J. Anat. (1977), **124**, 2, pp. 275–286
With 9 figures
Printed in Great Britain

Syncytial knots and intervillous bridges in the human placenta: an ultrastructural study

CAROLYN J. P. JONES AND H. FOX
Department of Pathology, University of Manchester

(*Accepted* 16 *July* 1976)

INTRODUCTION

The term 'syncytial knot' is applied to a focal aggregation, or clumping, of syncytial nuclei on the outer surface of a tertiary placental villus; such knots are rarely seen in the immature placenta, but gradually increase in number throughout gestation, and at term are present on between 10 and 30% of the terminal villi (Fox, 1965). Syncytial knots differ from syncytial 'sprouts', which are present in early pregnancy and represent the first stage in the formation of new villi (Boyd & Hamilton, 1970), and from syncytial 'buds', which are seen, in gradually decreasing numbers, throughout gestation and are ingrowths or projections of small masses of trophoblast into the villous stroma (Boyd & Hamilton, 1964). The mode of formation and the function of syncytial knots are still far from clear, for they have been variously considered as a degenerative phenomenon (Tenney & Parker, 1940; Merrill 1963), an ageing change (Kubli & Budliger, 1963), a manifestation of syncytial hyperplasia (Shanklin, 1958; Aladjem, 1967), an indication of trophoblastic amoeboid activity (Baker, Hook & Severinghaus, 1944), and as a response to trophoblastic ischaemia or hypoxia (Wilkin, 1965; Tominaga & Page, 1966). Hörmann (1953) suggested that the knots represent an attempt to form intervillous bridges which could serve as an internal strut system to protect the villous capillaries from the effects of sudden changes in intervillous space pressure during labour. This view has received little attention but merits examination for, although the concept of the placenta as a labyrinthine organ is now of historical interest only, there is no doubt that intervillous syncytial bridges do exist in a high proportion of placentae (Peter, 1943; Boyd & Hamilton, 1970); such bridges often contain numerous nuclei and tend to resemble elongated syncytial knots.

We have examined the fine structure of both syncytial knots and syncytial intervillous bridges with the aim of elucidating the nature and function of the knots and their role in intervillous bridge formation.

MATERIALS AND METHODS

Eighty eight placentae were examined; these were collected on a random basis and although many were from normal pregnancies, others were from pregnancies complicated by pre-eclampsia, maternal diabetes mellitus, materno-fetal rhesus incompatability or maternal essential hypertension. All the placentae were, however, from pregnancies that had extended into the third trimester, most being from full term gestations, but some were from pregnancies that had extended up to, or beyond, the 42nd week of gestation.

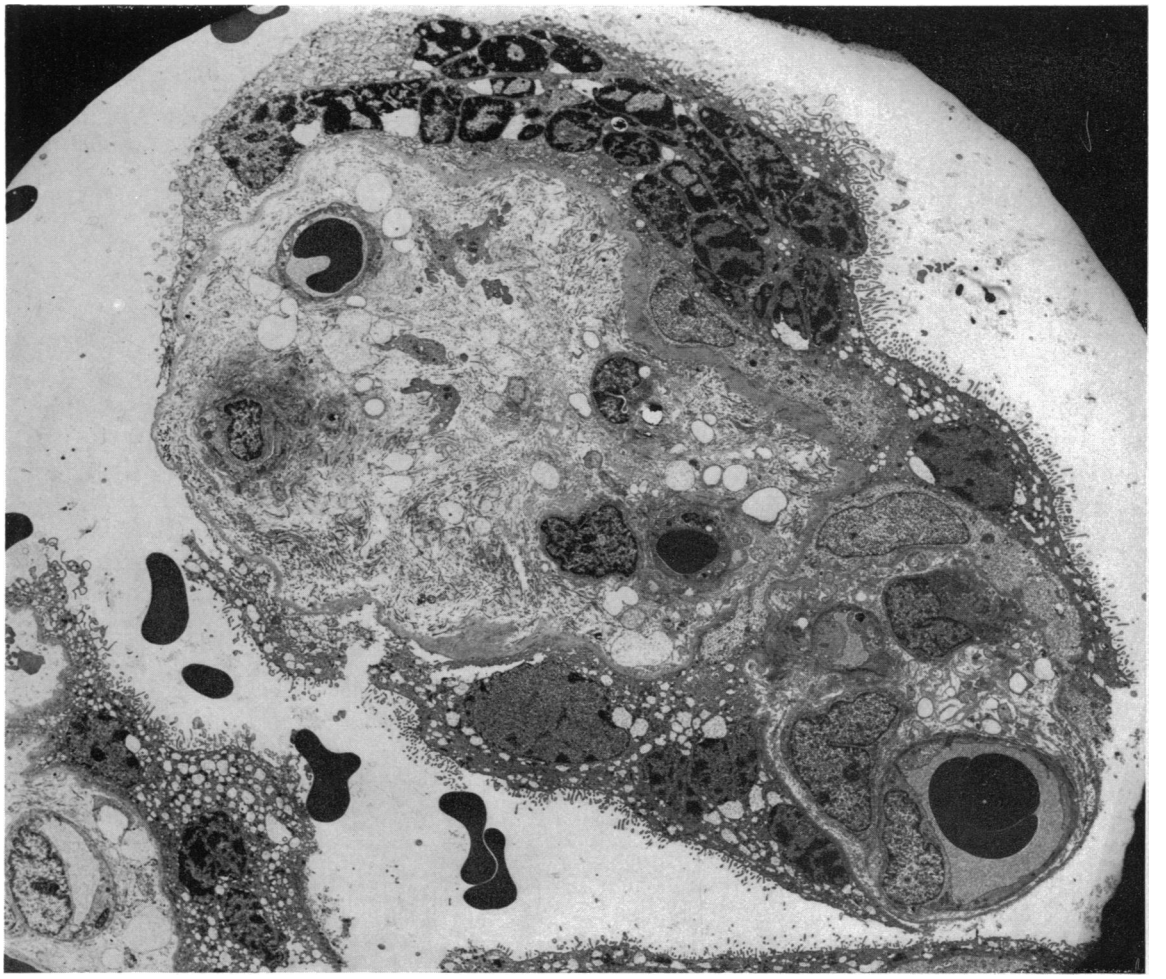

Fig. 1. Low power electron micrograph of a terminal villus bearing a syncytial knot (at top). The electron density of the aggregated nuclei within the knot contrasts with the dispersed chromatin pattern of the non-aggregated nuclei. × 2000.

