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Observations on the fine structure of the normal porcine liver

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INTRODUCTION

The fine structure of the porcine liver has received scant attention hitherto, the few existing studies being largely concerned with foetal or neonatal tissue (Grasso, Ackerman & Knouff, 1959; Ackerman, Grasso & Knouff, 1961; Bischoff, Richter & Stein, 1969). In these studies, interest has centred mainly on haematopoiesis and on the transition from foetal to early neonatal stages of development. In recent years the pig has been increasingly used as an experimental animal, especially in the field of liver transplantation (Peacock & Terblanche, 1967; Hunt, 1967; Hobbs *et al.* 1968; Calne *et al.* 1969), owing to its similarity to man in having no hepatic vein sphincters, in being omnivorous, and in having a similar blood biochemistry (Peacock & Terblanche, 1967).

In view of this increasing usage and to provide a basis for future investigations into the subcellular pathology of the transplanted or otherwise altered porcine liver, it is necessary that adequate documentation should exist of its fine structural morphology. The present report gives an account of the fine structure of the normal adult pig liver, together with brief consideration of the tissue in the newborn.

MATERIALS AND METHODS

Samples of hepatic tissue were taken from six young adult and two neonatal (12 h) pigs (*Sus domestica*) which had been killed by stunning. The samples were removed, immediately after death, into ice-cold cacodylate-buffered 4% glutaraldehyde, pH 7·2 (Sabatini, Bensch & Barrnett, 1963), in which they were finely minced. Fixation was continued for a total of 4 h, after which the tissue was washed for 16 h at 0–4 °C in cacodylate-buffered 0·25 M sucrose and postfixed for 2 h in phosphate-buffered 1% osmium tetroxide (Millonig, 1961*a*). The fixed tissue was dehydrated in ethanol and embedded in Epon 812, essentially by the method of Luft (1961). Thin sections were cut on a Sorvall MT-1 Porter–Blum ultramicrotome, using glass or Ge-Fe-Ri diamond knives. They were mounted on naked copper grids, stained with lead tartrate (Millonig, 1961*b*) and examined in a Philips EM 300 electron microscope at an accelerating voltage of 80 kV.

Histological preparations were made from hepatic tissue fixed in buffered formol-saline, paraffin sections being stained with haematoxylin and eosin.

OBSERVATIONS

Histology

Histological preparations of both adult and neonatal pig liver show the tissue to be divided into well-defined lobules by thick, prominent interlobular septa. Otherwise the appearance does not differ from that of mammalian hepatic tissues in general.

Electron microscopy

The overall fine structural organization of the porcine hepatocyte (Figs. 1, 2) resembles, in general, that of the hepatocytes of many other mammalian species (reviewed by David, 1964; Oudea & Domart-Oudea, 1967). The cytoplasm contains an abundance of glycogen rosettes, aggregated into typical glycogen areas. The glycogen is usually associated with small irregular vesicles of the agranular endoplasmic reticulum. Distinctly tubular elements of the latter organelle are rarely observed. The cisternae of the granular endoplasmic reticulum are short and frequently tortuous in profile and are present either as single elements or as small arrays.

The pericanalicular cytoplasm contains Golgi zones which are often extensive and always enclose numerous coarse granules within their saccules and vesicles. Small numbers of lysosome-like bodies are often present near the Golgi apparatus (Fig. 2).

The fibrous interlobular septa of the porcine liver are thicker than those of most other species. In electron micrographs they are seen to consist mainly of large bundles of collagen fibres (Fig. 3), embedded in an amorphous matrix. Fibroblasts and macrophages are present among the collagen. The bases of the adjoining hepatocytes rest directly on the septal material, without the presence of any intervening basement membrane. Their basal surfaces are highly irregular in outline.

Much of the lumen of the porcine hepatic sinusoid is occupied by large cytoplasmic processes originating from Kupffer cells (Fig. 4). Typically, these possess large dense bodies which appear to belong to the lysosomal group of organelles, and which contain monoparticulate glycogen in addition to partially lysed cellular debris.

The hepatic cell surface which borders on the space of Disse possesses numerous small microvilli. The lateral cell surfaces are smooth and closely apposed, except at the bile canaliculi where they also possess microvilli (Fig. 5). Juxta-luminal cellular attachments are present, resembling the junctional complexes described by Farquhar & Palade (1963), in that the bile canaliculus is limited by a zonula occludens, distal to which is a zonula adhaerens and a macula adhaerens or desmosome. The bile canaliculi appear to be identical to those observed in the hepatic tissue of other mammalian species (David, 1964, Fawcett, 1955; Oudea & Domart-Oudea, 1967).

The most unusual and characteristic feature of the porcine liver is the common presence of large lacunae in the hepatic cell cytoplasm. These are not visible in histological preparations, but in electron micrographs they appear as large spaces, enclosed by a single membrane, which contain a homogenous, finely granular material (Figs. 5, 6). They are either irregular or round to oval in outline. The limiting membrane does not possess recognizable microvilli and direct continuity of the lumen with either the space of Disse or the bile canaliculi was not observed. The cytoplasm

