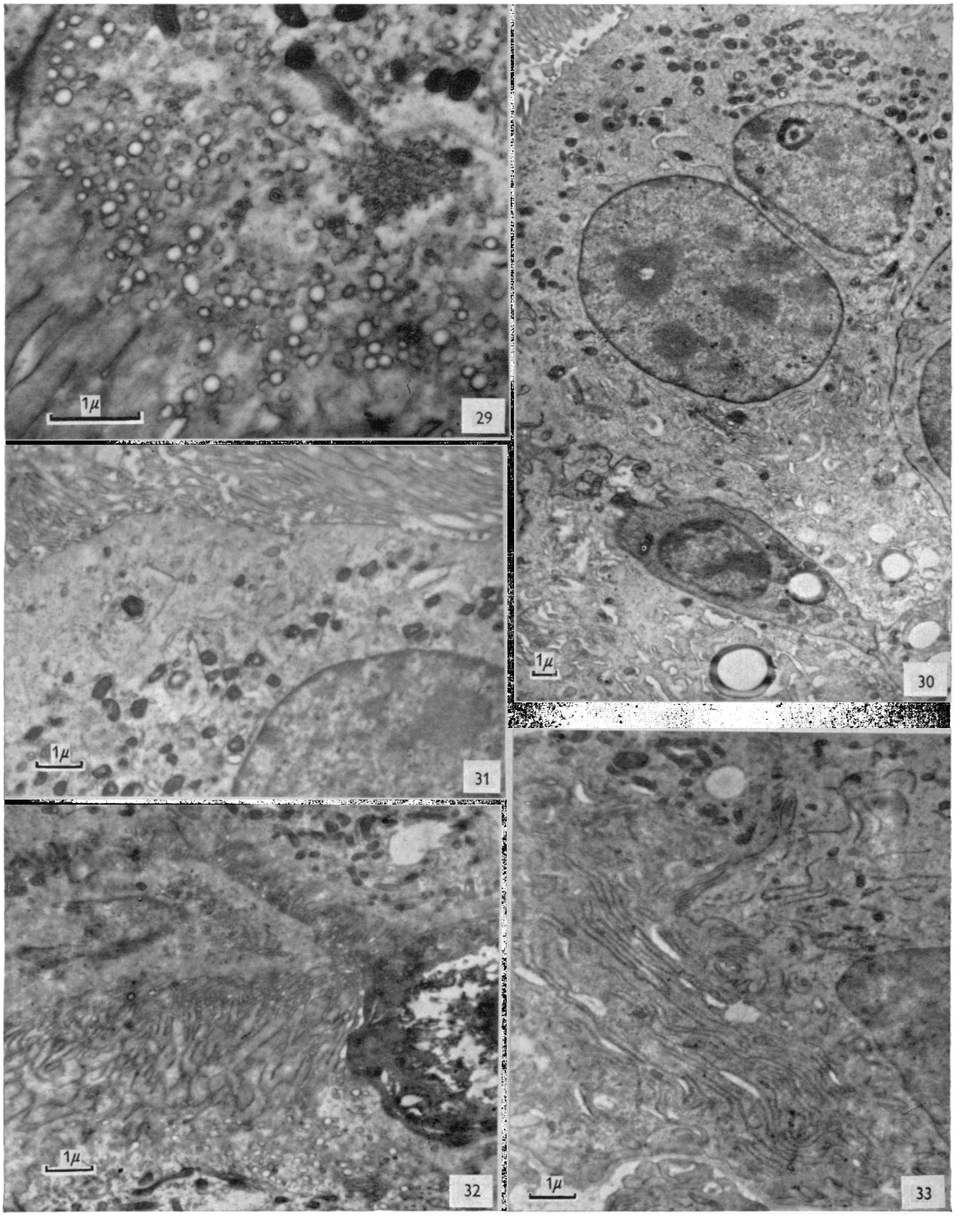
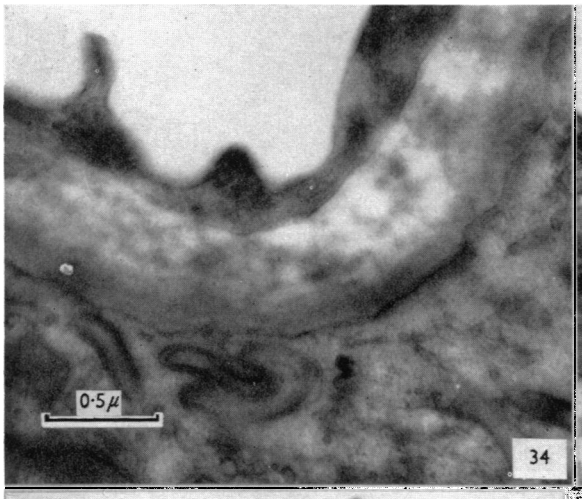