The placentae were obtained immediately after delivery and a small piece of villous tissue was excised from beneath the centre of the basal plate. This was quickly diced into small fragments with a razor blade on dental wax and fixed for 4 hours at room temperature in 2·5% glutaraldehyde in 0·1 M cacodylate buffer at a pH of 7·4. The fixative was then decanted and the tissue rinsed three times over a 24 hour period in 0·1 M cacodylate buffer (pH 7·4) containing 0·003 M calcium chloride. Post-fixation was carried out using 1% osmium tetroxide in 0·1 M cacodylate buffer (pH 7·4) at 4 °C for 1 hour. After a brief rinse in buffer the tissue was dehydrated in ascending concentrations of alcohol, embedded in Taab resin and polymerized at 60 °C for 36–48 hours. Sections were cut at a thickness of 0·5 μm on an LKB ultramicrotome and stained with toluidine blue for examination at the light microscopic level. Areas containing tertiary villi which were free of perivillous fibrin but in which there were either syncytial knots or intervillous bridges were selected for

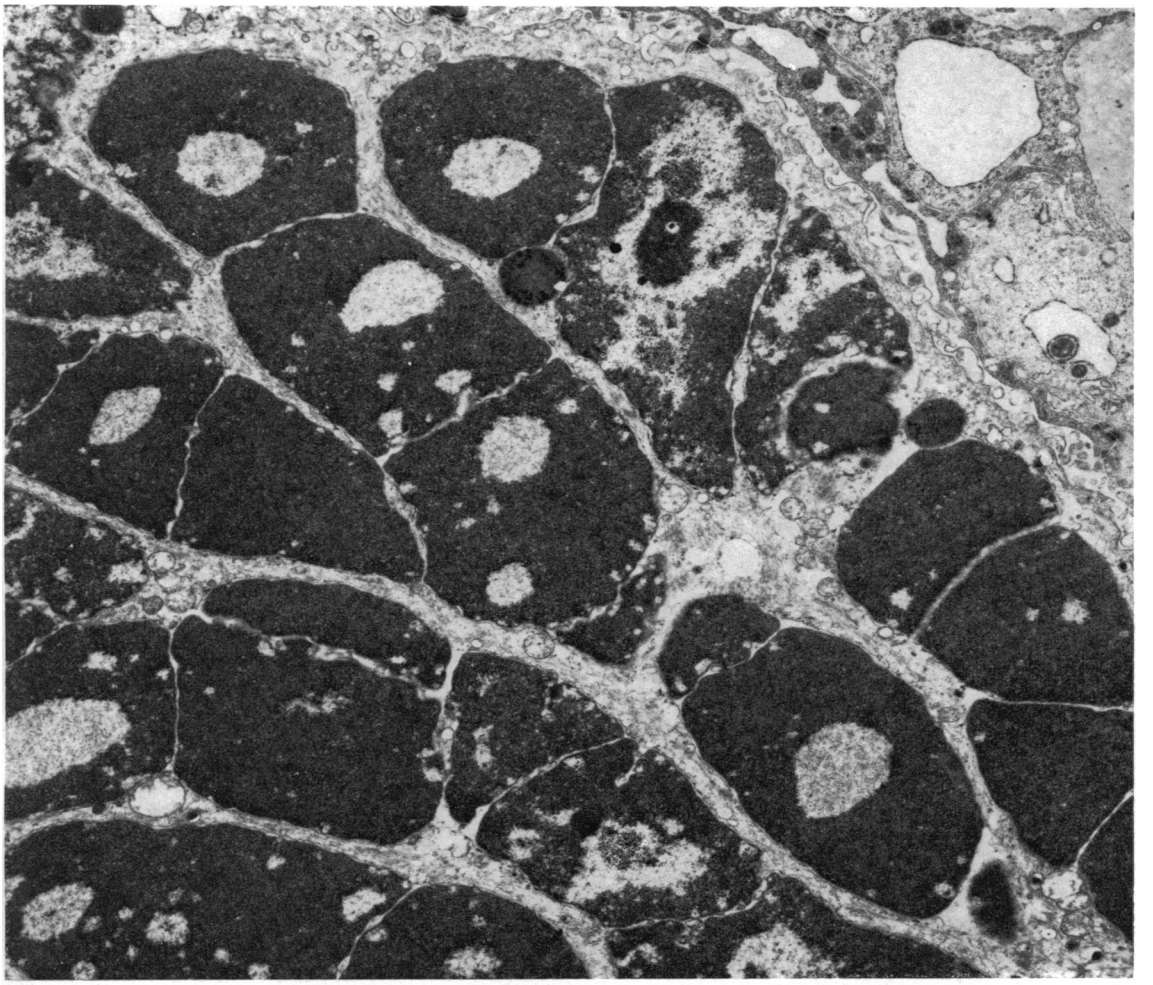

Fig. 2. Electron micrograph of aggregated nuclei in a syncytial knot. ×10000.

ultrathin sectioning, and grids were stained with uranyl acetate and Reynolds' lead citrate prior to examination in an AEI EM6B electron microscope.

Alkaline phosphatase activity was demonstrated using a technique previously described (Jones & Fox, 1976).

RESULTS

Syncytial knots

(*a*) *Nuclei*

Non-aggregated nuclei in a villus bearing a syncytial knot tended to have dispersed chromatin which formed small, irregular, electron-dense clumps beneath the nuclear membrane and throughout the nuclear substance; the outline of these nuclei was generally crenated.