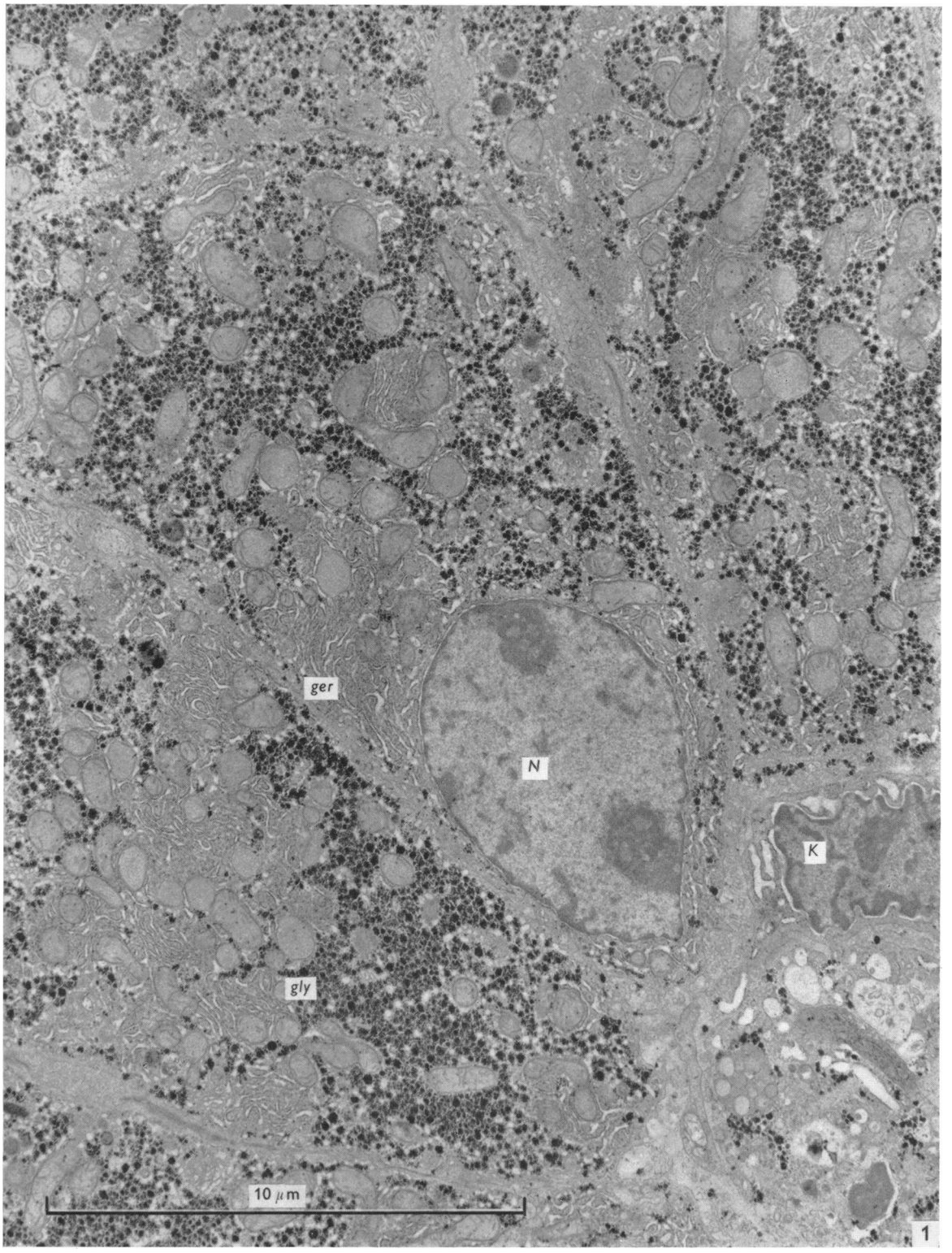


Fig. 1. Low-power electron micrograph of adult porcine liver showing the general disposition of cell contents. Typical glycogen areas (*gly*) and irregular arrays of the granular endoplasmic reticulum (*ger*) are present in the cytoplasm. The nucleus (*N*) contains two nucleoli. A sinusoid is present at the lower right-hand side of the field, and contains a typical porcine Kupffer cell (*K*) with abundant cytoplasm.