HAMILTON, HARRISON AND YOUNG—ASPECTS OF PLACENTATION IN CERTAIN CERVIDAE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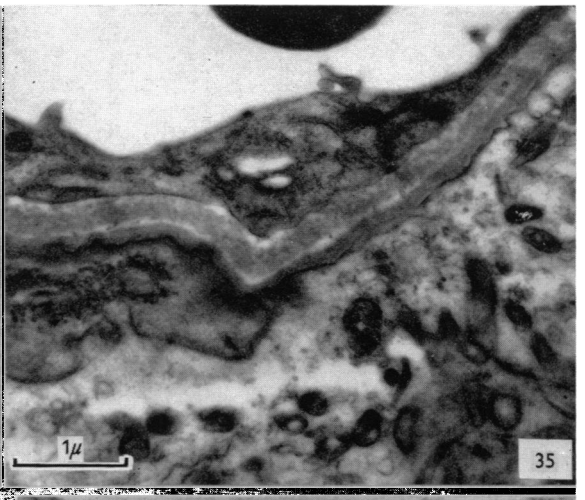
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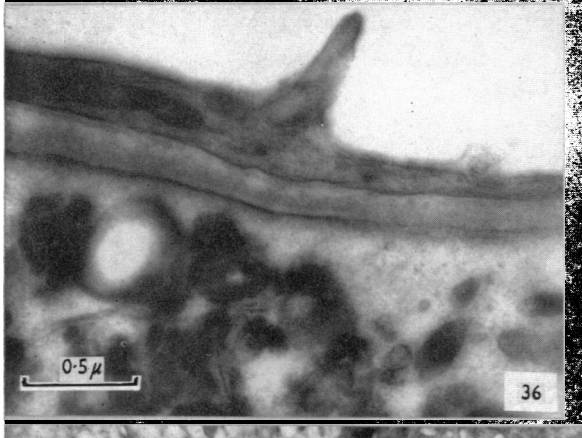
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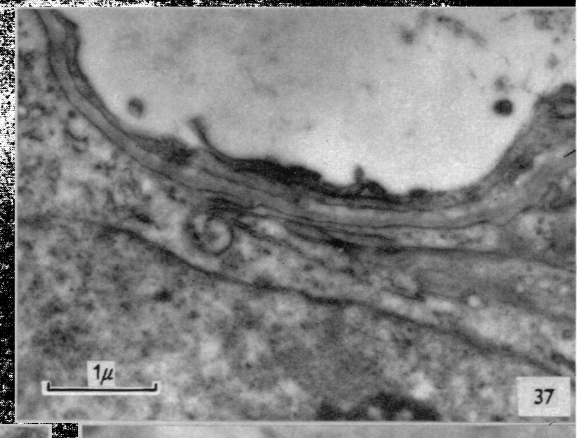
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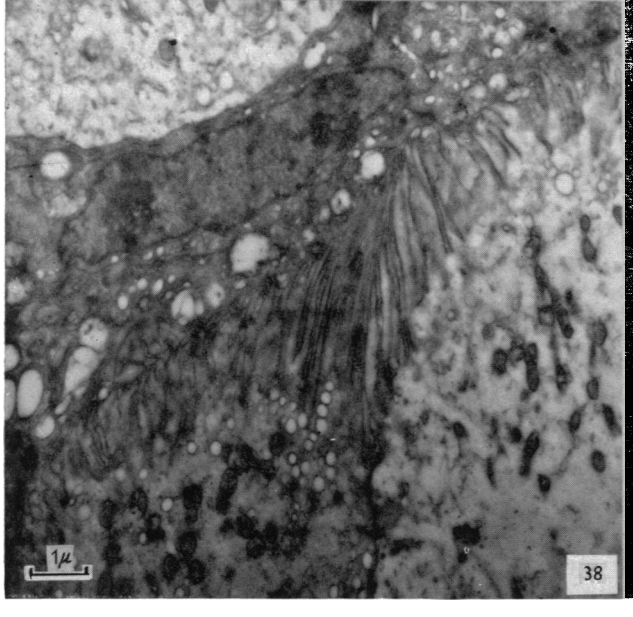
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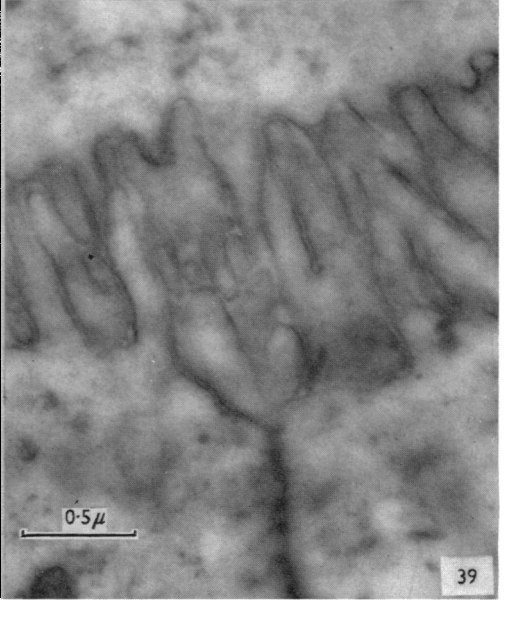
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PLATE 4