By contrast, the nuclei in a syncytial knot (Figs, 1, 2) generally had a smooth outline, whilst their content of electron-dense chromatin was proportionally increased considerably in relationship to total nuclear volume; very often the electron-lucent areas in a nucleus were seen only as small spaces beneath the nuclear pores and,

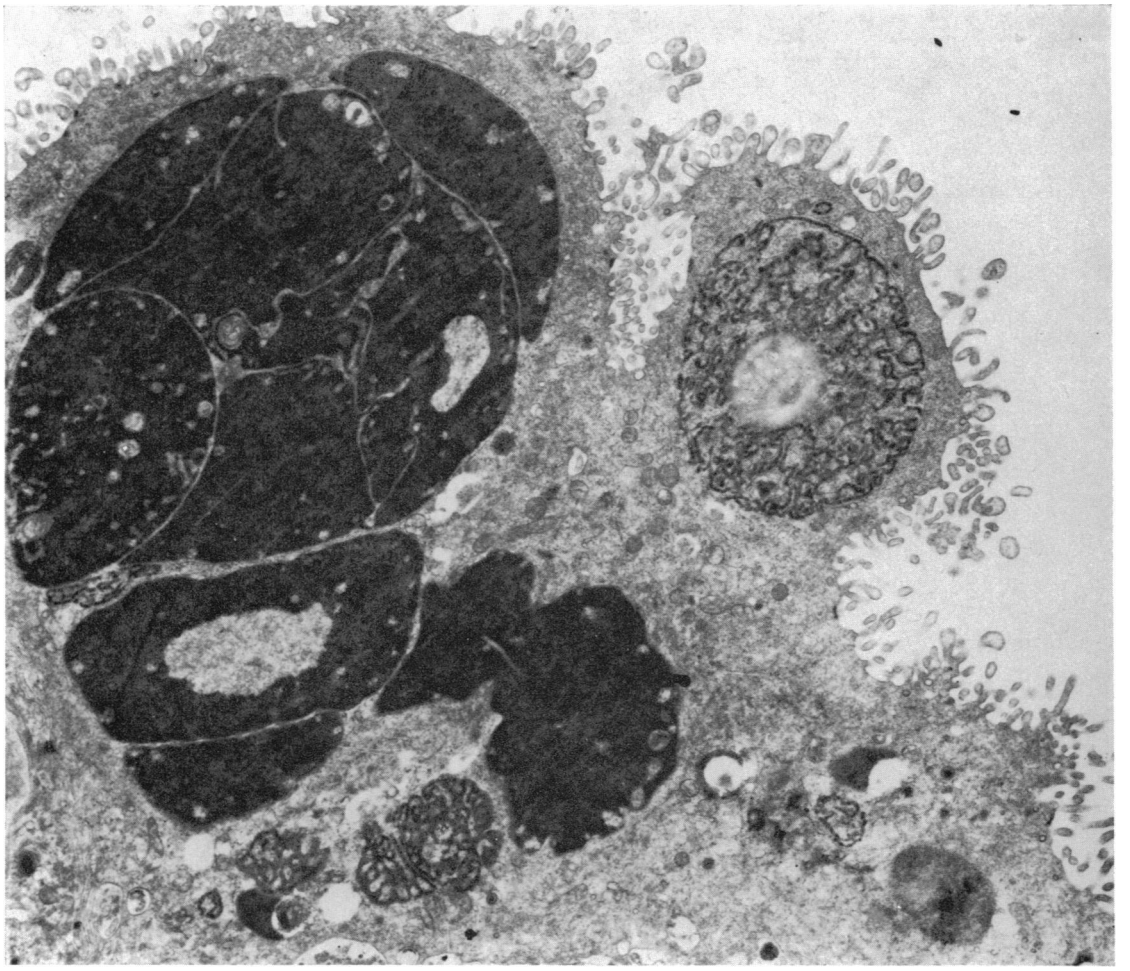

Fig. 3. Electron micrograph of nuclei in a syncytial knot. In several nuclei the chromatin is thrown into tight coils, whilst several autophagic vacuoles are seen in close proximity to partially fragmented nuclei (below). ×10000.

frequently, as a central, roughly circular, chromatin-free area (Fig. 2). In extreme examples the nuclei were almost entirely electron-opaque, with smooth outlines and were separated from each other only by narrow bands of cytoplasm; in some cases there even appeared to be fusion of the nuclear membranes. The central chromatin-free area sometimes contained a cluster of small electron-dense particles, the nature of which was obscure.

Occasionally, evidence of a further change was seen in the nuclei within a syncytial knot. The nuclear limiting membrane appeared to break down and the chromatin was thrown into dense coils (Fig. 3); in some instances, these tended to separate out to form a shell around a central, cylindrical, relatively electron-lucent area, whilst in others the coiled segments appeared to break down into fragments, these latter often in close proximity to autophagic vacuoles.

It has to be admitted that, although in general terms there was a marked difference in structure between aggregated and non-aggregated nuclei in a villus, occasional isolated non-aggregated nuclei were seen which showed the morphological features