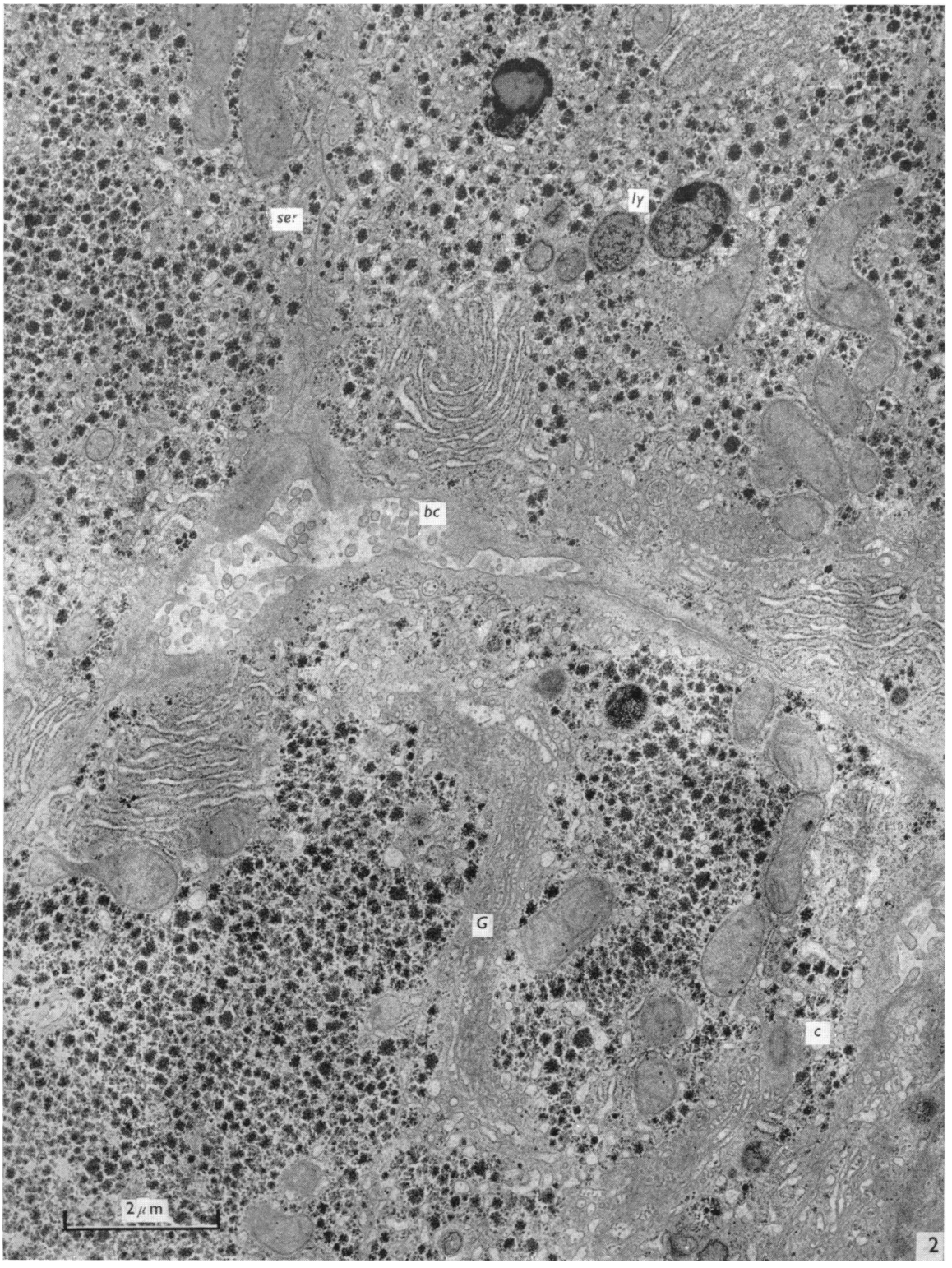


Fig. 2. Detail of cytoplasm of adult porcine hepatocyte. A bile canaliculus (*bc*) is shown, having numerous microvilli on its surface. The pericanalicular cytoplasm contains areas of glycogen interspersed with the rather irregular elements of the agranular endoplasmic reticulum (*ser*). Golgi zones (*G*), lysosomes (*ly*); small arrays of granular endoplasmic reticulum cisternae are also present. A centriole is present at the lower right of the field (*c*).

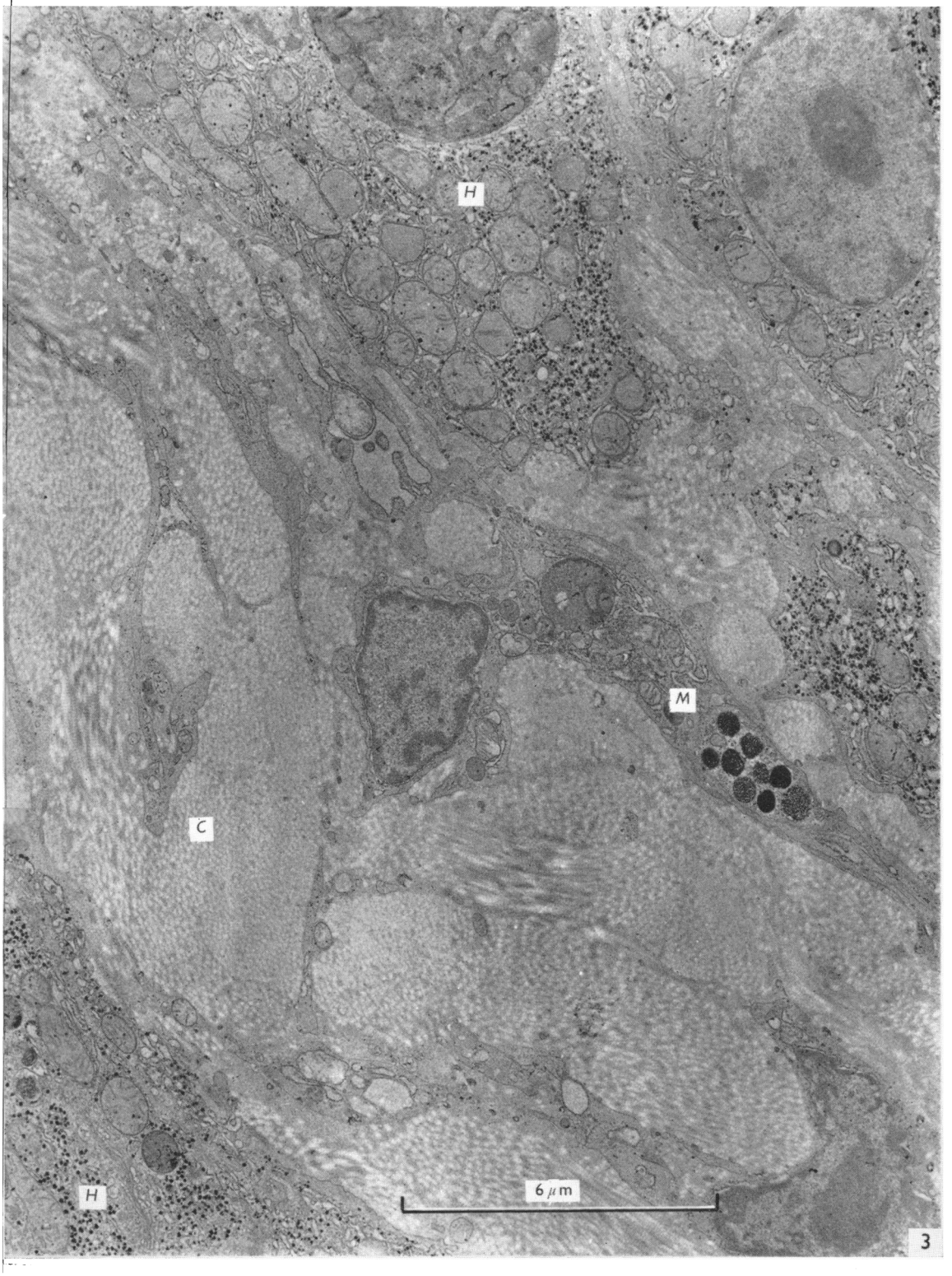


Fig. 3. Interlobular septum of adult pig liver. Thick bands of collagen (*C*) are shown, together with a macrophage (*M*). Adjoining hepatocytes (*H*) possess rather irregular basal surfaces and rest directly on the septum.

