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The existence during gestation of an immunological buffer zone at the interface between maternal and foetal tissues

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[Plates 10–13]

In mammalian pregnancy the trophoblast normally constitutes an uninterrupted boundary of foetal tissue in immediate contact with maternal tissue, including blood in some species, and is the decisive immunological barrier to rejection of the foetus as an allograft. The ability of the trophoblast to function as a barrier evidently results from its capacity to resist immunological attack by either alloantibody or alloimmune cells and to prevent immunocompetent cells from reaching and damaging the foetus but, as yet, there is no general agreement regarding the means by which it exercises these functions. In view of the dramatic hormonal changes that occur during pregnancy and the undisputed involvement of trophoblast in these endocrine events, the possibility exists of an interaction between the hormones of pregnancy and the immunological phenomena. The present account furnishes evidence that endocrine activity at the maternal surface of the trophoblast, the presumptive site of the immunological frontier between foetus and mother, may be a factor in its local survival at implantation. The placental hormones so far known that are capable of blocking the antigen receptor sites of the mother's lymphocytes and thus preventing the latter from reacting with the foetal antigens are the glycoprotein, human chorionic gonadotrophin (HCG) and the polypeptide hormone, human chorionic somatomammotrophin (HCS) or human placental lactogen (HPL), both of which are specific to the human placenta. The origin of these hormones, their spatial distribution and their probable interaction with placental steroid hormones are discussed. It is argued that the place of highest concentration of these hormones is on the surface of the syncytial microvilli and the adjacent caviolae of the apical plasma membrane, as well as on the surfaces of the persisting cytotrophoblastic cells of the basal plate (cytotrophoblastic shell), the cell islands and the septa – precisely where the immunological challenge of the foetal allograft to the maternal host occurs. An explanation is offered for the continuing production of the voluminous quantities of these hormones during human pregnancy.

Introduction

To the transplantation immunologist the habit of viviparity, and with it the establishment of a respiratory and trophic connexion between parent and offspring, is a much more complex relationship than is that between a tissue or organ homograft and an immunologically mature host. This emerges from the fact that viviparity, which is widespread in the animal kingdom, entails a parabiotic union between two different organisms of dissimilar genetic constitution, culminating in mammals with the elaboration of a placenta which shows its full range of complexity from the primitive yolk-sac (chorio-vitelline) placenta of the marsupials to the haemochorial condition of primates. Here trophoblast is in direct contact with, and actually bathed by, maternal blood as in conventional man-made grafts. In contrast to surgically created allografts, however, the foetus possesses a circulatory system which is normally fully independent of that of the maternal host, though occasionally permitting a covert exchange of

cellular elements of various kinds (a ready mechanism for sensitization) and, in some species (guinea-pig, rabbit, monkey and man), a transfer of passive immunity from mother to young.

Since it normally possesses some paternal genetic characteristics that differ from the maternal host, the conceptus is in every sense of the term a natural allograft, but one which yet escapes immunological destruction for the duration of pregnancy, despite its proven antigenicity and the intact immunological competence of the mother. Viviparity, as was first pointed out by Medawar (1953), thus presents the biologist with a set of fundamental problems concerning the relationship of pregnancy to immunological reactions and the fascinating enigma of the failure of the maternal immune mechanism to identify and reject the foetus as non-self. That rejection of such grafting in pregnancy is the exception rather than the rule indicates that a unique sequence of events obtains at the time of implantation.

Perhaps, then, the zoologist is right to regard intra-uterine development as the resultant of a series of compromises between the need to reduce the foeto–maternal barrier for more effective transport and, simultaneously, to retain it intact as a protection against allograft rejection. However, since pregnancy represents a *special state*, it must be emphasized at the outset that the immunological reactions of the mother against the products of conception should not be equated too rigidly with those shown against other tissues engrafted in her body; nor should it be supposed that the normal development of the foetus can be explained solely on the basis of the immunological reactivities of the gravid female and entirely apart from the endocrine framework in which viviparity has evolved.

Looking at the problem, this time, from the endocrinologist's point of view, it can be asserted with reasonable confidence that there is no category of behaviour that is more clearly influenced by endocrine secretions than are those associated with the initiation, maintenance and termination of pregnancy, to which both the foetus and its placenta contribute. Hence it is not beyond conjecture that the placental site, as an endocrine organ of pregnancy, contains some of the clues to the failure of the foetus and its membranes to provoke an effective immunological reaction from its mother.

While bearing in mind that a complete solution may possibly be contained in the irregular behaviour of certain hormones – oestrogens, progesterone, corticosterone and the gonadotrophic substances in the blood and urine of pregnancy – clearly associated with reproduction in man and in some other animals, it is proposed to deal here principally, but not exclusively, with some of the more outstanding questions which arise when the immunosuppressive role o the placental gonadotrophins is taken as the point of reference. From this viewpoint the question is: do chorionic gonadotrophins provide a perfect immunological insulation to the trophoblast, rendering it both incapable of provoking maternal sensitization and insusceptible to an existing state of sensitization, and if this is so, is the placental trophoblast of mammals fully equipped to exercise these functions? The gist of the answer is that chorionic gonadotrophins (HCG, HCS) will inhibit an immunological system dependent on antigen recognition and will synergize with steroids; furthermore, the implanting mammalian blastocyst has the ability to synthesize both oestrogens and gonadotrophins in sufficient quantities to be a factor in the local survival of the trophoblast. The ideas put forward are admittedly speculative, but evidence derived from a variety of sources suggests the wide occurrence of placental agents with both hormonal and immunosuppressive qualities.

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The presence of a barrier between mother and foetus

Of all possible safeguards of the conceptus against immunological attack, it now appears that in mammalian pregnancy the trophoblast, which normally constitutes an uninterrupted boundary of foetal tissue in immediate contact with maternal tissue – including blood in some species – is the decisive barrier to rejection of the foetus as an allograft. However, since there is evidence, both circumstantial (Billington 1969) and direct (Loke, Joysey & Borland 1971) for the presence of HL-A antigenic determinants on human trophoblast and on its malignant counterpart choriocarcinoma (Rudolph & Thomas 1971) it seems unlikely that the trophoblast cells themselves constitute such a barrier. It is, therefore, presumed with strong collateral evidence (Kirby, Billington, Bradbury & Goldstein 1964; Bradbury, Billington & Kirby 1965; Currie, Doorninck & Bagshawe 1968) that some form of immunologically inert material, closely associated with the maternal surface of the trophoblast, masks the histocompatibility antigens, and effectively insulates the foetus from the depredations of its mother. It must, therefore, operate against cellular immunity at both the afferent and efferent ends of the process.

On the basis of electron microscopic and histochemical studies of murine placentae, Kirby and his co-workers (Kirby *et al.* 1964; Bradbury *et al.* 1965; Kirby, Billington & James 1966), suggested that the insulating pericellular coating was a layer of 'fibrinoid' mucopolysaccharide material rich in hyaluronic acid and sialic acid (*N*-neuraminic acid). They argued that this layer of 'fibrinoid', which was conspicuously increased at the foeto–maternal junction of a hybrid foetus as compared with the condition when mother and foetus were antigenically identical, enveloped each trophoblast cell in the placenta, and acted as an immunological buffer zone. Subsequently, Currie *et al.* (1968) showed that digesting this mucopolysaccharide coating was sufficient to unmask transplantation antigenicity on murine trophoblast. The enzyme neuraminidase specifically removes only the sialic acid groups from the sialomucins, thus implying that the barrier to detection of antigen must depend on just the terminal residues of the glycoprotein molecule and not necessarily on any special properties of the molecule as a whole.

Although these findings appear to be consistent with the hypothesis that the postulated layer of mucopolysaccharide 'fibrinoid' material might act to mask the histocompatibility antigens on the cell surface and render the trophoblastic cell relatively non-antigenic on transplantation, there are reasons for thinking this interpretation insufficient. A prominent 'fibrinoid' barrier around trophoblastic cells cannot be demonstrated in the placentae of all mammals (Wynn 1969, 1971), nor can it be found around each of the cells of pure trophoblastic transplants (Simmons, Cruse & McKay 1967), i.e. in exactly those circumstances in which trophoblast fails to express its antigenicity. If, on the other hand, the acellular (sialomucin) surface layer on the trophoblast of murine and human placentae, described by Kirby *et al.* (1964), Currie & Bagshawe (1967) Bradbury *et al.* (1965) and Bradbury, Billington, Kirby & Williams (1969), could be identified with the sialoglycoprotein hormones, a plausible explanation would, it is suggested, be forthcoming to account for the immunological dispensations enjoyed by placental trophoblast and the cells of pure trophoblastic transplants (Simmons *et al.* 1967; Borland, Loke & Wilson 1974). Of special interest in this connexion is the intriguing situation encountered in the epitheliochorial placenta of the sow, mare and goat (figure 2, plate 10), where there is neither deposition of 'fibrinoid' nor the occurrence of extensive

necrosis (Amoroso 1952; Lawn, Chiquoine & Amoroso 1969), but convincing evidence of the production of gonadotrophic substances by chorionic tissue (Philipp, 1929; Catchpole & Lyons 1934; Forsyth 1972; Allen, Hamilton & Moor 1973).

A further shortcoming of the 'fibrinoid' sialomucin theory pertains to the conditions prevailing in haemochorial placentae (primates, rodents, rabbit). Histologically demonstrable fibrinoids are readily revealed in areas where trophoblast and endometrium are in intimate contact, but not normally around trophoblast bathed by maternal blood in the intervillous space of the human placenta (Wynn 1971), nor on the trophoblastic microvilli of rabbits (Tai & Halasz 1967), i.e. precisely where the trophoblast is most vulnerable to attack. If the microvillous surface of the placenta is to be regarded as the presumptive site of the immunological frontier between the foetus and its mother, the significant question is whether it is a purely chance occurrence that it is also the tissue that is so heavily implicated in the placental production of steroid and non-steroid hormones now regarded as agents of immunosuppression.

The survival of gestational trophoblast

In haemochorial placentae, foetal trophoblast is in direct contact with maternal blood for almost the whole of gestation and in catarrhine primates the association of trophoblastic tissue (syncytiotrophoblast) with large quantities of oestrogens, progestins and gonadotrophic hormones (HCG, HCS) is unmistakable. It is tempting, therefore, to speculate that the irregular behaviour during pregnancy of certain of these hormones, clearly associated with reproduction in man (and other animals) might furnish clues to the prolonged survival of gestational trophoblast, that is to say, the 40 weeks of generally undisturbed symbiosis between the human female and her foetus. In marsupials, on the other hand, the pre-attachment vesicle is enclosed in a shell membrane for a relatively large part of gestation during which time embryogenesis is very slow (Tyndale-Biscoe 1973). Only when this membrane ruptures, liberating the vesicle (intrauterine hatching), is the mother exposed to foetal antigens of the vascular yolk-sac, which for the majority of marsupials constitutes the only placental attachment. Since the exposure is confined to the interval between hatching and the birth of immature young, the survival of gestational trophoblast is of necessity brief; for although the total length of gestation is longer than the homograft rejection period, the interval of exposure from attachment to parturition (rejection) is always shorter (Tyndale-Biscoe, Hearn & Renfree 1974). In some species of *Perameles* (the bandicoots), which possess a complex, intimately apposed and highly efficient chorioallantoic placenta in addition to a yolk-sac placenta (figure 4, plate 11), although the young at birth are more advanced than other marsupials at this stage, gestation lasts only 12 days. This is shorter than in all other marsupials with the possible exception of the native cat (*Dasyurus viverrinus*). Worthy of comment also is the fact that the duration of pregnancy does not exceed the length of one oestrous cycle, the luteal phase being comparable in both. Hence, it may be assumed that the maintenance of pregnancy does not involve hormonal mechanisms other than those that participate in the regulation of the cycle; as yet there are few factual data on possible placental endocrine function in marsupials.