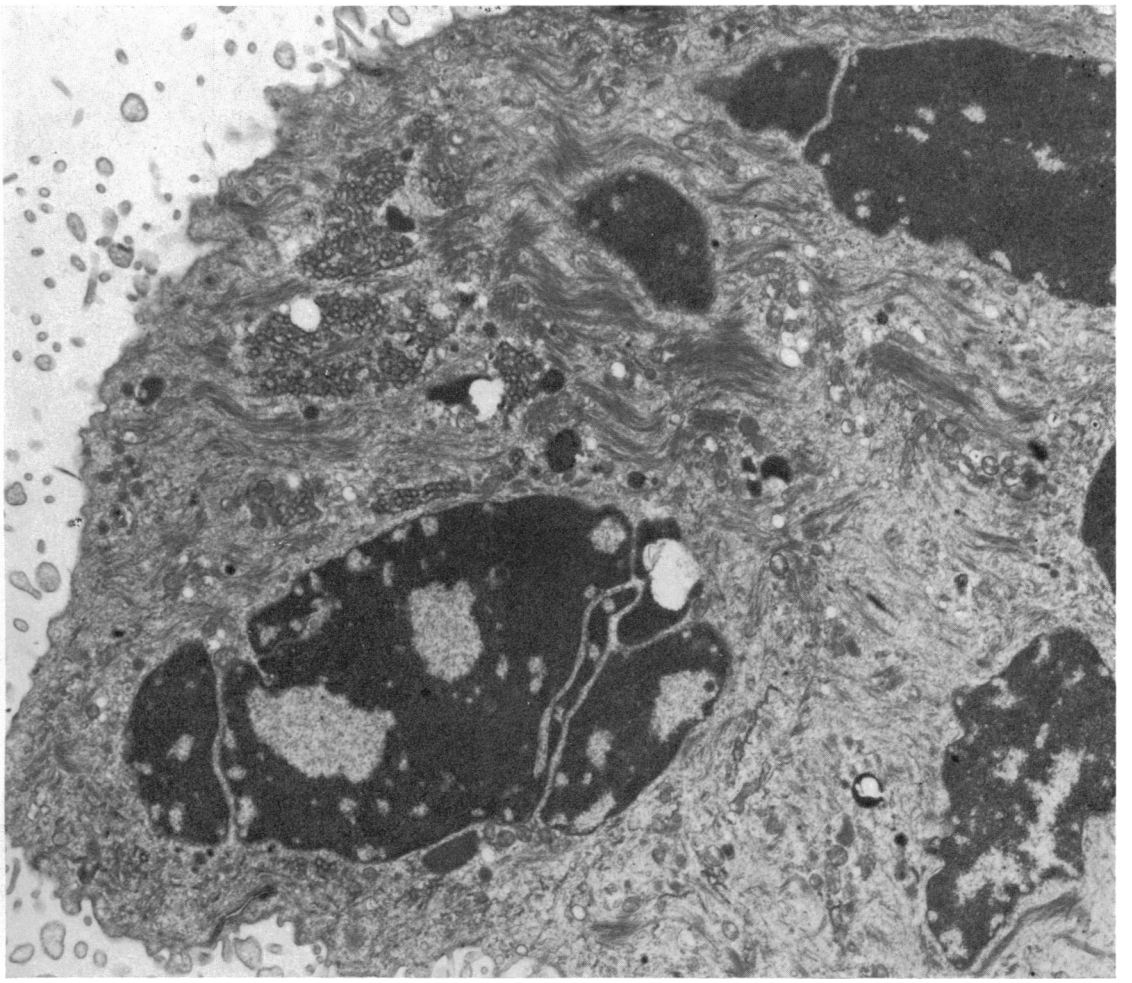

Fig. 4. Electron micrograph of a syncytial knot. Many bundles of cytoplasmic filaments are present. There are very few microvilli on the free syncytial surface of the knot. ×10000.

described as characteristic of those forming knots; the degree of such change was, however, rarely as marked as that seen in aggregated nuclei.

(*b*) *Cytoplasm*

The cytoplasmic changes seen in syncytial knots were of variable degree and extent, but certain features were consistently observed. In many knots there was a marked increase in the number of cytoplasmic filaments (Fig. 4) which were frequently associated with annulate lamellae (Fig. 5). These filaments formed thick bundles between the aggregated syncytial nuclei and were usually oriented in a plane parallel to the nuclear membrane. Mitochondria were usually sparse and tended to be small with few cristae. The endoplasmic reticulum was very variable in quantity but was often scanty and in occasional knots was markedly dilated to form cisternae filled with light, flocculent material. Occasionally, electron-dense inclusions were

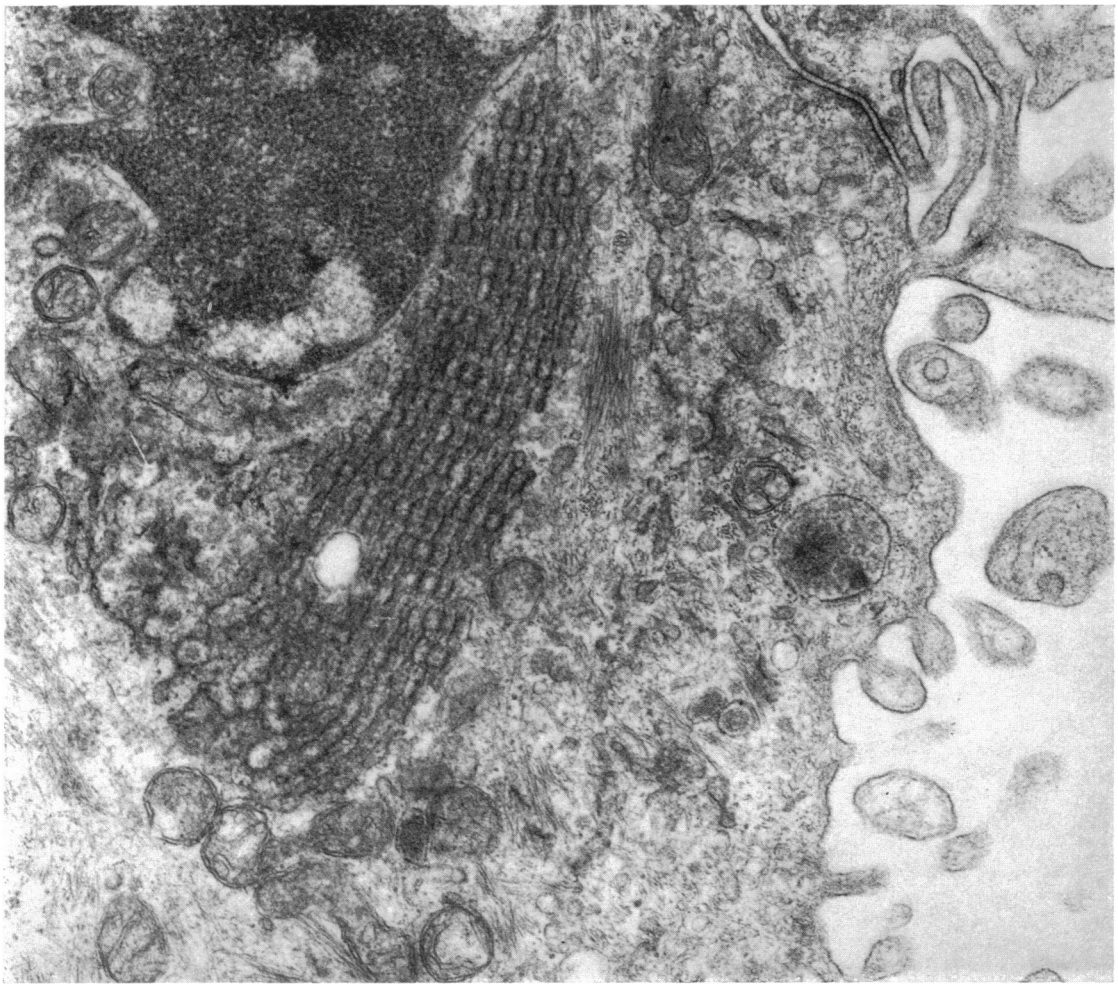

Fig. 5. Electron micrograph of a syncytial knot; stacks of annulate lamellae are present in the cytoplasm. ×40000.

seen in the cytoplasm; some of these were probably secretory droplets, being membrane-bound and homogeneous, but others contained numerous vesicles of variable electron density and appeared to be residual bodies. The number of 'secretory' droplets tended to be lower than in the cytoplasm of areas where the nuclei were not aggregated. Autophagic vacuoles were present in a few knots, and were occasionally numerous (Fig. 6).