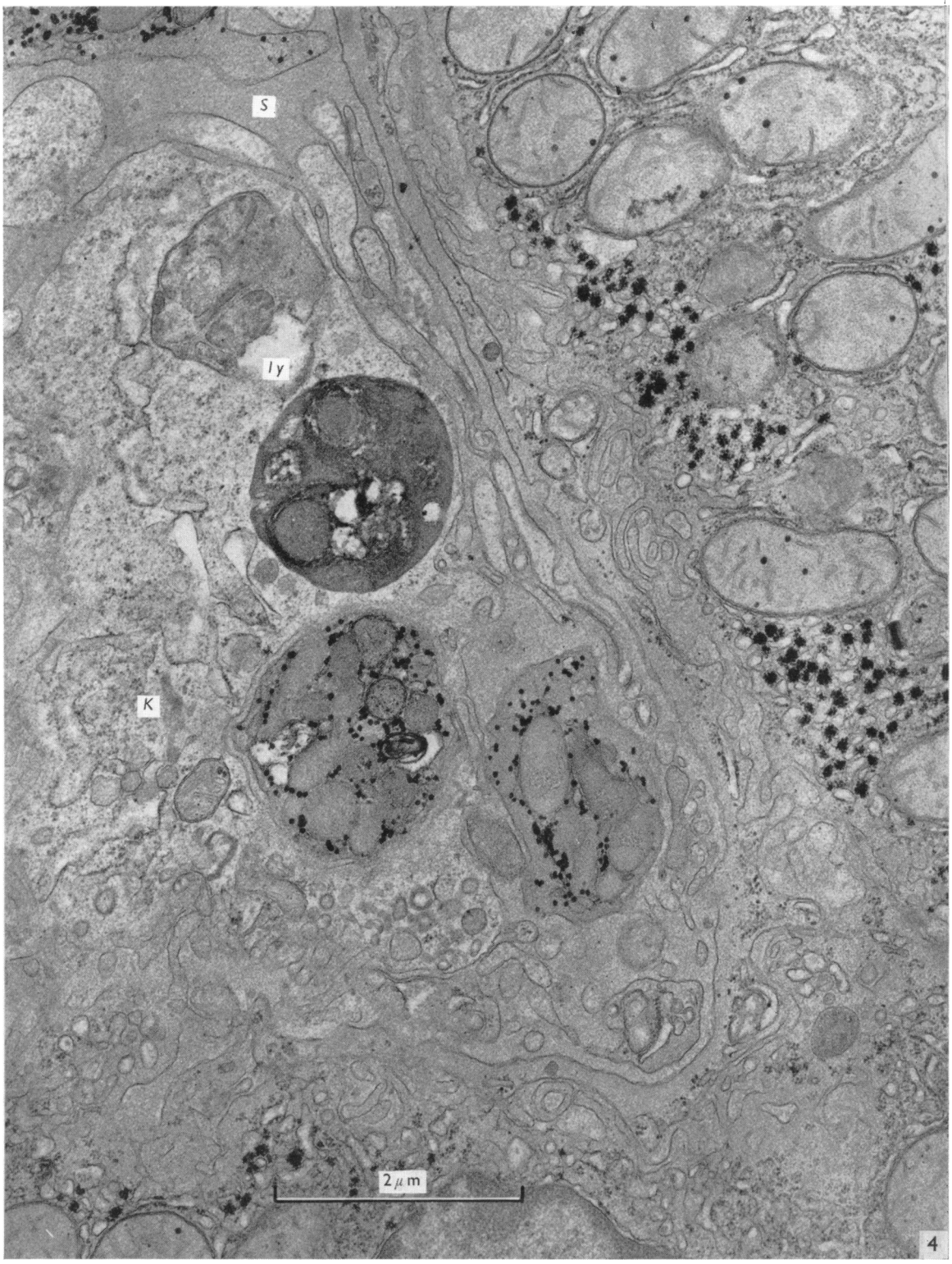


Fig. 4. Hepatic sinusoid of adult pig (*S*). The hepatic cell surfaces facing the narrow space of Disse possess numerous microvilli. A large portion of cytoplasm belonging to a Kupffer cell (*K*) is present in the sinusoidal lumen. This typically possesses large phagocytic vacuoles (*ly*) containing monoparticulate glycogen as well as partially lysed mitochondria and other cytoplasmic constituents.