The general absence of intimate fusion between foetal and maternal tissues in the majority of marsupials in contrast to the very intimate, though short-lived, attachment of the bandicoot's placenta suggests that while the vascularized allantochorionic placenta may have conferred advantages in efficient transport, it may also have necessitated a shortening of gestation to avoid

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FIGURE 1. Human. A section through the middle of the implantation site of a 9-day pregnancy. The blastocyst is almost completely embedded; the implanted portion shows various phases of trophoblast formation and in it there is extensive lacunar development. Some leucocytes are present in the lacunae, which may already be in open communication with the surrounding sinusoids. Many lymphocyte-like cells are present in the decidua. (Courtesy of Dr J. D. Ebert, Dept. of Embryology, Carnegie Institution of Washington.)

FIGURE 2. Goat. Part of a placentome from a goat 42 days pregnant. Most of the characteristics of the mature placentome have been established. The epithelia of the villus (trophoblast) and the crypt lining are closely interlocked by microvilli. The crypt lining consists of syncytial masses separated by lateral cell membranes; the foetal trophoblast is cellular. Magn. × 5000. (From Lawn, Chiquoine & Amoroso 1969.) Cf. figure 9, plate 13.

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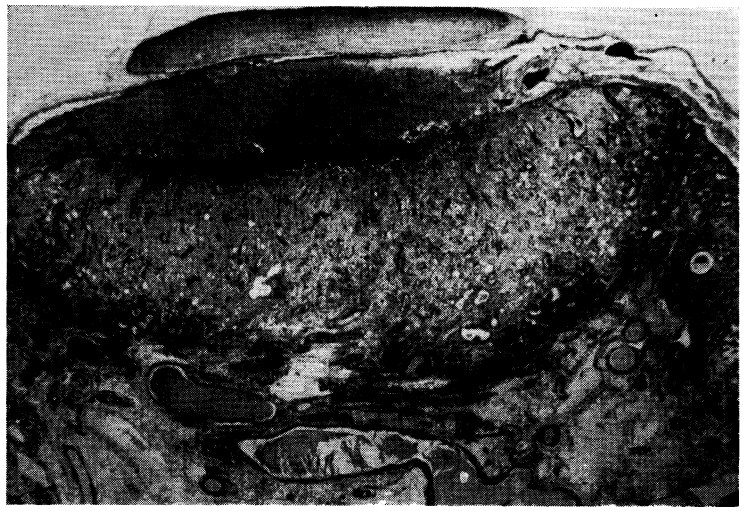

FIGURE 3. Endometrial cup from a mare killed on the 56th day of pregnancy. At this stage the cup, consisting of proliferated chorionic epithelial cells, projects from the surface of the uterus and is separated from the stroma by a dense layer of lymphocytes. Note also that the uterine epithelium over the surface of the cup is entirely absent, and that there is extensive autolysis of the cup tissue. The detritic coagulum which has accumulated in the uterline lumen is rich in gonadotrophic hormone (PMSG) and is the product of degenerating chorionic cells and glandular secretions, some of which has seeped beyond the margins of the cup. Magn. × 10.

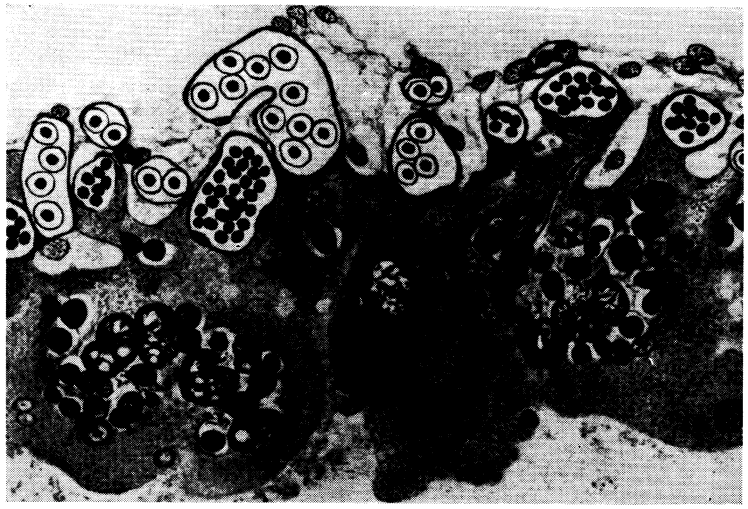

FIGURE 4. Bandicoot. The foetal-maternal junction in the mature allantoic placenta, which is really formed by the fusion of the allanto-chorionic trophoblast with the maternal syncytial layer which results from the proliferation of the uterine epithelium and the concomitant ingrowth of maternal capillaries; foetal and maternal blood vessels are in intimate apposition, but in places are separated by wisps of syncytial cytoplasm. Within the symplasma the enlarged nuclei are of foetal derivation and one gets the impression of an unstable relationship. Note the presence of lymphocytes. Magn. × 300.

The blocks for figures 3 and 4 were kindly provided by the Royal Society of Medicine.

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allograft reaction. 'Thus' as Tyndale-Biscoe (1973) puts it, 'bandicoots have not been able to exploit one adaptation for lack of another, and the result is the birth of young unmistakably marsupial in their immaturity'. If this proves to be the case and if eutherian embryos enjoy immunological privileges throughout gestation that are not enjoyed by marsupials, such changes must have been initiated in evolutionary history by a direct functional response of the trophoblast of the resident blastocyst to the uterine mucosa at the time the shell membrane was discarded as a protective device, and intrauterine hatching abolished. The direction of these changes is unmistakable; it is, as Medawar (1953) suggested, towards a complete endocrinological self-sufficiency of the conceptus and its trophoblast in respect of steroidogenic and gonadotrophic powers. From such considerations and in the light of the undisputed evidence of enhanced secretion of steroids and gonadotrophic substances in eutherian pregnancy, these hormones, whatever other functions they may exercise during pregnancy, have come under suspicion as affording an effective mechanism for the inhibition of the immunological reactivities of the mother.

Human placental hormones and the immunological phenomena of pregnancy

The balance of evidence at present indicates that the oestrogenic activity of blood during mammalian pregnancy is elevated in almost every species investigated, and an immunosuppressive role has been claimed for it. The marsupials are the only exception. It is ironical, however, that the one hormone which would seem essential for pregnancy – progesterone – is the least satisfactorily linked with the immune response, while the oestrogens and the gonadotrophic hormones which have to be accounted for (in human, simian and equine pregnancy) are rather an embarrassment than otherwise. Their quantity, measured in terms of activity, is so large and they so saturate the body that it would be simpler to regard them rather as products of metabolism, if such an explanation did not raise further difficulties.

Placental steroids

There are several hints that enhanced steroid production of the pregnant female has an effect on the development of homograft sensitivity. Notable among these is Medawar's (1953) discriminating analysis of the role of cortisone-like steroids in protecting the foetus from immunizing its mother.

There has been speculation that a high concentration of steroid hormones, produced locally by the invading trophoblast, prevents sensitization (Zipper, Ferrando, Saez & Tchernitchin 1966; Beer & Billingham 1971). This would clearly be most advantageous at the time of implantation and oestrogen, a known depressant of thymic function in small animals, might be thought likely to be involved. The demonstration of the steroidogenic properties of the unimplanted blastocyst of the pig (Perry, Heap & Amoroso 1973) and the strong indications of endocrine activity in the rabbit blastocyst and rat morula (Huff & Eik-Nes 1966; Dickmann & Dey 1974) focus attention on the precocious expression of trophoblast activity which could be a factor in its survival. This does not mean, however, that the participation of maternal progesterone can be lightly dismissed, since it is possible that a new test may yet reveal the true immunological activity of this endocrinologically powerful agent, long regarded as *the* hormone of pregnancy.

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Clearly, the type of evidence we have been considering, though suggestive, promises always to fall short of actual proof. However, since it is unlikely that a single factor will suffice to explain the success of the foetus as an allograft, it would be as well to keep an open mind.

Placental protein hormones

The case for the participation of gonadotrophins in overriding strong histocompatibility barriers is much stronger and centres on their role as inhibitors of lymphocyte transformation. The number of gonadotrophic hormones, so far known, that are capable of blocking the antigen receptor sites of the mother's lymphocytes and thus preventing the latter from reacting with foetal antigens is small. The best characterized and most thoroughly examined are the glycoprotein, human chorionic gonadotrophin (HCG) and the polypeptide human chorionic somatomammotrophin (HCS; or human placental lactogen, HPL), both of which are specific to the human placenta.

Human chorionic gonadotrophin

HCG is a glycoprotein of molecular mass 30000 (Got 1959; Got & Bourrillon 1960), which has no N-terminal and no G-terminal amino acids, but contains *N*-acetylneuraminic acid (NANA) and fucose, as non-reducing terminal units (Bahl 1969). It is now agreed that the presence of NANA is essential for the activity of the hormone to be expressed, as shown by its inactivation by neuraminidase treatment which releases all the sialic acid residues (Brossmer & Walter 1958). Recent chemical studies have demonstrated a dual chain structure for this hormone (Morgan & Canfield 1971). The amino acids composing these two non-identical subunits, designated HCG-α and HCG-β share many features seen in luteinizing hormone (LH) from bovine and ovine sources.

The human zygote apparently synthesizes HCG at a very early date and the hormone has been detected in the serum by specific radioimmunoassay as early as 8–10 days following probable ovulation (Wide 1969; Kosasa, Levesque, Goldstein & Taymor 1973). This is associated with the appearance of distinct trophoblastic lacunae in the early implanted blastocyst in open communication with maternal venous channels (figure 1, plate 10). It is not unreasonable to suppose that the hormone would be detected earlier at the site of its production (see below), that is to say, in the maternal tissues intimately associated with the peripheral trophoblast and within the trophoblastic lacunae before they open into the venous sinuses. Saxena, Hasan, Haour & Schmidt-Gollwitzer (1974) have shown that by using a radio-receptor procedure, pregnancy could be detected by assaying the gonadotrophin in plasma samples obtained from day 6 to 8 after conception in the human female, i.e. before the trophoblastic lacunae communicate with the maternal circulation. The source of this gonadotrophin-like substance in the blood similar to HCG or LH as early as day 4–6 is unknown. It appears to be specific for early pregnancy and may be secreted by the blastocyst; or a signal emanating from the blastocyst may sustain high levels of pituitary LH in early pregnancy until placental secretion of HCG is established.

Measurable amounts of HCG are thus excreted at the time of implantation, following which the hormone concentration rises sharply to a peak during the second and third lunar month, with an abrupt decrease thereafter to relatively constant values for the remainder of pregnancy. It is rapidly eliminated from the body after delivery, after spontaneous or therapeutic abortion, or cure of any of the pathological conditions with trophoblastic tumours (Hertz *et al.* 1959).

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Gestational choriocarcinoma is a unique malignancy in that it arises from the trophoblast of the placenta and, like the foetus, contains genetic material of paternal as well as maternal origin. It thus constitutes an allogenic graft in the maternal host, and rejection would be expected. Evidence now at hand suggests that gestational carcinoma develops, invades, and disseminates in the maternal host despite clear incompatibility with respect to strong transplantation antigens demonstrated both by leucocyte serotyping and by another *in vitro* test of histocompatibility, the mixed-leucocyte culture. The mixed-leucocyte reactivity further shows that the maternal lymphocytes can recognize these antigenic differences and respond to them *in vitro* (Rudolph & Thomas 1971). If this be true, the results are not consistent with the hypothesis that the survival and dissemination of postgestational choriocarcinoma depends upon histocompatibility between the malignant graft and the maternal host. Hence alternative mechanisms must be postulated. It is now well accepted that HCG estimations are a reliable and sensitive index for monitoring the response of patients to therapy. It is thus possible that the significant clinical remissions following methotrexate chemotherapy (Hertz *et al.* 1959) may yet be shown to be causally related to the dramatic fall in the levels of HCG that are reported.

Human placental lactogens

Human chorionic somatomammotrophin (HCS) is a polypeptide protein of molecular mass 20 000 which has only been found during pregnancy, and its production by the placenta appears to be independent of the presence of the pituitary or a foetus (Josimovich & Brande 1964; Josimovich & Astwood 1964). The hormone is secreted primarily into the maternal circulation and only small amounts are found in umbilical cord blood (Kaplan & Grumbach 1965). HCS is detectable consistently after six weeks of gestation, but has been demonstrated as early as the second week. The rising serum concentration, which reaches a peak after 34 weeks of gestation, is followed by a significant drop before parturition and before spontaneous abortion (Saxena, Emerson & Selenkow 1969; Saxena 1971).