(*c*) *Syncytial plasma membrane*

In many knots the syncytial limiting membrane did not differ in any way from that of the syncytium in areas where the nuclei were not aggregated. In a proportion, however, there was a noticeable decrease in the number of microvilli on the plasma membrane, particularly towards the 'apex' of the knot. (Fig. 4). In areas where microvilli were diminished in number there was also usually a marked decrease in pinocytotic pitting. Alkaline phosphatase activity on the plasma membrane of the knots was usually normal both in distribution and degree.

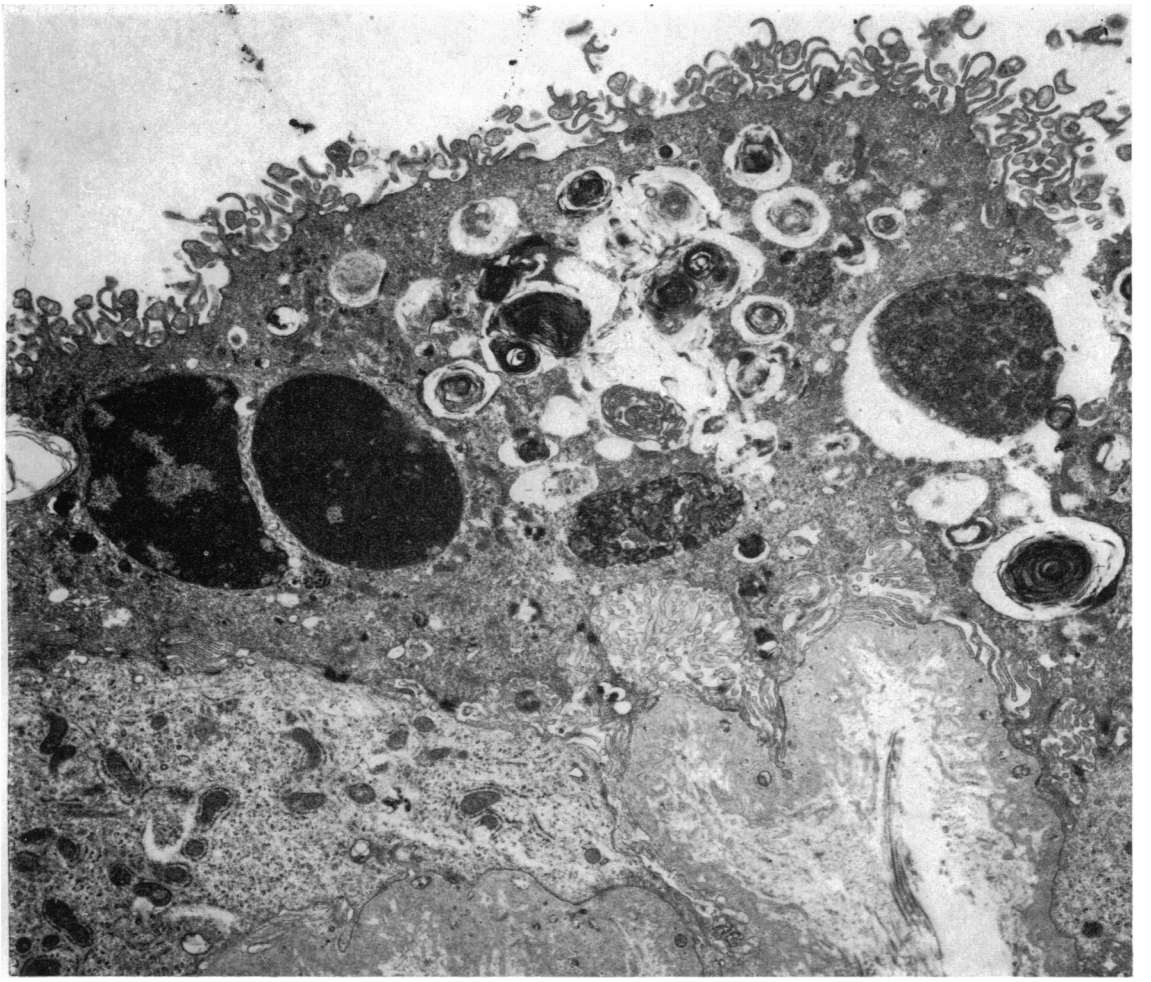

Fig. 6. Electron micrograph of a syncytial knot in which there are many autophagic vacuoles. × 7500.

Intervillous bridges

Occasional intervillous bridges were seen which were free of nuclei. Most bridges contained, however, an abundance of aggregated nuclei (Fig. 7) and these invariably showed the same features as the nuclei of syncytial knots, being electron-dense with a central electron-lucent area and having smooth outlines. Coiling of chromatin was, however, only rarely seen in the nuclei of an intervillous bridge and there was no evidence of breakdown of nuclear limiting membranes.

The cytoplasm in an intervillous bridge also showed features identical to those seen in the cytoplasm of syncytial knots and, though having few mitochondria and little endoplasmic reticulum, contained an abundance of bundles of cytoplasmic filaments and many stacks of annulate lamellae (Fig. 8). Autophagic vacuoles and residual bodies were not seen in the bridges and 'secretion' droplets were rarely present. Alkaline phosphatase activity was normal along the outer syncytial borders of intervillous bridges; in one case (Fig. 9) staining for alkaline phosphatase activity demonstrated clearly the formation of a bridge by the fusion of syncytial outgrowths from adjacent villi, these outgrowths containing a number of aggregated nuclei.