Fig. 17. Photograph of the chorionic sac of a fallow deer pregnant with a foetus 18 mm. in C.R. length. Note the developing cotyledons bearing short villi. × ¼.

Fig. 18. Photograph of the chorionic sac of a fallow deer pregnant with a foetus 25 mm. in C.R. length. Note atrophic tips to the sac. × ¼.

Fig. 19. Photomicrograph of a caruncle of a fallow deer pregnant with a foetus 12 mm. in C.R. length showing early crypt formation. H. and E. × 60.

Fig. 20. Photomicrograph to show a stage in crypt formation later than that shown above. The fallow deer foetus was 22 mm. in C.R. length. × 60.

Fig. 21. Photomicrograph of developing villus and crypt in the caruncle of a fallow deer 27 mm. in C.R. length. The section has been treated by the PAS technique which demonstrates the binucleate cells clearly. × 100.

Fig. 22. Photomicrograph of the developing crypts in the caruncle of a fallow deer pregnant with a foetus 33 mm. in C.R. length. Note the hypertrophied lining of the crypts' ends. H. and E. × 30.

PLATE 5

Fig. 23. Photomicrograph of the main stem villi and crypt mouth of a placentome of a roe deer (foetus 91 mm. in C.R. length). Compare the tips of the maternal septa with those shown in fig. 24. H. and E. × 60.

Fig. 24. Photomicrograph of the atrophic terminal portions of the maternal septa of a placentome of a fallow deer (foetus 290 mm. in C.R. length). The inset shows a portion of the tip of one septum enlarged. H. and E. × 30; inset × 60.

Fig. 25. Photomicrograph of part of the placentome of a reindeer (foetus 260 mm. C.R. length). H. and E. × 30.

Fig. 26. Photomicrograph of part of the placentome of a reindeer (foetus 290 mm. C.R. length). H. and E. × 50.

PLATE 6

Fig. 27. Electron micrograph of placentome of a fallow deer pregnant with a 200 mm. C.R. foetus, showing foetal-maternal junction. Foetal trophoblast cells above with line of mitochondria. Microvilli interlocking with maternal syncytium below. × 3900.

Fig. 28. As above showing foetal-maternal junction with included degenerating cell. Foetal trophoblast on the right and maternal syncytium on left. × 3900.

PLATE 7

Fig. 29. Electron micrograph of placentome of a fallow deer pregnant with a 200 mm. C.R.-length foetus, showing small vesicles at apex of foetal trophoblast cell. Microvilli on left. × 7350.

Fig. 30. As above showing maternal giant cell. × 1950.

Fig. 31. As above showing apex of maternal giant cell with few microvilli. × 3600.

Fig. 32. As above showing junctional zone with included dead cell on right. Small vessels and microvilli of foetal trophoblast cell below. × 3600.

Fig. 33. As above showing maternal crypt cell with endoplasmic reticulum and folded plasma membrane. × 3600.

PLATE 8

Fig. 34. Electron micrograph of placentome of a fallow deer pregnant with a 200 mm. C.R.-length foetus, showing maternal capillary and basement membrane. Lumen of capillary with process of endothelium above, cytoplasm and unfolded plasma membrane of crypt cell below. × 14700.

Fig. 35. As above showing maternal capillary and basement membrane. × 7350.

Fig. 36. As above showing foetal capillary and its basement membrane. Small process of foetal endothelium projecting into lumen of capillary. × 14700.

Fig. 37. As above showing apex of foetal capillary. × 7350.

Fig. 38. As above showing apex of foetal columnar trophoblast cell with microvilli, small vesicles and line of mitochondria. × 3600.

Fig. 39. As above showing interlocking of microvilli at foetal-maternal junction. Plasma membrane between two foetal trophoblast cells is seen. × 14700.