The concentration of HCS in maternal serum in late pregnancy, as measured by immunoassay, reaches levels higher than that of any other known protein hormone at any time and may be a thousand times that of growth hormone (Grumbach, Kaplan, Sciarra & Burr 1968). For this reason its measurement becomes relatively easy, and it has been suggested that its level in maternal serum may be a sensitive and practical indicator of placental function and foetal well-being. As with human chorionic gonadotrophin, HCS disappears very rapidly from the maternal serum after removal of the placenta; half of it is gone after 15 min and 90 % in 2 h (Kaplan, Gurpide, Sciarra & Grumbach 1968; Beck and Daughaday 1967). It has been calculated that only about 0.001 % of the estimated production of HCS is excreted in the urine of pregnant women. This may be the result of rapid metabolic degradation and might well be regarded as a safety mechanism which prevents over-saturation with the hormone.

Immunosuppressive role of HCG and HCS

As late as 1944 there were, surprisingly enough, no clearly demonstrable effects of HCG on mother or foetus, and Levin, commenting on the state of affairs then prevailing, remarked that 'until we have more evidence, it may be just as well to consider the appearance of HCG as a gift of nature to make easier the obstetrician's task in the diagnosis of early pregnancy. This much we know is true'. In retrospect, this sweeping statement does not seem justified, yet the

continued secretion of HCG during pregnancy and long after the time when the corpus luteum has lost its endocrinological importance remains a puzzling fact, and its physiological significance during the later stages of pregnancy is not fully understood. In taking stock of the present position it will become apparent why a straightforward statement cannot be made.

Simply because of their sheer quantity, HCG and HCS have come under suspicion as fulfilling an important role in protecting the mother from developing a state of transplantation immunity to the alien histocompatibility antigens of her foetus. The main theme in a large number of investigations (e.g. those of Kaye & Jones 1971; Kaye, Jones & Ing 1972; Jenkins, Acres, Peters & Riley 1972; Kaye 1973*a*, *b*; Contractor & Davies 1973; Adcock *et al.* 1973) dealing with this aspect of foetal survival has been the evaluation of the nature of the two hormones as regards their ability to modify the transformation (proliferation) of lymphocytes that occurs when they are confronted with the standard laboratory antigen, phytohaemagglutinin (PHA).

Basically what was found was that when PHA was added to lymphocytes together with HCG or HCS, the lymphocytes' transformation was significantly less than when PHA was given alone (Contractor & Davies 1973); in the case of HCG the effect was both reversible and without cytotoxicity (Adcock *et al.* 1973; Marz, Bahl & Mohn 1973). This shows that the hormones can indeed suppress this particular immunological reaction. HCS had about twice the suppressive action of HCG per unit mass and the effects of the hormones are reasonably specific.

However, that result could indicate that the hormones were masking the lymphocytes by competing with the PHA for their receptor sites or that they were simply reacting with PHA and preventing it from exerting its mitogenic action (Powell 1974). The latter action – simply knocking out a particular antigen by reacting with it – would obviously be much less far-reaching in its implications than the former. The proof that the hormones compete with the PHA lies in the essentially complete inhibition (up to 100%) of the lymphocyte activation when either hormone was added to the lymphocytes before the addition of the lectin. In other words, the hormones were far more effective when allowed to get at the lymphocytes before the PHA was added, suggesting that their main effect was indeed on the lymphocytes, and not on the antigen (Contractor & Davies 1973). Adcock *et al.* (1973) had tentatively suggested that the foetus is accepted because HCG represents trophoblastic surface antigen and blocks the action of maternal lymphocytes. The facts, such as they are, do not support this view. Subsequently, Adcock (1974) agreed that the effect of HCG was likely to be a direct one upon the lymphocytes although the earlier data presented by Adcock *et al.* (1973), would certainly not have permitted them to make that distinction.

That HCG also inhibits mixed lymphocytes in culture (this involves stimulation of lymphocytes by foreign lymphocytes rather than PHA) has been demonstrated beyond cavil (Kaye & Jones 1971; Jenkins *et al.* 1972). Using the mixed lymphocyte reaction, Jenkins and his associates showed that HCG, in concentrations which are achieved in the serum in early pregnancy, will inhibit an immunological system dependent on antigen recognition; the hormone is presumably present in yet higher concentrations at its site of release in the placenta. Kaye & Jones (1971) and Jenkins *et al.* (1972) further suggest that a similar immunosuppressive role may be exercised *in vivo* and may be particularly important in protecting the foetal allograft in early pregnancy. There is also strong circumstantial evidence (see below) for thinking that the continued secretion of HCS, best-known for its somatomammotrophic properties, is a

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further placental specialization for subduing the immunological reactions of the mother or protecting the foetus from the ravages of marauding maternal lymphocytes during the later stages of gestation. This idea, though it lacks factual support, has the merit of proposing for the first time the working of a factor that is peculiar to primate pregnancy.

Equally far-reaching are experiments of Borland and his co-workers (Loke *et al.* 1971; Borland *et al.* 1974), who analysed the killing effect of rabbit lymphocytes sensitized against trophoblast target cells and showed that human trophoblast cells were not susceptible to this killing action. If, however, the HCG layer around the trophoblast cell was first removed by enzymatic action then they were killed by the sensitized lymphocytes. Where, on the other hand, such trophoblast cells were treated with HCG after exposure to the enzyme, their resistance to specific lymphocyte damage was restored. It is also a matter of some importance that HCG has the same effect upon transplanted human trophoblast cells in guinea-pigs; treatment of trophoblast cells with neuraminidase resulted in the loss of their immunological immunity, but subsequent treatment of the cells with the hormone restored the protection (Borland *et al.* 1974).

On the assumption that HCG and HCS do indeed inhibit the maternal lymphocytes, then presumably – since they circulate in the maternal blood – they should suppress other maternal cell-mediated immune responses and not simply those directed at the foetus. The available evidence points in that direction. There are, for example, the experiments of Lewis *et al.* (1966) and those of Jenkins & Hancock (1972), who demonstrated reduced mixed lymphocyte reactivity between maternal and paternal lymphocytes during pregnancy, while mixed lymphocyte reactivity between maternal and unrelated male donor cells was unaffected. More recently Jones & Curzen (1973) have shown that mixed lymphocyte reactivity between cells from unrelated pregnant women is less than that between cells from unrelated non-pregnant donors. However, they were careful to point out that while the results of Lewis *et al.* (1966) and those of Jenkins & Hancock (1972) suggest that the reduction is specific only for stimulation by paternal histocompatibility factors, their own results imply that there must also be some non-specific reduction of maternal lymphocyte reactivity. Other evidence that maternal lymphocyte reactivity is reduced during pregnancy is provided by Finn *et al.* (1972) and by Purtilo, Hallgren & Yunis (1972), who reported that PHA-induced lymphocyte transformation is significantly less in pregnant than in non-pregnant women.

Placental protein hormones in other mammals

The question which must now concern us is whether, in general, the placenta of eutherian mammals secretes gonadotrophic and lactogenic substances which lend it similar properties to that of pituitary tissue. It must be said at once that it is gradually becoming apparent that enhanced secretion of gonadotrophins during pregnancy is of much more general occurrence than was formerly thought. There is a good deal of information pointing more and more to the placental trophoblast as a source of these hormones, 'but' as Newton (1938, p. 419) put it, 'the unifying conception which would make everything fall into place is [still] elusive'.

HCG-like hormones

Non-human primates

Our knowledge of placental gonadotrophin production in non-human primates, though limited to a small number of species, suggests that chorionic gonadotrophins are a regular

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accompaniment of pregnancy in Old World monkeys, and although one may cite references that alternately affirm (Hampton, Levy & Sweet 1969; Castellanos & McCombs 1968) or deny (Hobson 1971) that this is true of some New World monkeys, the balance of evidence indicates that this may indeed be so. Chlorionic gonadotrophin has been detected in blood or urine of the macaque (rhesus, and stumped-tailed monkey), baboon, chimpanzee, and gorilla at about the expected time of implantation (for references see Tullner 1974), and in the urine of New World marmosets and squirrel monkeys as early as the second or third week of pregnancy (Hampton *et al.* 1969; Nathan, Rosenblum, Limson & Nelson 1966).

Among the catarrhine monkeys chorionic gonadotrophin (MCG) has been most extensively studied in the rhesus monkey (*Macaca mulatta*). The hormone has been reported in the uterine venous blood of this species as early as the eighth or ninth day of pregnancy, which is about the time of implantation and coincides also with the time the trophoblastic lacunae communicate with the maternal circulation (Meyer 1972). As with HCG, serum and urinary levels of MCG rise rapidly with highest concentrations from the 18th to 25th day of gestation, falling rapidly thereafter. Although the hormone was not measurable in serum beyond 40 days (Hodgen, Vaitukaitis, Ross & Tullner 1974), Hobson (1972) reported small amounts throughout pregnancy by bioassay of 96 h pooled urine samples, following the rapid decline from peak levels. These results are at variance with those of Hodgen, Dufau, Cott & Tullner (1972) who failed to detect the hormone in blood or urine in late pregnancy, but these investigators did not employ pooled samples of urine. At the moment it seems inadvisable, in view of these results, coupled with the transient appearance in quantity of hormone in the macaque, to stress the negative results which have been reported for other apes and monkeys. In the case of large animals it is possible that sufficiently systematic and continuous blood and urine examinations have not been made, while in small animals the quantities of hormone may be too little for detection, or may, as in the macaque, appear in quantity for only a very brief period.

The specific identification of chorionic gonadotrophin in tupaiids or other insectivorans, strepsirhines (lemurs, galagos), or *Tarsius*, remains still to be accomplished. On morphological evidence, however, there is at least a suspicion that developmental structures, peculiar to implantation in these species, such as the gland-free endometrial pads of *Tupaia*; the trophoblastic plaques of *Loris*, regarded by Butler (1967) as remnants of a temporary attachment of giant cell trophoblast; the localized, but transient, invasive giant cell trophoblast of *Galago senegalensis*; and the nodular mass of *Tarsius*, believed by Hill (1932) to be of trophoblastic derivation (but denied by Luckett 1971) may all represent chorionic specializations genuinely involved in placental endocrine activities resembling those of the specialized endometrial cups of the mare (see figure 3, plate 11).

Other mammals

Soon after the discovery by Aschheim & Zondek (1927) of a gonadotrophic substance in the blood and urine from women in early pregnancy (*A–Z* test), Philipp (1929) reported that the domestic pig's placenta gave a positive *A–Z* reaction; Menzani & Gentile (1934) reported positive though irregular tests from the urine of cows between the 38th and 150th day, and the existence of a gonadotrophic substance in the serum of the pregnant mare (Cole & Hart 1930; Zondek 1930; Catchpole & Lyons 1934; Rowlands 1938) is well established. The pregnant mare, sow and cow (and the giraffe, see below) show that the production of gonadotrophic hormone during pregnancy is not confined to animals with a haemochorial placenta.

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A gonadotrophic substance has been demonstrated also in the placenta of rats (Pencharz & Long 1933; Astwood & Greep 1938; Astwood 1941; Averill, Ray & Lyons 1950; Ray, Averill, Lyons & Johnson 1955; Mathies 1967), and mice (Newton & Beck 1939; Cerruti & Lyons 1960) and a luteotrophic function has been claimed for it. Evidence of an early luteotrophic ('anti-luteolytic') effect of the conceptus in the sheep has been furnished by Moor & Rowson (1966), through experiments in which conceptuses had been removed at various times after mating. It has been shown also that the urine of the pregnant giraffe contains a gonad-stimulating substance (Wilkinson & de Fremery 1940) whose elimination from the urine ceases before term (Amoroso 1955). As judged by the extensive follicular development and luteinization of the foetal ovaries, the biological properties of this hormone appear to be closer to those of HCG than to PMSG (see below).