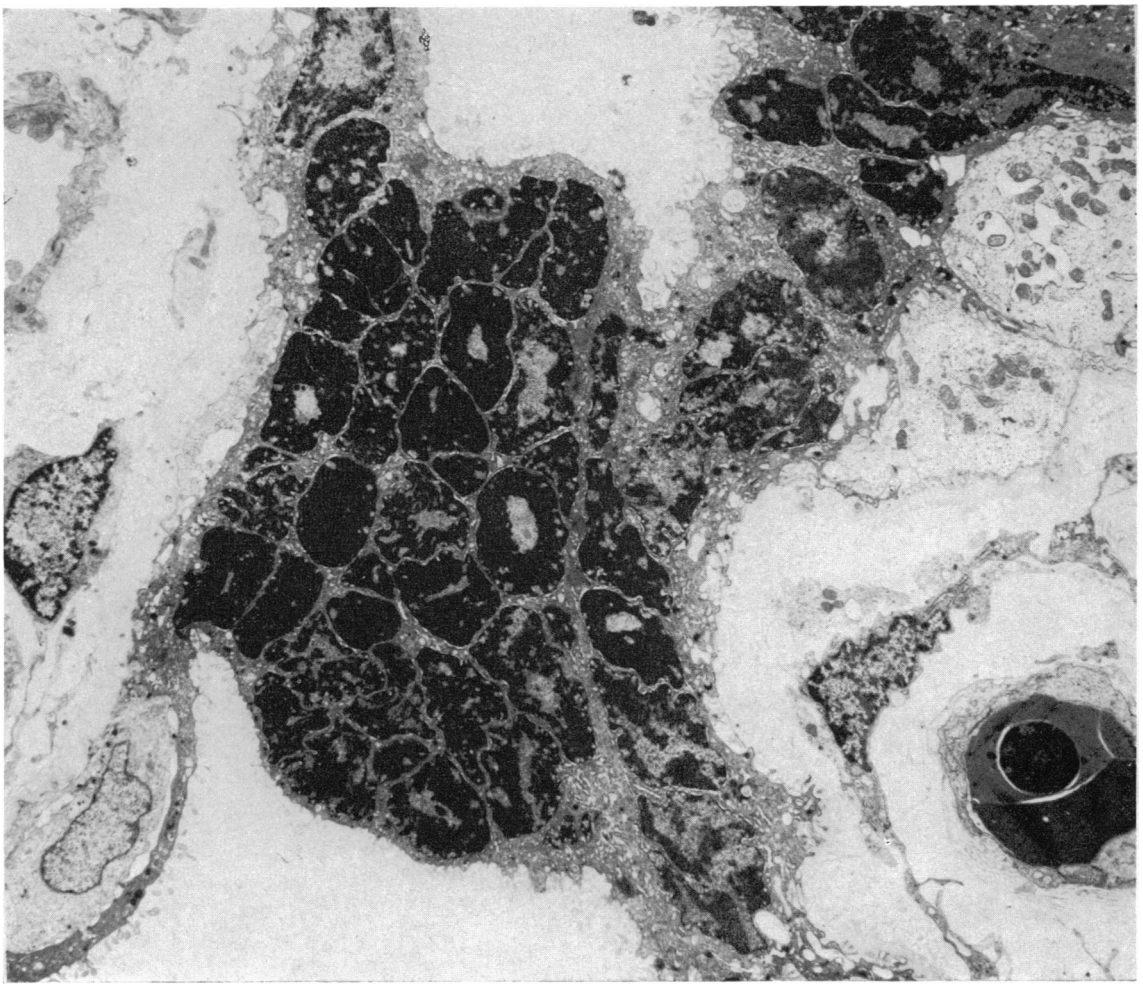

Fig. 7. Electron micrograph of an intervillous bridge. × 5000.

DISCUSSION

The fine structure of the nuclei within a syncytial knot differs so markedly from that of most of the non-aggregated nuclei in the same villus as to make it clear that the formation of a syncytial knot is not due simply to a random mechanical aggregation of nuclei. On the other hand, the finding of occasional non-aggregated nuclei showing similar changes to those seen in clustered nuclei indicates that the morphological abnormalities in the nuclei of a syncytial knot are not solely a consequence of their aggregation.

It is clear that most of the nuclei within a syncytial knot are undergoing degenerative changes, and the fact that these knots appear principally towards the end of pregnancy (Fox, 1965; Boyd & Hamilton, 1970) suggests that the nuclear abnormalities are an expression of nuclear senescence. Martin & Spicer (1973) have demonstrated that ageing changes do occur in human syncytiotrophoblastic nuclei and have suggested that these are due to a programmed senescence; the changes they described were, however, seen in non-aggregated nuclei, and fell far short of those seen in the nuclei