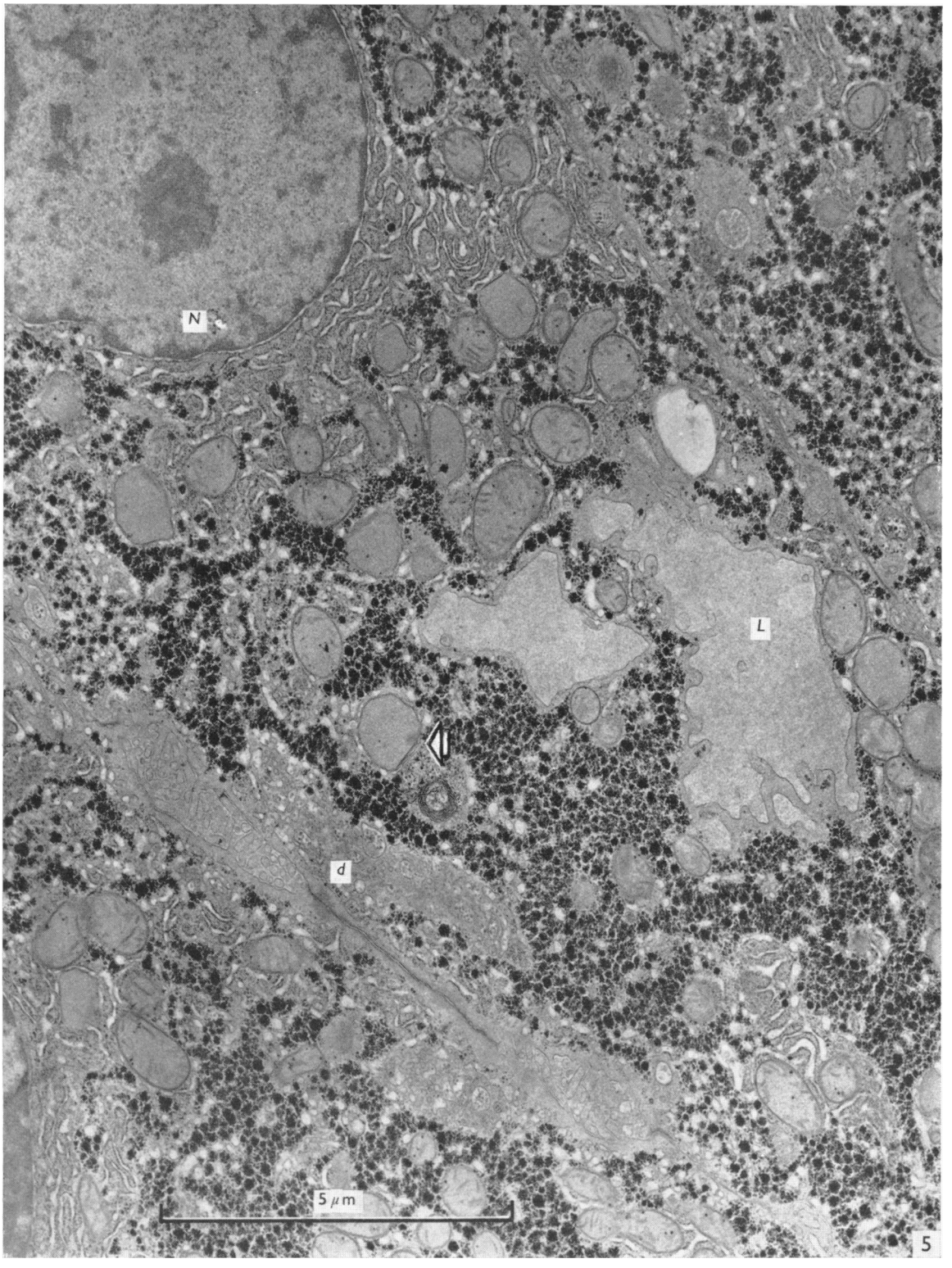
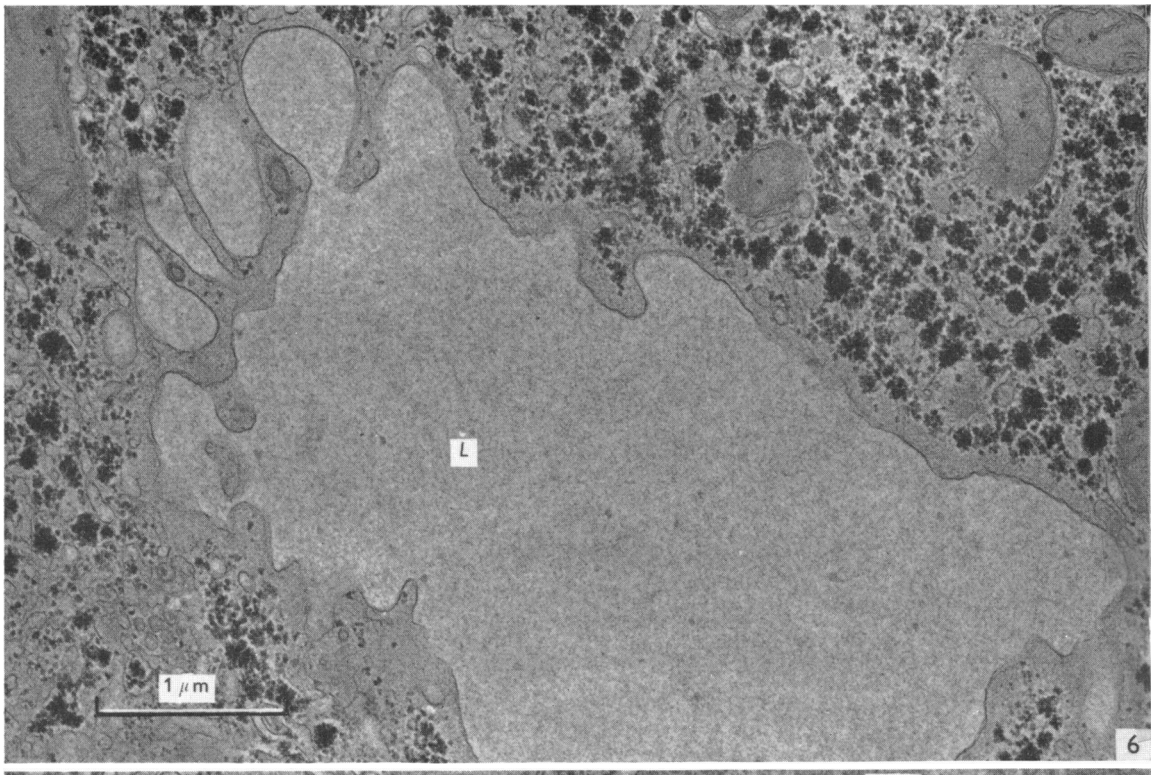


Fig. 5. Adult porcine hepatocytes. The unusual cytoplasmic lacunae (*L*) are shown here. Their large size may be judged by comparison with the nucleus (*N*). A desmosome (*d*) is present near the bile canaliculus and a 'faceted' microbody is shown (arrow).



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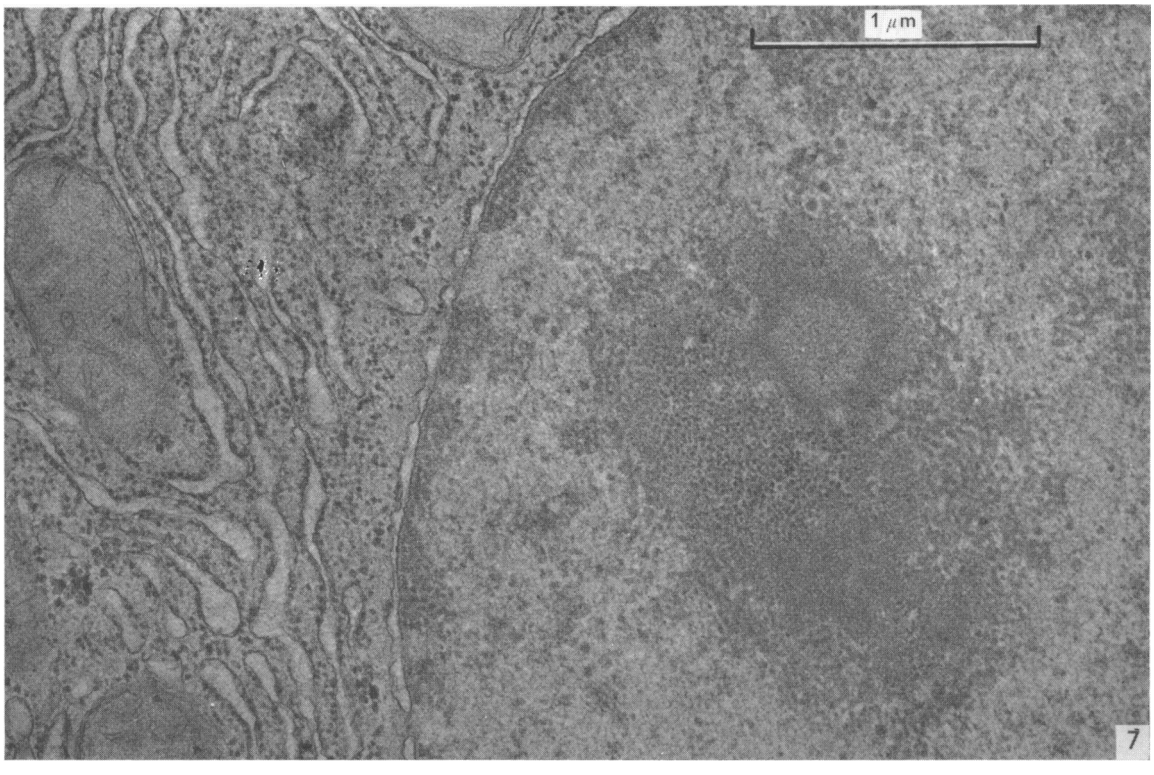