There is some evidence for the secretion of LH-like material by the rabbit blastocyst as measured by radioimmunoassay (Fujimoto, Woody & Dukelow 1973), and a gonadotrophin similar to HCG or LH has been detected in the blastocyst fluid by a radio-receptor assay as early as $4\frac{1}{2}$–$5\frac{1}{2}$ days after ovulation (Haour & Saxena 1974). The concentrations of the HCG- or LH-like hormone in the blastocyst fluid were tenfold higher than that in the blood of pregnant rabbits at a corresponding stage of pregnancy. At present, however, it would be stretching a point to suggest that the hormone is secreted by the peripheral trophoblast of the blastocyst wall, since an HCG- or LH-like material was also detected in the uterine fluid.

There is some circumstantial evidence to suggest the occurrence of a gonadotrophin in other mammals, e.g. the guinea-pig (Davies, Dempsey & Amoroso 1961), and nilgai (*Boselaphus*) (Amoroso 1955), and possibly the African elephant (Perry 1953; Amoroso 1955), but whether or not the biological activity of the postulated gonadotrophins is similar to HCG or the luteotrophic principle in the rat's placenta has not been established.

Placental lactogen in various animals

The monkey placenta secretes a material (MCS) which, like HCS, combines prolactin-like activity with some of the biological characteristics of a growth hormone (Grant, Kaplan & Grumbach 1970). There is some suggestion also that the foetal component of the cotyledons of the goat's placenta produces a lactogenically active material which was not detectable by a radio-immunosasay for pituitary prolactin (Forsyth 1972). This deduction was based on co-culture experiments of foetal and placental tissues from goats during the second half of gestation with explants of mammary parenchyma from strain A mice. Kelly, Robertson & Friesen (1974) have described the production of a placental lactogen by the sheep's placenta.

The presence of a substance, or substances, possessing mammogenic, lactogenic, luteotrophic and crop-stimulating properties has been detected in the rat placenta, although it is as yet uncertain whether this material is identical with prolactin (Astwood & Greep 1938; Averill *et al.* 1950; Canivenc 1952; Canivenc & Mayer 1953; Ray *et al.* 1955; Matthies 1967, 1968; Cohen & Gala 1969; Shani, Zimbelman, Khazen & Sulman 1970); there is also evidence for the presence of a somatotrophin-like substance in rat placenta. Compared with pituitary mammotrophin (prolactin), the rat placenta is potent in regard to its luteotrophic, mammotrophic and lactogenic properties, but weak in its crop-stimulating activity. In the mouse placenta a prolactin-like substance has been detected (Cerruti & Lyons 1960; Kohmoto & Bern 1970), while a haemagglutination-inhibition method has been used to demonstrate material cross-reacting with HCS in placental fractions of the monkey, rat, dog, pig, horse

sheep, rabbit and cow (Gudson, Leake, vanDyke & Atkins 1970). Considered in the light of the generally accepted view of the existence of a prolactin-like hormone in many species of lower vertebrates, these observations remind us that endocrinological control evolves by the adoption of new functions by existing agents rather than the emergence of new hormones to fulfil specific roles.

Pregnant mares serum gonadotrophin (PMSG)

PMSG was discovered independently by Cole & Hart (1930) and Zondek (1930) in the serum of pregnant mares, but it was not until 13 years later that Cole & Goss (1943) provided good evidence that the hormone is secreted by the endometrial cups (figure 3, plate 11) the trophoblastic origin of which is now well established (Allen & Moor 1972; Allen *et al.* 1973). As early as 1934, however, Catchpole & Lyons had reported finding the hormone in the allantochorion, and like Glud, Pederson-Bjergard & Portman (1933), drew attention to the fact that it suddenly appears in the blood at the time when the maternal and foetal tissues first make intimate contact. The sudden appearance and rise in concentration of the hormone in the serum occurs between the 35th and 41st days of pregnancy, and after reaching a peak at about 70 days it drops precipitately, disappearing from the circulation gradually after about 120 days (Allen 1969*a*).

PMSG has the highest carbohydrate and sialic acid content among gonadotrophins; glucose seems to be present in small amounts. The hormone exhibits two types of gonadotrophic activity, FSH-like and LH-like, but all attempts to separate these two activities have failed (Legault-Démare & Clauser 1961). However, for the full expression of its biological activity, the integrity of the carbohydrate moiety of PMSG seems to be necessary, since the hormone can be totally inactivated by digestion with neuraminidase (Walter & Brossmer 1966).

Description of plate 12

Figure 5. Human. Surface view of a terminal villus and a syncytial sprout from an 11 week old placenta. Straight and bulbous microvilli project into the intervillous space. In many places the microvilli are matted in appearance. Magn. ×2750. (From Dempsey & Luse, 1971.)

Figure 6. Human. Indirect immunoreaction with PO for the visualization of HCS. Villi of a 5-month placenta fixed in AFA and imbedded in paraffin. The reaction (black on the print) is localized exclusively in the syncytial cytoplasm. The nuclei are always negative. Note the scalloped appearance of the positively reacting syncytium due to the non-reaction of very numerous Langhans' cells (L). S, syncytiotrophoblast. Magn. ×280. (From de Ikonicoff & Cedard 1973.)

Figure 7. Human term placenta fixed with glutaraldehyde followed by osmium tetroxide and stained with non-immune rabbit serum in place of anti-HCG rabbit serum. In this control preparation the apical plasma membrane covering the microvilli (mV) and the ergastoplasmic cisternae (e) lack deposits of peroxidase reaction production (cf. figure 8). Some large cytoplasmic granules are the only structures that contain dense deposits indicative of intrinsic peroxidase. Other similar structures (arrows) reveal light or no deposits. Magn. ×17000. (Reproduced from Dreskin, Spicer & Green 1970.)

Figure 8. Human term placenta fixed with glutaraldehyde followed by osmium tetroxide and stained with rabbit antiserum to HCG. A portion of syncytiotrophoblast displays electron opaque deposits of peroxidase reaction product indicative of immunostaining for HCG. These deposits, covering the outer or maternal surface of the apical plasma membrane and filling cisternae of ergastoplasm, have a finely particulate character. The deposits of reaction product appear to show a fine periodicity on the surface of the apical plasma membrane and tend to concentrate on the luminal surface of the rough endoplasmic reticulum. Caviolae at the apical plasma membrane are immunostained. Thick annular profiles of immuno-reactive material, bridging between microvilli, are possibly related to ghostlike outlines observed in morphological preparations. The outer surface of the folded basal plasma membrane discloses light immunostaining. Peroxidase reaction product is not evident in profiles presumed to represent the small variety of cytoplasmic granules, or in Golgi elements (g) mitochondria (m) or basement membrane (bm). Magn. ×28000. (Reproduced from Dreskin *et al.* 1970.)

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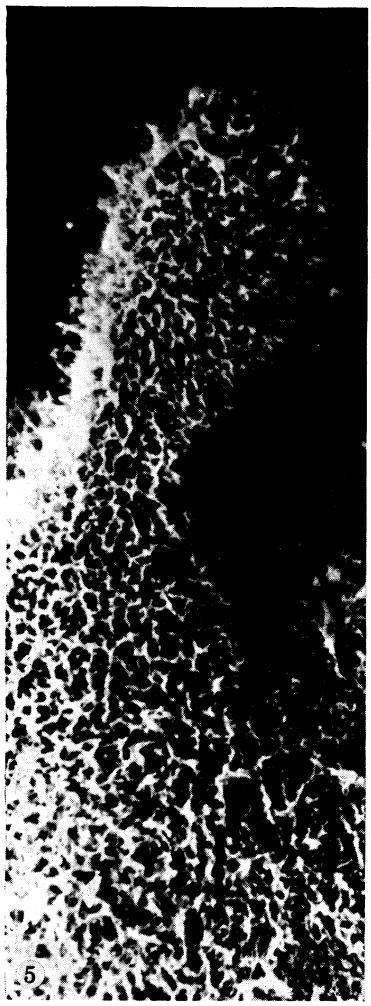

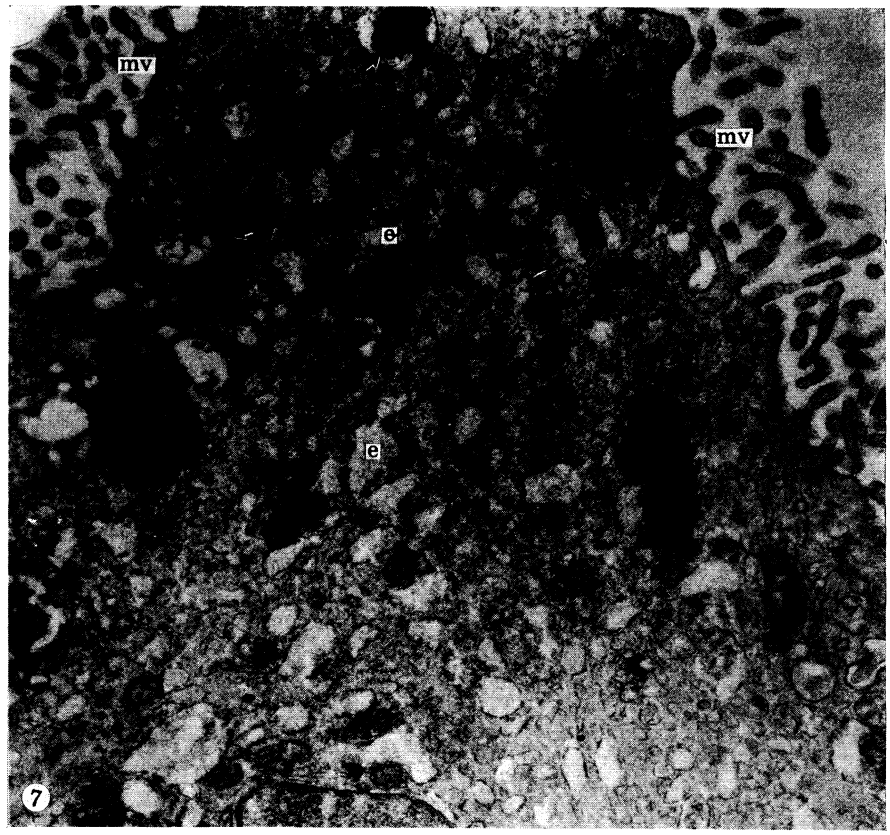

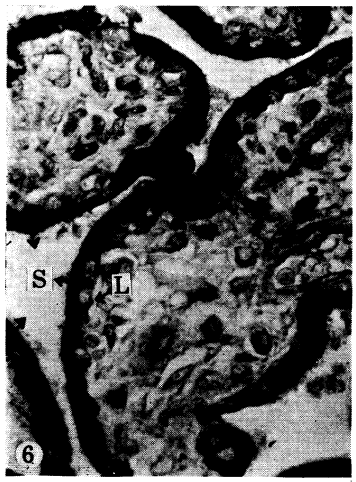

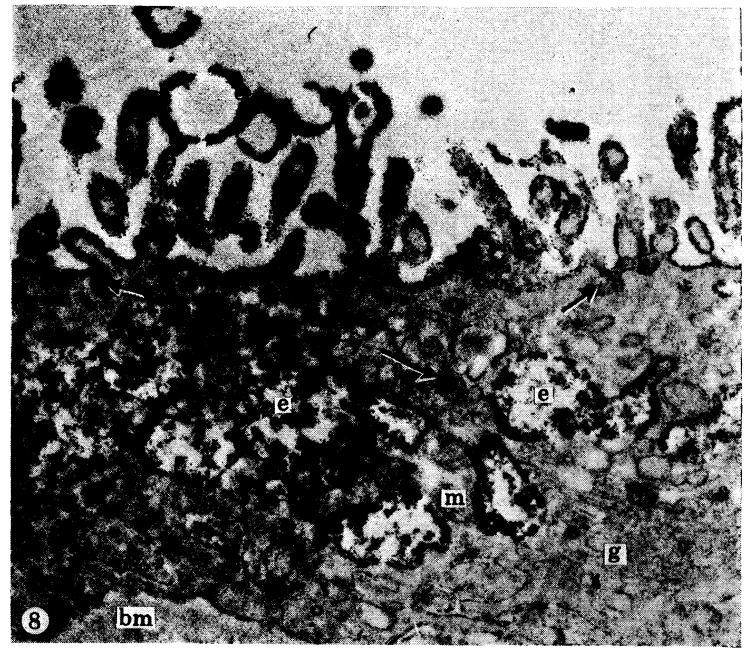

FIGURES 5–8. For description see opposite.