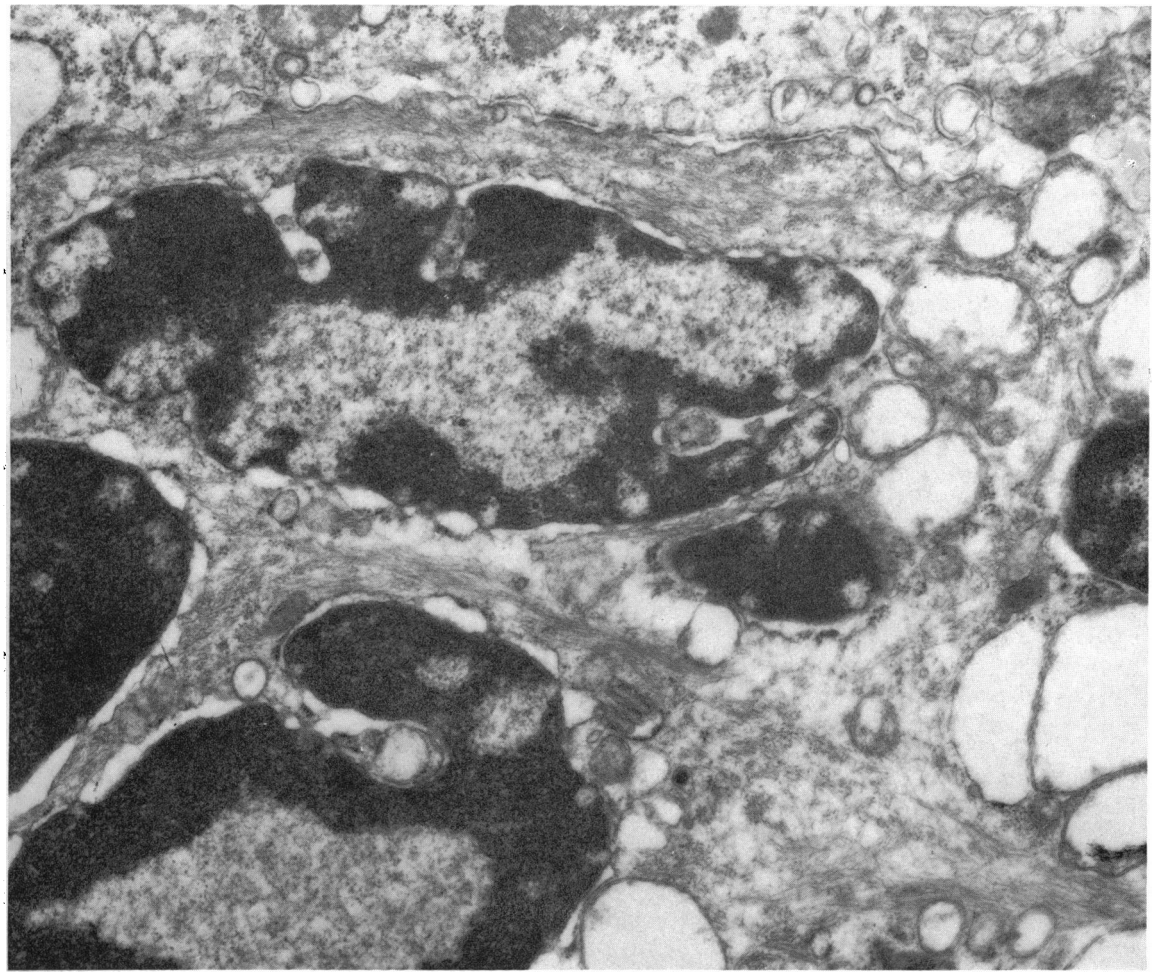

Fig. 8. Electron micrograph of an intervillous bridge. Bundles of filaments and occasional annulate lamellae are present in the cytoplasm. ×27500.

of a syncytial knot. It has to be borne in mind that the syncytiotrophoblast is derived from the cytotrophoblast, which can be considered as the germinative zone of the trophoblast; mitotic activity within the cytotrophoblastic cells can easily be observed during the first trimester and continues, though to a progressively diminishing extent, into the third trimester. It therefore follows that the syncytial nuclei within any individual villus are formed at varying stages of pregnancy and are, in respect to age, a heterogeneous population. If trophoblastic nuclear senescence is a programmed event it would therefore be expected that in the villi of the mature placenta a proportion of the syncytial nuclei, namely those formed during the earliest stages of pregnancy, will show ageing changes, whilst others, formed at a later stage of gestation, will show little, or no, evidence of senescence. Teleologically, there is an obvious benefit to be derived in removing senescent nuclei from areas of metabolically active syncytium and aggregating the effete nuclear material together in one area of the villus: the syncytial knots may well be considered a form of sequestration of unwanted, aged nuclei. If it is assumed that as nuclei age they are, at least partially,

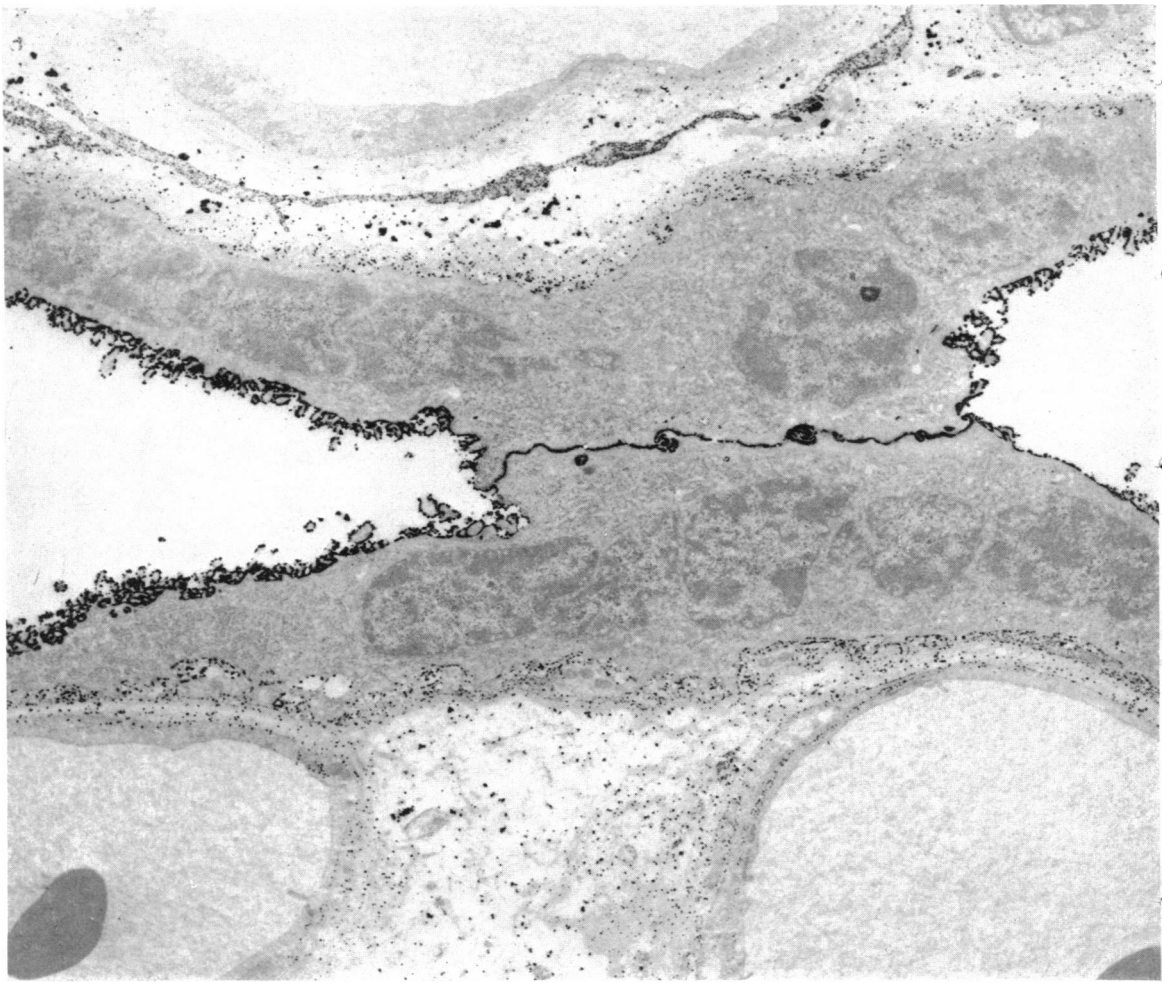

Fig. 9. Electron micrograph of an intervillous bridge which has been stained for alkaline phosphatase activity. It can be seen that the bridge is being formed by the fusion of syncytial outgrowths from adjacent villi. × 5000.

replaced by fresh nuclei derived from the cytotrophoblast, it follows that villi bearing syncytial knots should, on average, have more syncytial nuclei than those lacking such structures; that knot-bearing villi do indeed have an excess of syncytial nuclei has been shown by Fox (1965) and by Gerl *et al.* (1973).