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adjacent to the membrane is devoid of organelles, as is the plasma membrane at the space of Disse.

The mitochondria resemble those of the hepatocytes of the rat. The microbodies, however, generally lack a distinct nucleoid, thus resembling those of man. Forms of microbody are occasionally observed which possess a single linear 'facet' on their surfaces (Fig. 5). Lipid droplets are frequently present in the cytoplasm, sometimes in direct contact with mitochondria, as has been observed previously by other workers (David, 1964; Palade, 1959).

The nuclei are round or oval in section and possess one or more prominent nucleoli. The latter are often skein-like in appearance, with well-defined pars amorpha and nucleolonema (Fig. 7). The perinuclear cisternae are interrupted by numerous typical nuclear pores. The morphology of the hepatocyte during mitosis (Fig. 8) is rarely observed, but the mitotic apparatus resembles that of other cell types with respect to its spindle tubules and chromosomes.

Examination of the hepatocytes from neonatal porcine livers reveals little gross difference between their fine structure and that of adult cells. There is, however, a relative scarcity of glycogen and agranular endoplasmic reticulum, and there are very few microbodies. On the other hand, there are more free ribosomes, which are disposed as polysomal aggregates. The Golgi zones are small, contain relatively little granular material and are associated with fewer lysosomes. The granular endoplasmic reticulum is present as dispersed, anastomosing cisternae (Fig. 9), parallel arrays being uncommon: its disposition is more diffuse than in the adult. The main difference between adult and neonatal hepatocytes is observed at the cell surface, where the surfaces of adjoining cells are frequently covered by interdigitating processes (Fig. 10). Prominent desmosomes are common, more so than in the adult, and the peripheral cytoplasm also sometimes contains numerous microtubules. The other organelles of the neonatal hepatocyte resemble those of the adult.

DISCUSSION

The general resemblance of the fine structure of the adult porcine hepatocyte to that of other mammalian hepatocytes has been noted. Since the fine structure of the liver has been extensively reviewed by David (1964) and Oudea & Domart-Oudea, (1967), discussion will be confined mainly to those features which appear to be characteristic of the pig.

In its typical distribution and anastomosing form the granular endoplasmic reticulum in the pig liver cell differs from that in rat or mouse liver cells (Bruni & Porter, 1965; Fawcett, 1955), where large parallel arrays of cisternae are common. It does, however, bear a resemblance to the granular endoplasmic reticulum of human liver (Brown, Delor, Greider & Frajola, 1957; Wills, 1968*a*, *b*). Similarly, the agran-

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Fig. 6. Detail of cytoplasmic lacuna, showing the homogeneity of the finely granular contents.

Fig. 7. Adult porcine hepatocyte nucleus showing the typical nucleolar morphology and the perinuclear cisternae, interrupted by nuclear pores. The perinuclear cytoplasm contains characteristically irregular cisternae of the granular endoplasmic reticulum.