(*Facing p.* 354)

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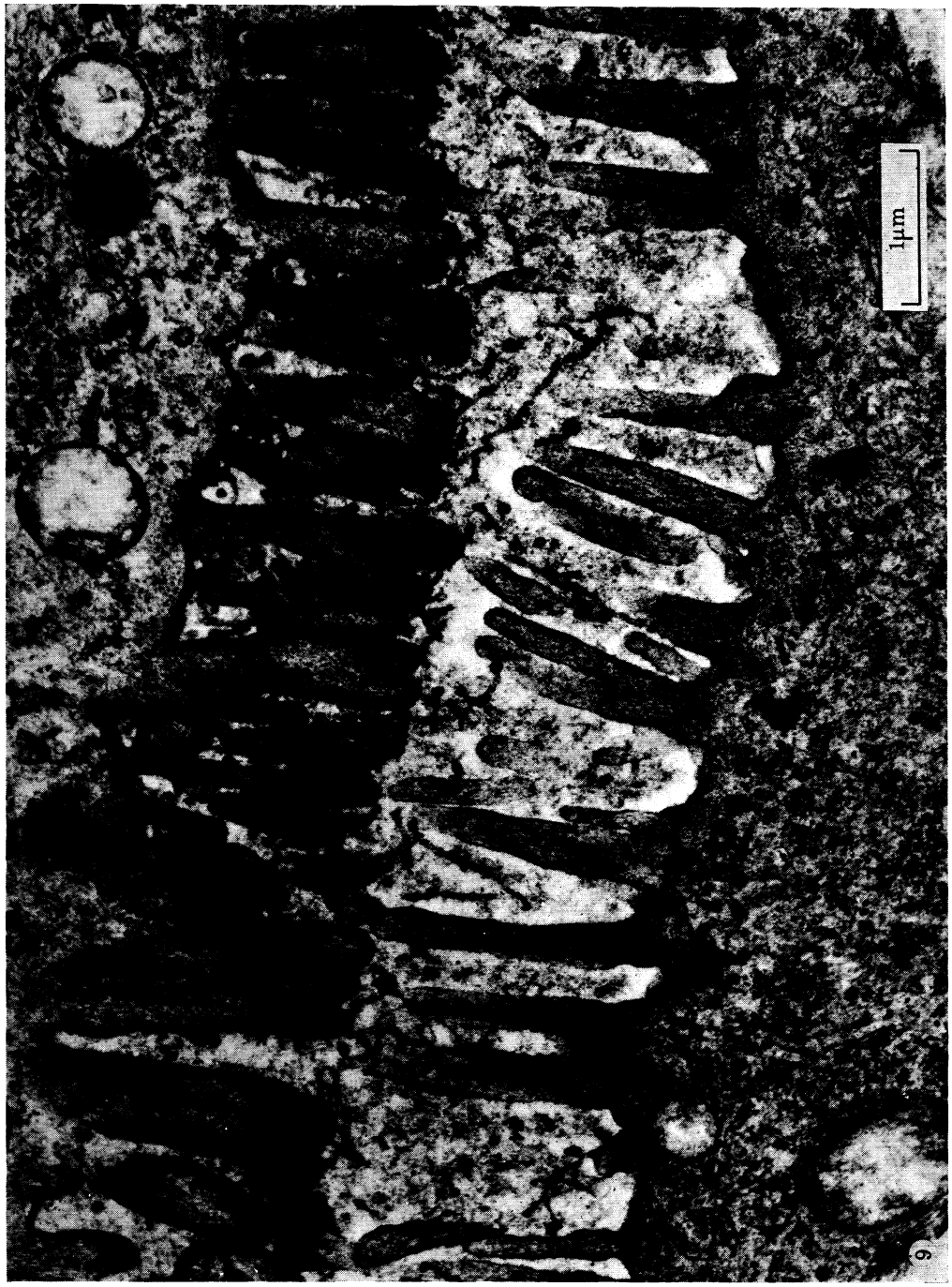

FIGURE 9. Artificial separation of the placenta of the cow at 265 days gestation. The darkly staining cell membrane of the foetal side of the placenta contrasts with the lightly staining membrane of the maternal side. Magn. ×45000.

(By courtesy of Dr D. H. Steven.)

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Although there is, as yet, no direct evidence that PMSG inhibits lymphocyte transformation, the possibility must be considered because of the dramatic effect of the hormone in inducing thymic atrophy (Evans & Simpson 1934) in intact but not in gonadectomized rats.

Gonadotrophins with similar properties to those of PMSG have also been detected in the serum of the donkey (Samodelkin 1940; Zondek & Sulman 1945; Calisti & Oliva 1955; Oliva & Chiechini 1961), zebra (Zondek 1935) and fallow deer (Unterberger 1932) and there is a high probability that the placenta of the rhinoceros also secretes such a hormone. A series of experiments on hybrid donkey and horse pregnancies brings out the point that the genotype of the foetus may influence hormone production; the concentrations of PMSG in mares carrying a mule foetus were much lower than in mares carrying a horse foetus, or in donkeys with donkey foetuses, whereas donkeys carrying a hinny foetus had the highest concentrations of all (Bielanski, Ewy & Pigoniowa 1955; Clegg, Cole, Howard & Pigon 1962; Allen 1969*b*). The reasons for these differences remain obscure.

The localization and site of synthesis of HCG and HCS

Localization

Perhaps the most obvious route by which the human mother might become sensitized against the trophoblast or other foetal tissues is through direct contact between maternal blood and the syncytial covering of the chorionic villi. The actual degree of contact would appear to be more than sufficient to provide generous amounts of antigen to the mother (Amoroso 1952); and when one takes into account the vast increase in surface area contributed by the microvilli of the syncytial brush border as revealed by the electron microscope, the total surface of the placenta must be very considerable indeed. The tremendous area of the free surface of the microvilli that is exposed to the circulating blood is best appreciated by examining a scanning electron micrograph of this surface (figure 5, plate 12). If the gonadotrophins are to act as an effective immunological barrier in the human placenta they should be demonstrable as a continuous investing layer wherever trophoblast is exposed to maternal tissues, as in the intervillous space and basal plate (cytotrophoblastic shell).

There is now fairly general agreement that the synthesis of HCG and HCS is dependent upon the presence of chorionic tissue, but whether in the syncytiotrophoblast of chorionic villi exclusively or also in the cytotrophoblast of Langhans cells remains equivocal. There is much evidence, from immunofluorescent studies, in favour of production of HCG and HCS by the syncytiotrophoblast (Midgley & Pierce 1962; Sciarra, Kaplan & Grumbach 1963; Thiede & Choate 1963; Currie, Beck, Ellis & Read 1966; Beck, Gordon, Donald & Melvin 1969; Mason *et al.* 1969; de Ikonicoff and Cedard 1973), but there is sure evidence also that HCG cannot all be so derived.

A dual origin of the hormone from the cytotrophoblast and syncytiotrophoblast in both immature and mature placentae is suggested by the immunofluorescent studies of Thiede & Choate (1963). They showed its presence throughout the trophoblast (i.e. in the syncytium, in Langhans cells and in the cell islands and cell columns) of the immature placenta but were unable to detect HCG in the Langhans cells of the term placenta. These results are not, however, in entire agreement with the more recent studies of de Ikonicoff & Cedard (1973) with the light microscope (figure 6, plate 12). Using both the indirect and direct method of labelling with peroxidase, they failed to detect either HCG or HCS in Langhans' cytotrophoblast from

six weeks of gestation to term; positive reactions for the hormones were found exclusively in the syncytial cytoplasm of the chorionic villi in all placentae studied. In the present state of doubt, one might say confusion, about the production of HCG by the Langhans cells of the chorionic villi of the placenta, it is important to realize that many other investigators (e.g. Wislocki & Bennett 1943; Dempsey & Wislocki 1945; Wislocki 1951; Thomsen & Willemsen 1959; Dallenbach-Hellweg & Nette 1963; Boyd & Hamilton 1970) find HCG predominantly in the cytotrophoblastic cells of the basal plate (cytotrophoblastic shell), in the placental septa and in the cell islands, with little in the Langhans cells. While Wilkin & Oosterwijck (1967) believe that the syncytiotrophoblast is the main if not the only site of production of HCG, they do, however, add that the cytotrophoblast of the basal plate, columns, islets and septa may participate in the production of the hormone.

In this connexion it may perhaps be well to recall evidence advanced in favour of the actual production of gonadotrophic hormone by the placenta, on the basis of tests of the decidua and basal plate in abortions and abnormal types of pregnancy. Philipp & Huber (1936) argued that if the placenta picks up the hormone from the blood stream, then there should be a sharp transition between the decidual and placental concentrations owing to the selective action of the placenta. This is not found. The decidua near the placenta shows a relatively high concentration, while that which is remote, i.e. in the non-pregnant horn of a bicornuate uterus, or the endometrium in tubal pregnancy, contains none. The simplest explanation is that the hormone is issuing from, and not going into, the placenta (basal plate). This evidence is clearly not conclusive, since other explanations are possible, but it remains the best hypothesis.

The careful studies of Loke & Borland (1970) using monolayer cultures, suggest that young cytotrophoblast cells may be involved in the production of HCG, thus confirming prior findings established by means of tissue culture (Sengupta 1935; Gey, Seegar & Hellman 1938; Jones, Gey & Gey 1943; Stewart, Sano & Montgomery 1948). It is an interesting commentary that in every instance where tissue culture techniques have been used to identify the source of HCG, it is cytotrophoblastic cells that have been implicated. On the basis therefore of the presumed derivation of the syncytiotrophoblast from cytotrophoblast, as revealed by both autoradiographic and electron microscopic studies (Midgley, Pierce, Deneau & Gosling 1963; Pierce, Midgley & Beals 1964; Enders 1965) the occurrence of the hormone in the cytotrophoblast of Langhans cells in the immature placenta (Thiede & Choate 1963) may be tentatively interpreted as indicating that the production of HCG may start in the cytotrophoblast somewhat before its transformation into syncytiotrophoblast (Benirschke & Driscoll 1957). It is perhaps well to remember also that the cytotrophoblastic cells of the terminal villi are relatively undifferentiated, in keeping with their primary role as a germinal source for the syncytiotrophoblast, and that these cells are considerably reduced in late stages of pregnancy and are frequently absent in places (Amoroso 1952; Burgos & Rodriguez 1966; Boyd & Hamilton 1967). Another possible link with transitional cytotrophoblast centres around the production of large quantities of HCG in choriocarcinoma (Patillo, Gey, Delfs & Mattingly 1968). It is probably unsafe, however, to draw conclusions about the normal placenta from its most extreme pathological derivative.

Synthesis

In two recent studies, Dreskin *et al.* (1970) and Hamanaka *et al.* (1971) have traced, by immunofluorescent techniques (PO) applied to electron microscopy, newly synthesized HCG in the syncitium of full-term human placentae. Their interpretation of the results is that

HCG is synthesized on the ribosomal-studded membranes of the syncytiotrophoblast and migrates via the dilated cisternae of the ergastoplasm to the rough coated vesicles of the apical plasma membrane (figures 7 and 8, plate 12). Presumably, at this stage, the HCG molecule is about to leave the cell – possibly through some concentration site such as the Golgi apparatus (Hamanaka *et al.* 1971; but this route is denied by Spicer and his co-workers, Dreskin *et al.* 1970; Martin, Spicer & Smythe 1974) – and through unknown forces in part adheres to the surfaces of the syncytial microvilli and adjacent caviolae of the apical plasma membrane. Attachment of the hormone to the surface of microvilli and adjacent caviolae represents the terminal event in its localization, so this sialomucin coat could significantly interfere with the immune reaction. The mechanism for the binding of the hormone to these sites remains unknown, and there is as yet no adequate model which can be used to describe polyelectrolyte behaviour (Noda, J., Tzuge, T. & Nagasawa, M. 1970 cited by Gibbons 1972). Nevertheless, the sequence of events obviously suggests a convenient design for concentrating the hormone on the surface of the chorionic membrane. In this situation, however, it is conceivable that the hormone could be carried off rapidly by the maternal blood which (in haemochorial but not in epitheliochorial placentae) washes over the chorionic epithelium. It is suggested, therefore, that the local concentration is maintained by enhanced secretion of the hormone, and that the 'overflow' into the urine is a natural consequence of the excessive quantities of gonadotrophin produced.