It has been clearly shown that an excessive number of syncytial knots is found on villi which are inadequately perfused by the fetal circulation (Fox, 1965). This is seen most strikingly when arrest of fetal blood flow to a localized group of villi occurs; thus, following thrombotic occlusion of a fetal artery in the human placenta, a marked excess of syncytial knots is seen in the localized group of villi supplied by the obstructed vessel (Fox, 1965), whilst exactly similar findings have been noted in the monkey placenta after experimental ligation of a fetal artery (Myers & Fujikura, 1968). This response to villous hypovascularity could indicate that under such circumstances the sequestration of aged nuclei is accelerated or accentuated so as to utilize optimally the amount of trophoblast available for transfer of oxygen and nutrients

across the syncytium; alternatively, it could be argued that a diminished fetal blood flow causes an accelerated ageing of the syncytial nuclei.

In a proportion of syncytial knots the aggregated nuclei appear to disintegrate, presumably due to lysosomal activity. In many instances, however, the syncytial knots appear to be utilized to form intervillous bridges. If such bridges are needed, and their undoubted presence in many placentae suggests that they are, then it would appear reasonable for the effete material in a syncytial knot to be used for this purpose. The marked proliferation of cytoplasmic filaments in a syncytial knot can be considered as a preparation for the assumption by these structures of a mechanical role; the annulate lamellae which are seen with some frequency in syncytial knots may possibly be involved in the synthesis of the cytoplasmic filaments, for such organelles appear to be related to protein synthesis (Wischnitzer, 1970; Benzo, 1974). That syncytial knots from adjacent villi do fuse to form intervillous bridges appears almost certain, for not only do the bridges usually have a fine structure which is identical to that of a syncytial knot, but we have demonstrated, by means of the alkaline phosphatase reaction, actual fusion of knots to form a bridge. The bridges themselves, with their tightly packed nuclei, sparsity of cytoplasmic organelles and rich content of cytoplasmic filaments, appear admirably suited for a mechanical role and this lends considerable support to Hörmann's theory (1953) that they form an internal strut system.

SUMMARY

An electron microscopic study has shown that the syncytial knots of the villi of the human placenta contain aggregated nuclei which exhibit marked degenerative changes; within the cytoplasm there is an abundance of cytoplasmic filaments and many stacks of annulate lamellae.

It is suggested that syncytial knots are a sequestration phenomenon in which senescent nuclear material is aggregated and removed from metabolically active areas of the syncytiotrophoblast.

Intervillous bridges appear to be formed chiefly by fusion of syncytial knots from adjacent villi, and it seems reasonable that the effete material in a syncytial knot should be used for this purpose. The intervillous bridges have a fine structure which suggests that they have a mechanical function, and this lends support to the theory that they form an internal strut system within the placenta.

This work was supported by a grant from the Research Grants Committee of the United Manchester Hospitals.

REFERENCES

ALADJEM, S. (1967). The syncytial knot: a sign of active syncytial proliferation. *American Journal of Obstetrics and Gynecology* **99**, 350–358.

BAKER, B. L., HOOK, S. J. & SEVERINGHAUS, A. E. (1944). The cytological structure of the human chorionic villus and decidua parietalis. *American Journal of Anatomy* **74**, 291–325.

BENZO, C. A. (1974). Annulate lamellae in hepatic and pancreatic beta cells of the chick embryo. *American Journal of Anatomy* **140**, 139–142.

BOYD, J. D. & HAMILTON, W. J. (1964). Stromal trophoblastic buds. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **71**, 1–10.

BOYD, J. D. & HAMILTON, W. J. (1970). *The Human Placenta*, Cambridge: Heffer.

FOX, H (1965). The significance of villous syncytial knots in the human placenta. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **72**, 347–355.

GERL, D., EICHHORN, H., EICHHORN, K.-H. & FRANKE, H. (1973). Quantitative Messungen synzytialer Zellkernkonzentrationen der menschlichen Plazenta bei normalen und pathologischen Schwangerschaften. *Zentralblatt für Gynäkologie* **95**, 263–266.

HÖRMANN, G. (1953). Ein Beitrag zur funktionellen Morphologie der menschlichen Placenta. *Archiv für Gynäkologie* **184**, 109–123.

JONES, C. J. P. & FOX, H. (1976). An ultrahistochemical study of the distribution of acid and alkaline phosphatase in placentae from normal and complicated pregnancies. *Journal of Pathology* **118**, 143–151.

MARTIN, B. J. & SPICER, S. S. (1973). Ultrastructural features of cellular maturation and aging in human trophoblast. *Journal of Ultrastructure Research* **43**, 133–149.

MERRILL, J. A. (1963). Common pathological changes of the placenta. *Clinical Obstetrics and Gynecology* **6**, 96–109.

MYERS, R. E. & FUJIKURA, T. (1968). Placental changes after experimental abruptic placentae and fetal vessel ligation of rhesus monkey placenta. *American Journal of Obstetrics and Gynecology* **100**, 846–851.

PETER, K. (1943). Placenta-Studien. I. Zotten und Zwischen-zottenräume zweier Placenta aus den letzten Monaten der Schwangerschaft. *Zeitschrift für mikropisch-anatomische Forschung* **53**, 142–174.

SHANKLIN, D. R. (1958). The human placenta: a clinico-pathologic study. *Obstetrics and Gynecology* **II**, 129–138.

TENNEY, B. & PARKER, F. (1940). The placenta in toxemia of pregnancy. *American Journal of Obstetrics and Gynecology* **39**, 1000–1005.

TOMINAGA, R. & PAGE, E. W. (1966). Accommodation of the human placenta to hypoxia. *American Journal of Obstetrics and Gynecology* **94**, 679–685.

WILKIN, P. (1965). *Pathologie du Placenta.* Paris: Masson.

WISCHNITZER, S. (1970). The annulate lamellae. *International Revue of Cytology* **27**, 65–100.