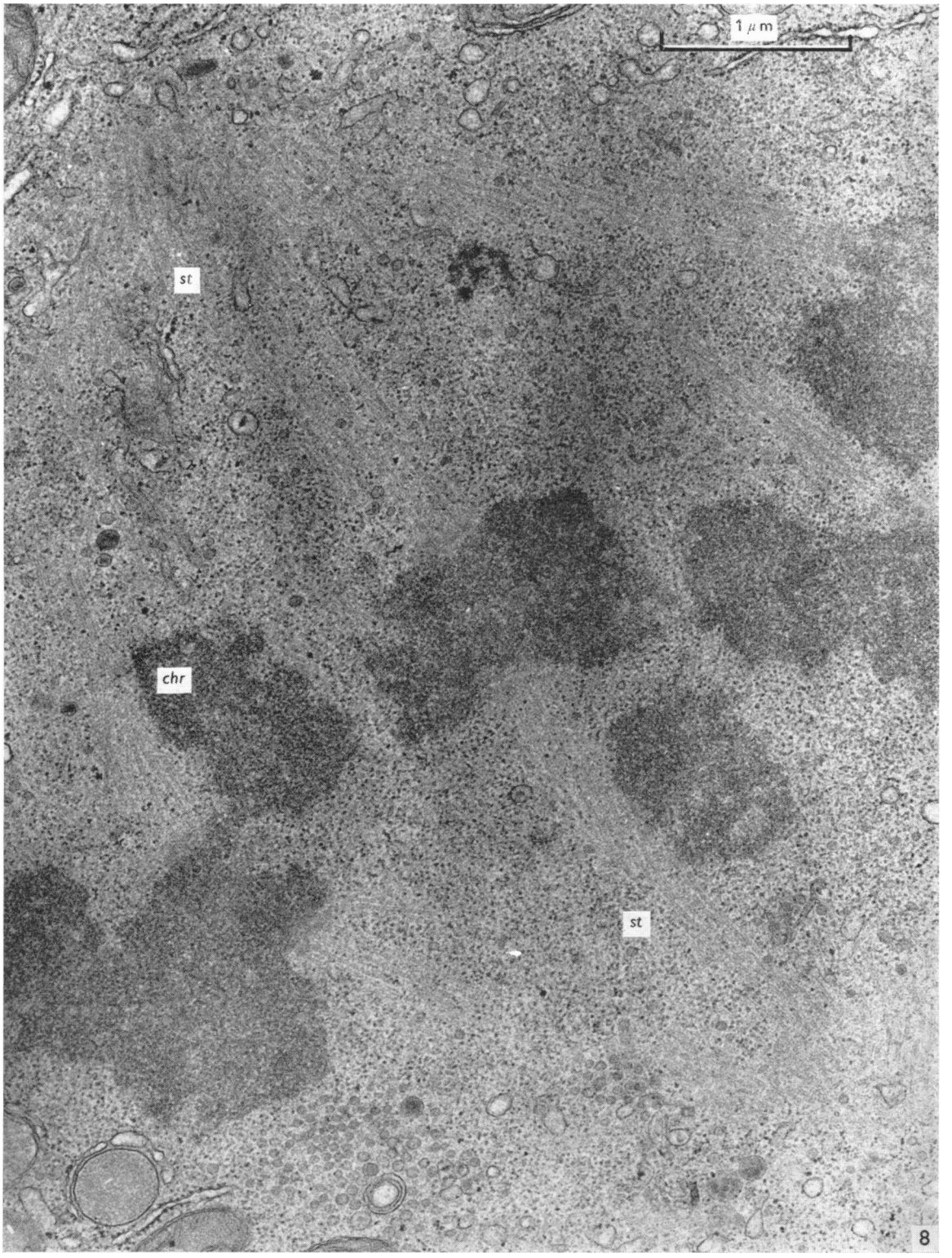

Fig. 8. Adult porcine hepatocyte in mitosis, showing the chromosomes (*chr*) and the spindle tubules (*st*).

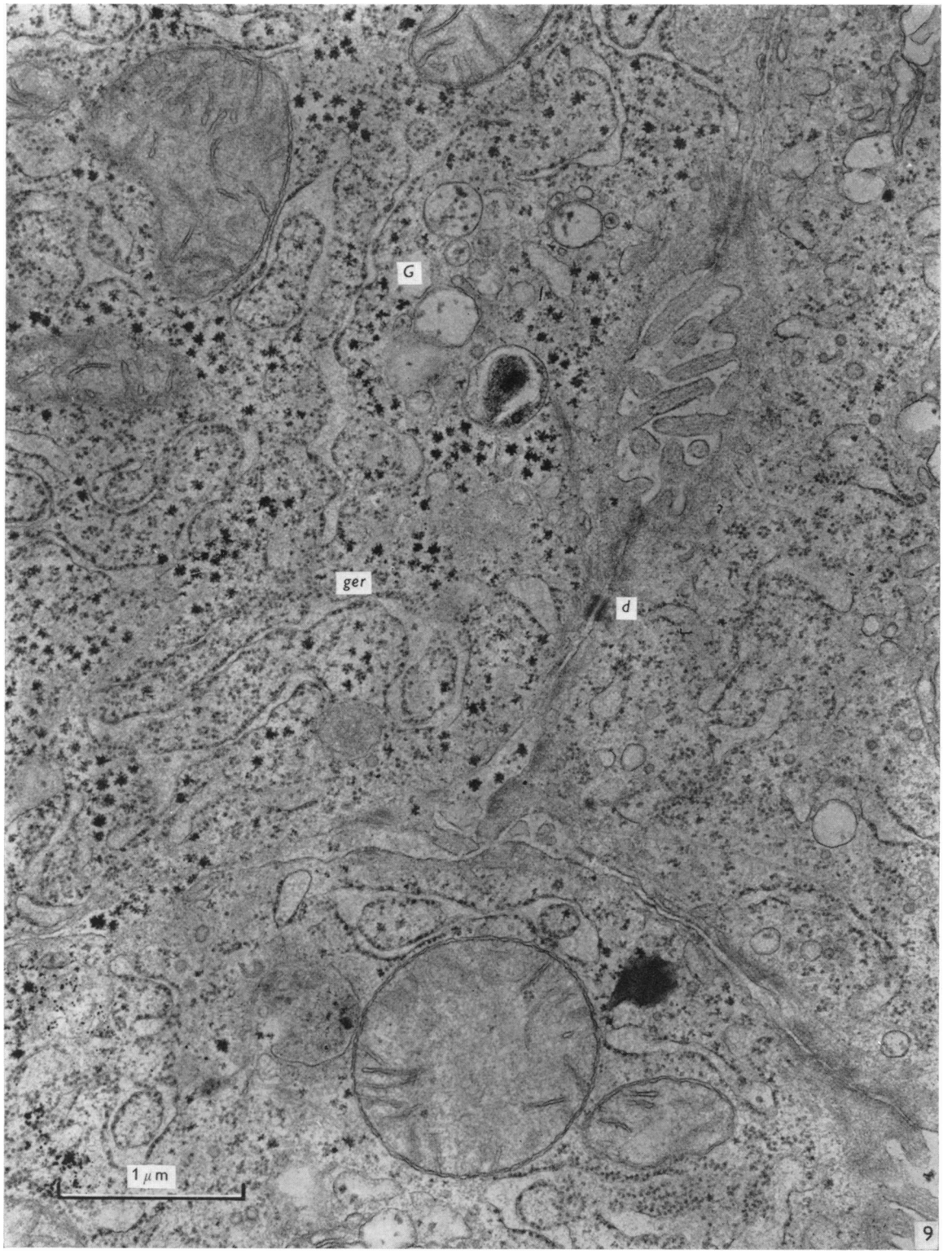

Fig. 9. Twelve-hour neonatal porcine hepatocyte cytoplasm. The granular endoplasmic reticulum (*ger*) is more irregular in form and more frequently anastomosing than that of the adult. Glycogen is relatively scarce and the agranular endoplasmic reticulum appears to be absent. A Golgi zone (*G*) containing granular material is present near a bile canaliculus. A prominent desmosome is shown (*d*).