The only inquiries as to the origin of chorionic gonadotrophin in non-human primates are the ultrastructural studies in the rhesus monkey by Pierce *et al.* (1964) and Luckett (1970). Their findings support the view that chorionic gonadotrophin is a product of the syncytiotrophoblast. Taking into account the nearly identical pattern in the fine structure of the terminal villi of the rhesus monkey (Pierce *et al.* 1964; Luckett 1970) and human (Boyd, Boyd & Hamilton 1968), it may be presumed that the whole process of synthesis is similar in the two species. The syncytiotrophoblast also appears to be involved in the synthesis or metabolism of steroid hormones, but the cytological pathway for this process is unclear (Luckett 1970).

The immuno-hormonal complex

On the basis of their results, Contractor & Davies (1973) have proposed a hypothesis that might explain the acceptance of the blastocyst by the uterus in human pregnancy. They emphasize the fact that two of the many interesting features of the implanting blastocyst are that it becomes surrounded by lymphocyte-like cells (figure 1, plate 10) and that it soon acquires the ability to secrete HCG, and shortly thereafter HCS. They conclude that the mechanism for non-rejection may be under the control of these placental polypeptide hormones, and they further suggest that a drop in hormone output could trigger parturition by removing protection from the foetus. Serum HCS concentrations fall quite significantly before spontaneous abortion, a fact that could be taken to suggest that these abortions also constitute an immuno-rejection process, resulting from loss of control over the (normal) cell-mediated response to the allograft. This extension of the hypothesis, to cover parturition and abortion as well as implantation and survival, is open to the objection that parturition is generally normal among intensively inbred individuals and their genetically identical offspring.

In considering the points of view of Kirby and his co-workers (Kirby *et al.* 1964, 1966; Bradbury *et al.* 1965, 1969) and those of Currie *et al.* (1968) together with those expressed in

the present account, this much can be said by way of harmonizing them. They all postulate the existence of a peritrophoblastic layer of sialo-mucopolysaccharide material which fulfils an immunological masking or concealing role. Beyond this, however, it would seem that specific hormones may suppress the immunological reactions between mother and foetus. Immunological tolerance and 'enhancement', on the other hand, no longer seem to have the importance once ascribed to them in this regard. 'Enhancement' requires that both circulating antibodies and sensitized cells in the immunized recipient are in direct contact with the antigens of the graft, and in normal pregnancy few maternal lymphocytes colonize the foetus. Edwards (1972) considers that this precludes the possibility that 'enhancement facilitation', in the accepted sense, plays an important part in protecting the foetus against rejection.

These opinions, in effect, bring us back to the sialoglycoprotein hormones and the ways in which they could exert an effect. HCG and HCS are regular components of the viscous sialomucoproteins secreted by, and covering, the surface of the human trophoblast throughout its extent – i.e. where the trophoblast adjoins the basal decidua or is bathed by circulating maternal blood in the intervillous space. They are thus appropriately placed to modify profoundly immune reactions of the type under consideration.

The specific identification of glycoprotein hormones in the trophoblast of the early conceptus, in mammals other than the higher primates, is avowedly imperfect but, as we have shown, chorionic gonadotrophins have been described in a number of species. Indeed, there is reason to suppose that the placentae of all eutherian mammals produce a luteotrophic principle at the time of implantation (Amoroso 1965, 1968) and although it is unlikely that immunological protection of the foetus depends on an identical mechanism throughout the group, it is probable that it evolved by the exploitation of existing properties of the chorion. A complex mosaic of functional units must exist at the glycocalyx of the trophoblast; some of these units may be identified with the export of the steroid and protein hormones that play so prominent a part in gestation, and with the acidic mucopolysaccharide and hyaluronic acid recently shown to be present on the maternal surface of the human placenta (Martin *et al.* 1974). The development of the chorion in placental mammals is all the more impressive when one considers that in origin it was a thin non-glandular membrane that served the respiratory requirements of embryos living within the confines of a shelled egg. The process whereby the chorion took on steroidogenic and gonadotrophic activities may well have been the initial adaptation leading to the failure of the immunological mechanisms of the mother to identify and reject the foetus in the same way as other foreign material.

Note added in proof (April 1975)

Recent work by L. D. Wiley (1974 *Nature, Lond.* **252**, 715–716) suggests the occurrence of a gonadotrophin on the surface of the morula of the mouse, prior to implantation, after superovulation with HCG. Wiley used an antiserum to HCG, applied to the embryos *in vitro*, and indirect immunofluorescence. He postulates an immunosuppressive role for the hormone.

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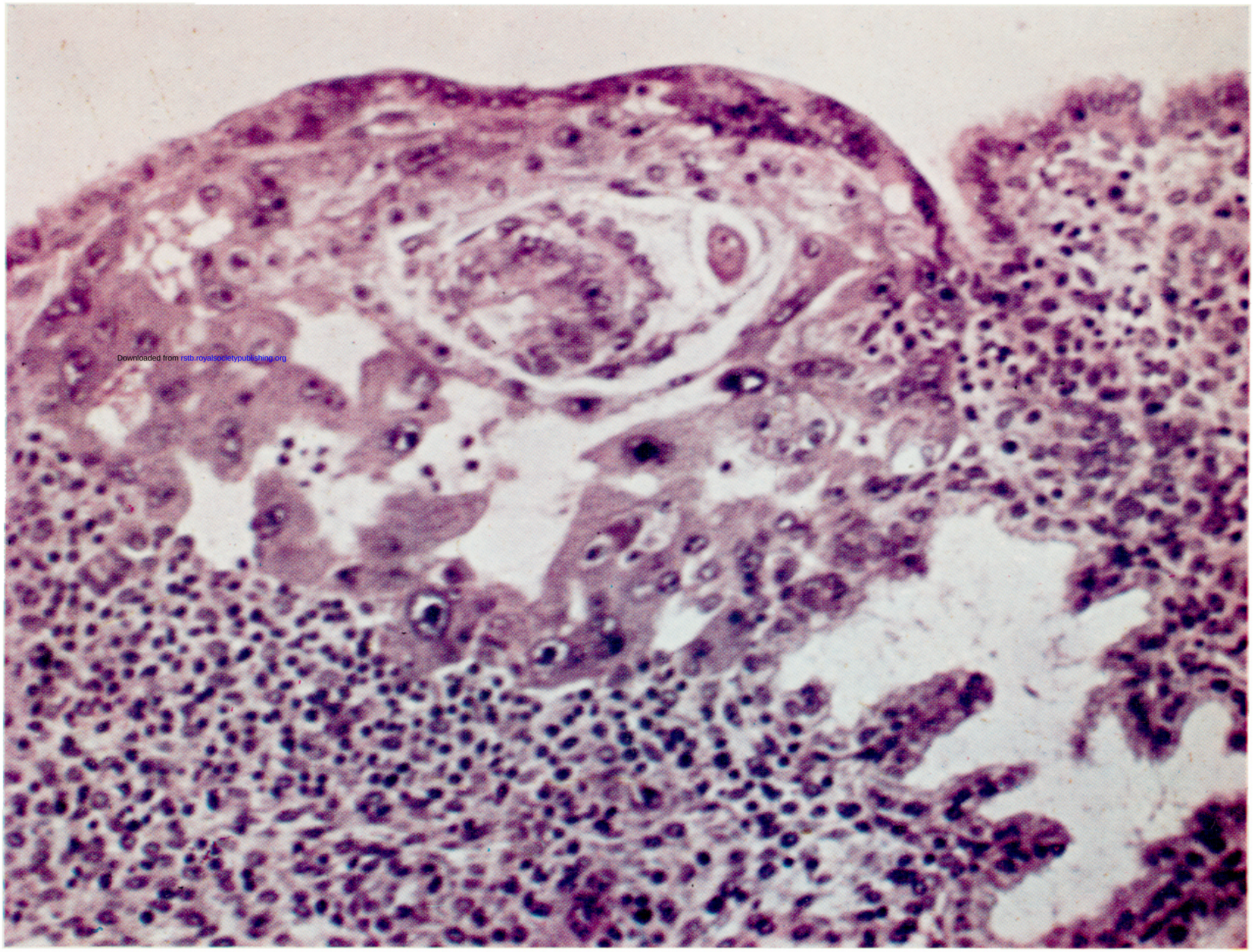

FIGURE 1. Human. A section through the middle of the implantation site of a 9-day pregnancy. The blastocyst is almost completely embedded; the implanted portion shows various phases of trophoblast formation and in it there is extensive lacunar development. Some leucocytes are present in the lacunae, which may already be in open communication with the surrounding sinusoids. Many lymphocyte-like cells are present in the decidua. (Courtesy of Dr J. D. Ebert, Dept. of Embryology, Carnegie Institution of Washington.)

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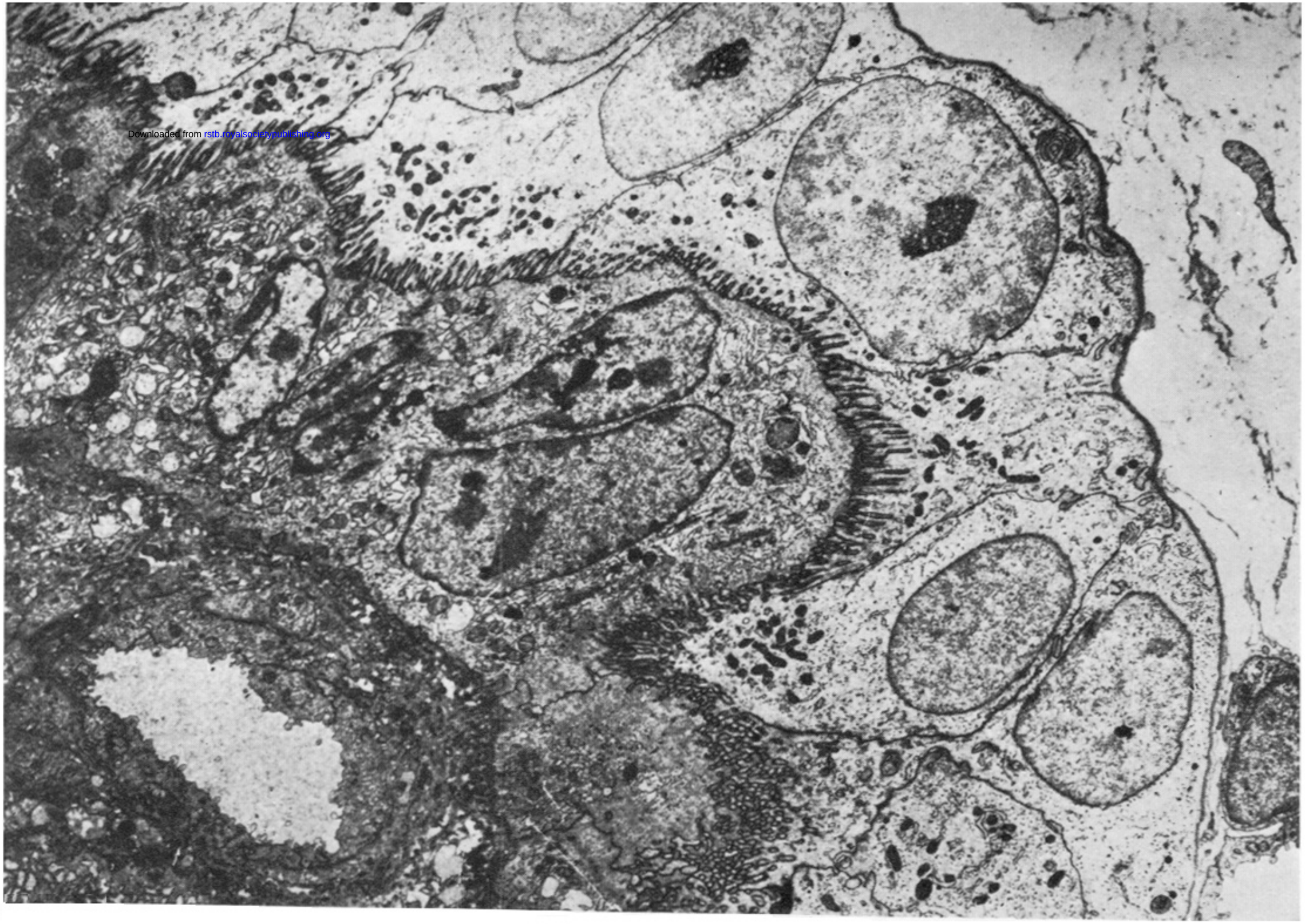

FIGURE 2. Goat. Part of a placentome from a goat 42 days pregnant. Most of the characteristics of the mature placentome have been established. The epithelia of the villus (trophoblast) and the crypt lining are closely interlocked by microvilli. The crypt lining consists of syncytial masses separated by lateral cell membranes; the foetal trophoblast is cellular. Magn. ×5000. (From Lawn, Chiquoine & Amoroso 1969.) Cf. figure 9, plate 13.

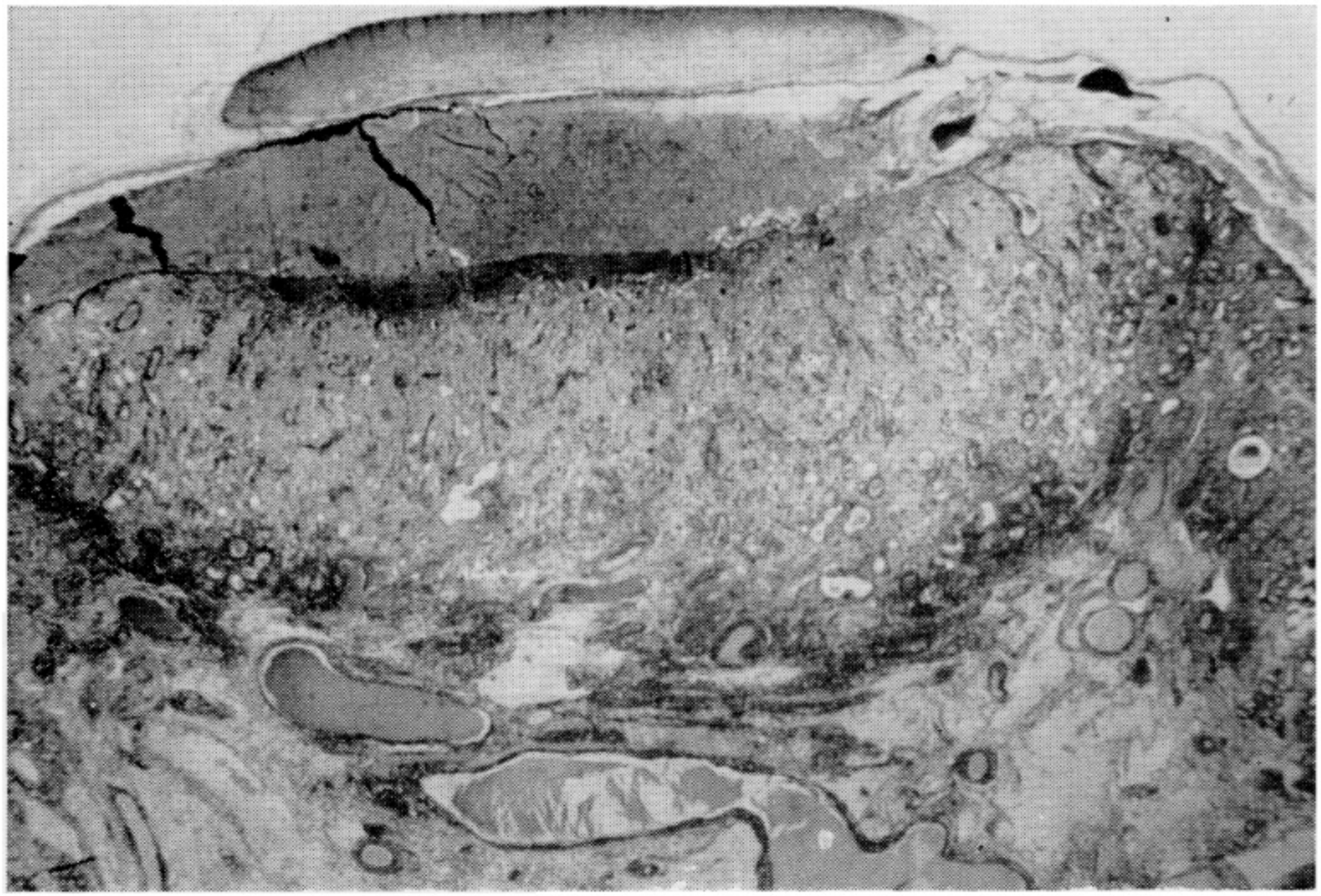

FIGURE 3. Endometrial cup from a mare killed on the 56th day of pregnancy. At this stage the cup, consisting of proliferated chorionic epithelial cells, projects from the surface of the uterus and is separated from the stroma by a dense layer of lymphocytes. Note also that the uterine epithelium over the surface of the cup is entirely absent, and that there is extensive autolysis of the cup tissue. The detritic coagulum which has accumulated in the uterline lumen is rich in gonadotrophic hormone (PMSG) and is the product of degenerating chorionic cells and glandular secretions, some of which has seeped beyond the margins of the cup. Magn. × 10.

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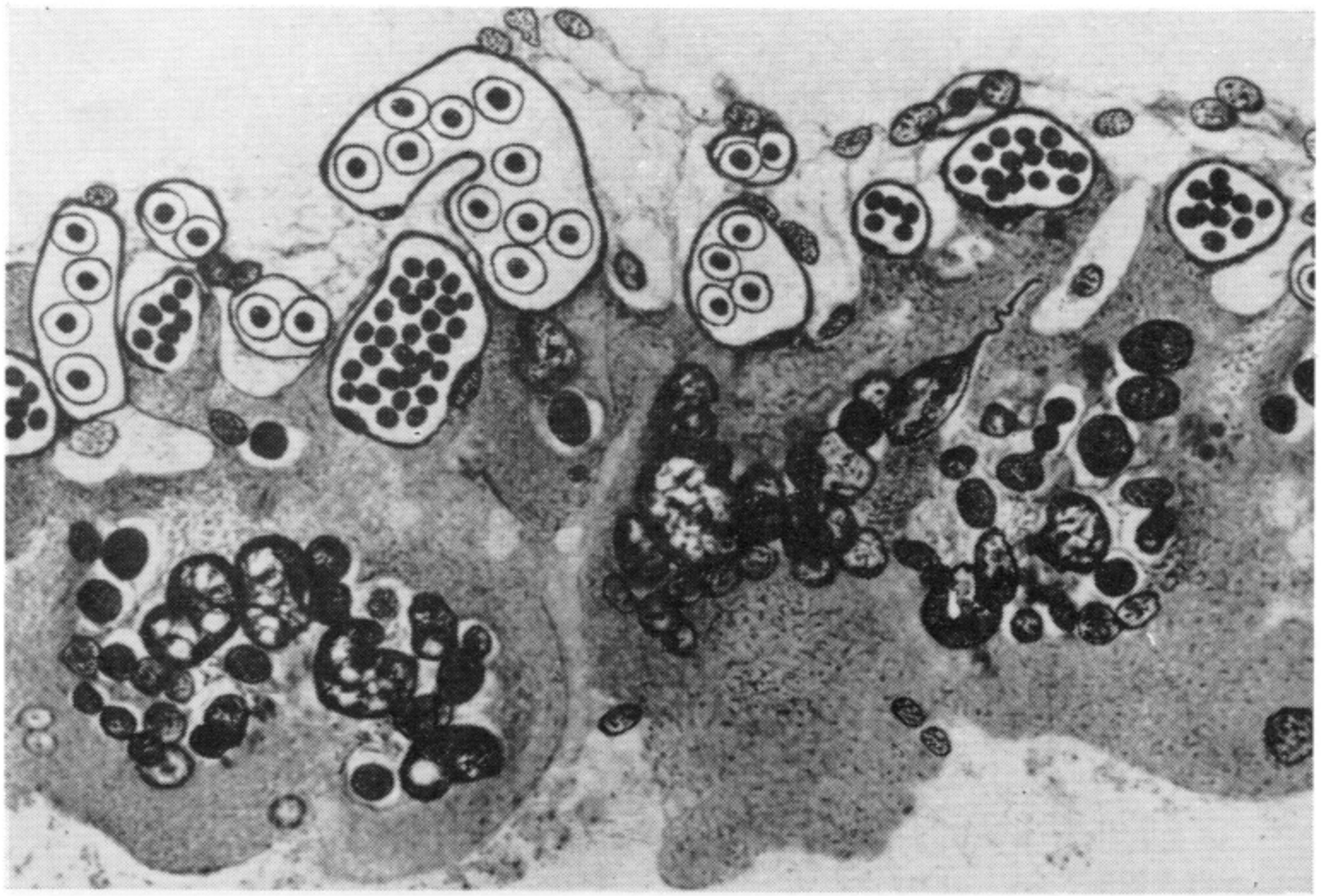
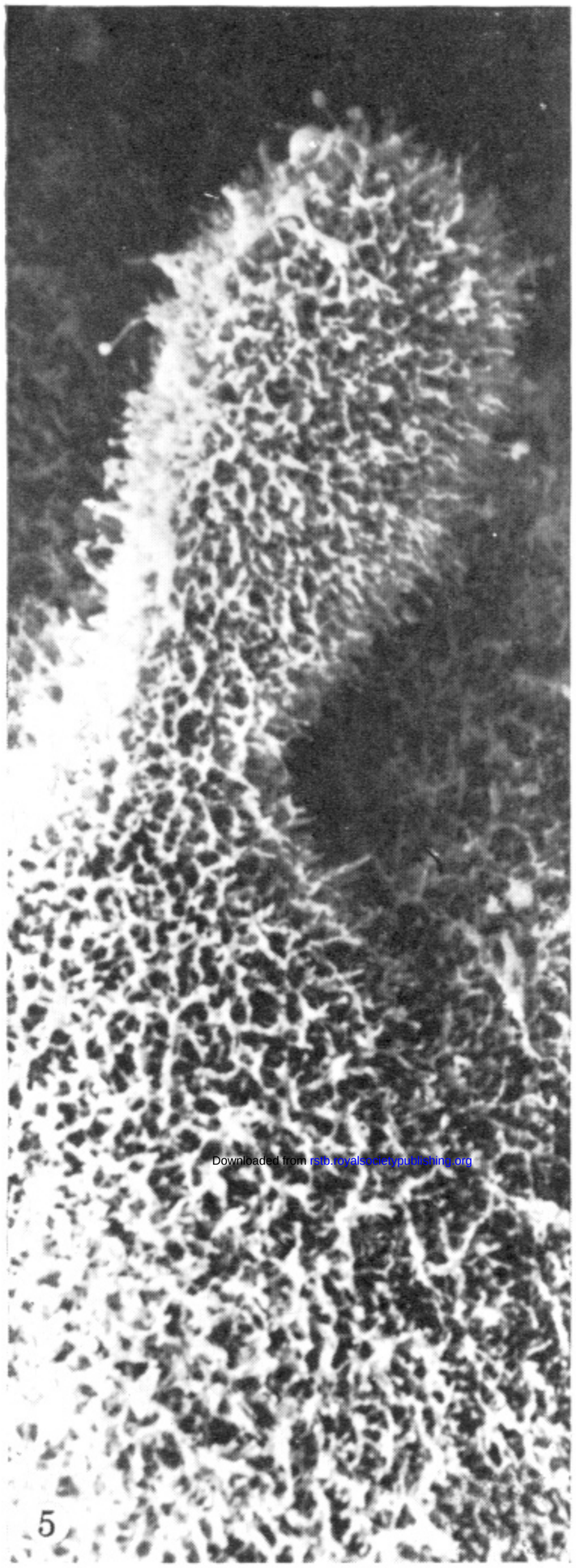


FIGURE 4. Bandicoot. The foetal-maternal junction in the mature allantoic placenta, which is really formed by the fusion of the allanto-chorionic trophoblast with the maternal syncytial layer which results from the proliferation of the uterine epithelium and the concomitant ingrowth of maternal capillaries; foetal and maternal blood vessels are in intimate apposition, but in places are separated by wisps of syncytial cytoplasm. Within the symplasma the enlarged nuclei are of foetal derivation and one gets the impression of an unstable relationship. Note the presence of lymphocytes. Magn. × 300.

The blocks for figures 3 and 4 were kindly provided by the Royal Society of Medicine.



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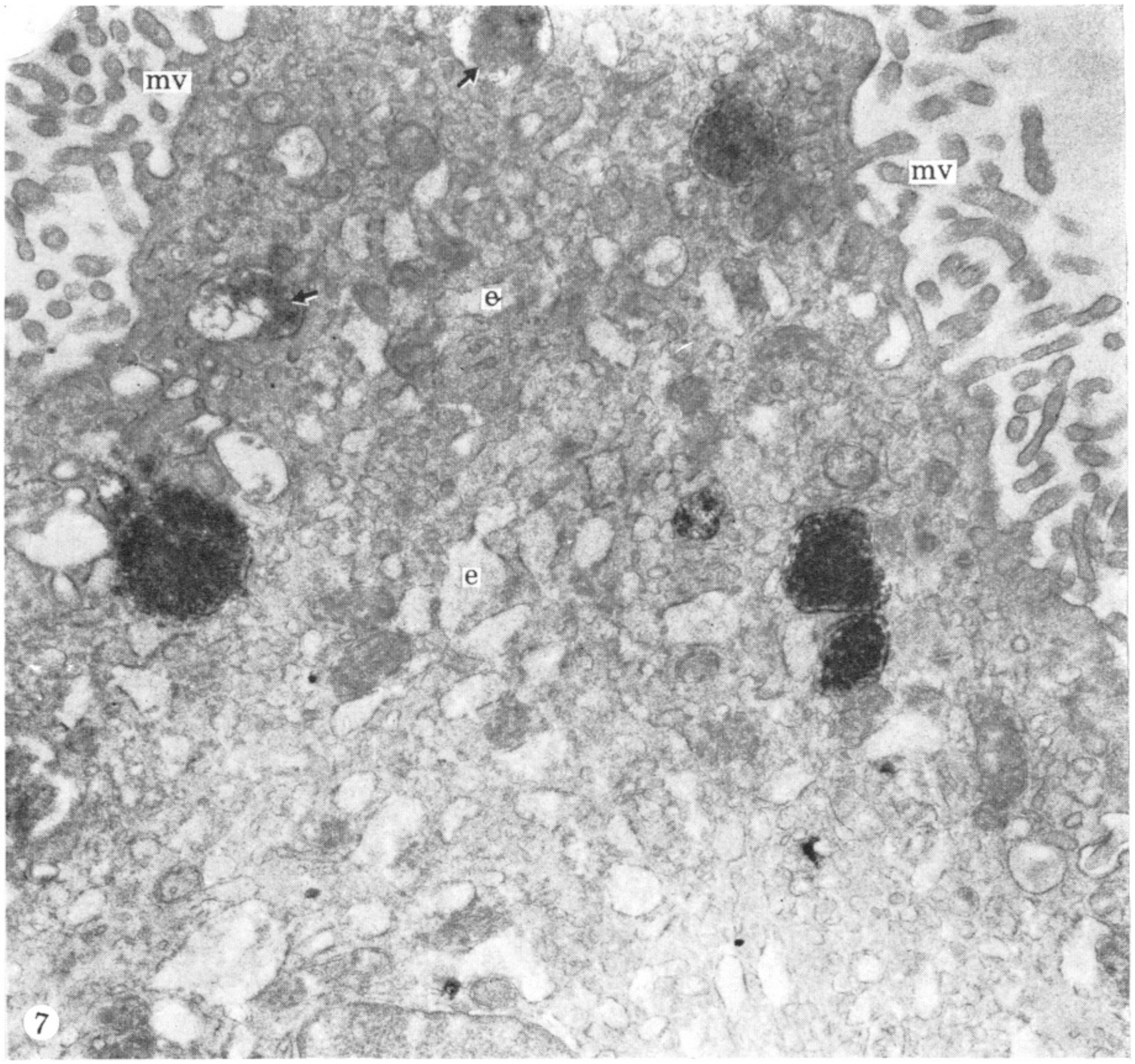

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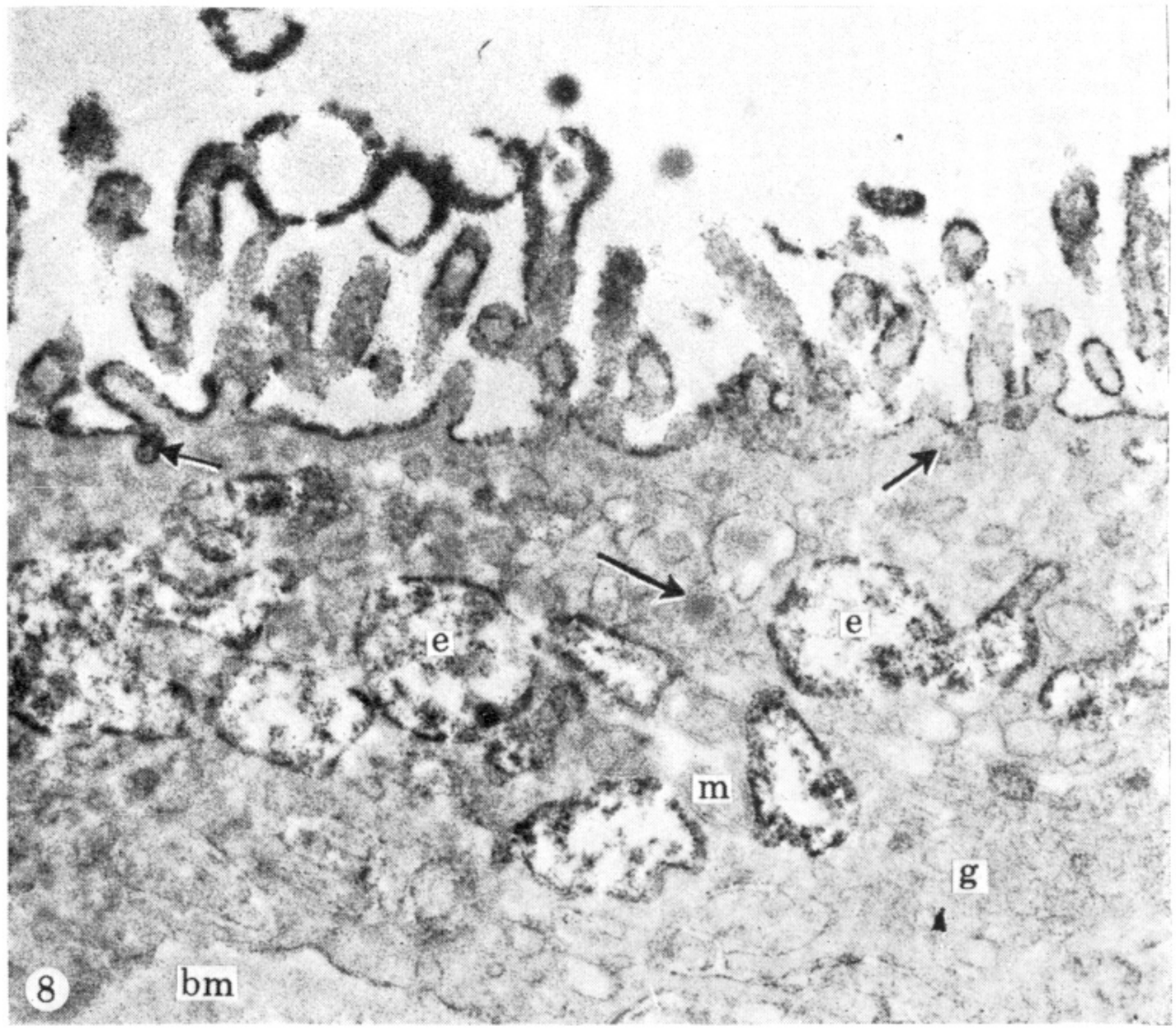

8

FIGURES 5–8. For description see opposite.

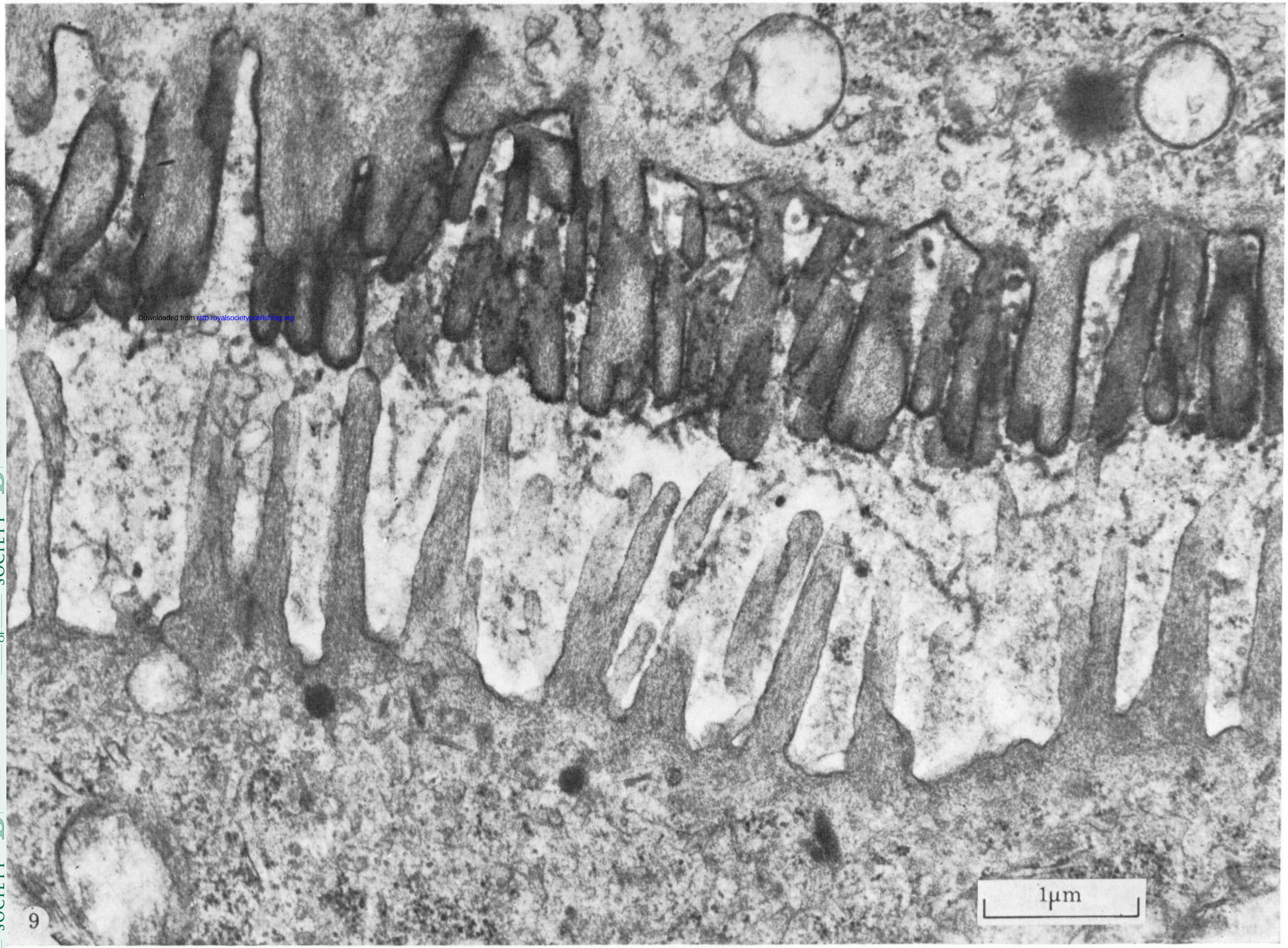

FIGURE 9. Artificial separation of the placenta of the cow at 265 days gestation. The darkly staining cell membrane of the foetal side of the placenta contrasts with the lightly staining membrane of the maternal side. Magn. ×45000.

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