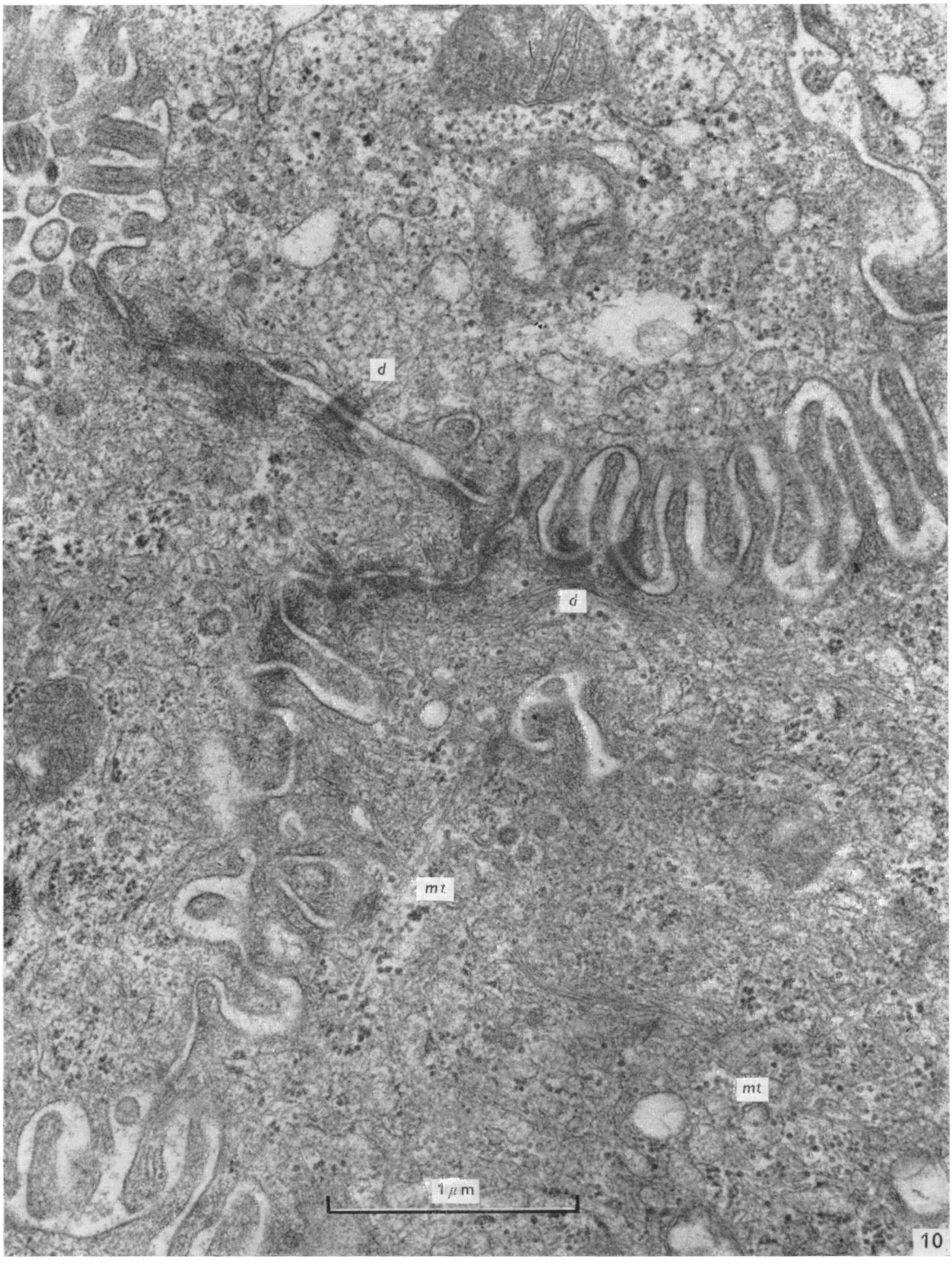

Fig. 10. Twelve-hour neonatal pig liver. The junction between three hepatocytes is shown. The apposed cell surfaces possess irregular interdigitating processes and desmosomes are frequently present (*d*). The cytoplasm contains microtubules (*mt*), apparently orientated at random.

ular endoplasmic reticulum of the pig hepatocyte resembles the human organelle, and lacks the narrow, branching tubular form observed in the rat.

The large cytoplasmic lacunae (Figs. 5, 6) which are present in many pig liver cells do not appear, solely on the evidence obtained, to be mere invaginations of the cell surface. They do not bear microvilli, the contents are homogenous in appearance and never include any cellular debris which might otherwise suggest that they are extensions of the space of Disse. However, although continuity of the lacunar lumen with the space of Disse or the bile canaliculi has not been observed, the possibility cannot be excluded that one or other may in fact occur, especially in view of the absence of organelles from the cytoplasm adjacent to both the lacunar membrane and the plasma membrane, which raises the probability that the lacunae may be derived from, if not continuous with, the space of Disse. The connexion, if present, may be transient in nature or so small that it is not easily obtained within a thin section. It is difficult, otherwise, to relate these structures to any of the normal cytoplasmic organelles. Their function remains obscure, but they appear to be unique to the porcine hepatocyte. The significance of the faceted microbodies (Fig. 5) is also obscure.

The appearance of the sinusoids of the pig liver is unusual. Characteristically, much of the lumen is occupied by cytoplasmic processes of the Kupffer cells, which contain distinctive phagosomes. In other species the hepatic sinusoids generally possess only a thin lining of Kupffer cell cytoplasm. The relatively narrow sinusoidal lumen of the pig, observed during this study, might be expected to impede the flow of blood. It is possible, however, that the lumen is wider during life, and the Kupffer cell cytoplasm more attenuated, and that partial contraction of the sinusoids may occur at the time of death, when tissue is taken for preparation for electron microscopy. Even the most gentle handling of the exposed liver of the anaesthetized pig rapidly results in readily visible areas of localized ischaemia. This does not appear to occur in most other species, and it might explain the unusual appearance, under the electron microscope, of the liver sinusoids in the pig.

The fine structural changes occurring in porcine hepatocytes during the transition from foetal to early neonatal life have been described by Bischoff *et al.* (1969). In general, the observations made here on the 12 h neonatal liver are broadly similar to theirs. However, the surface interdigitations and the abundance of desmosomes, frequently observed in the present study, have not previously been described. The common occurrence of numerous cytoplasmic microtubules also appears to have escaped notice. It is possible that these may represent the remains of the mitotic spindle.

Although the Golgi zones of the neonate are small relative to the adult, the presence within their elements of some granular material and their association with at least the occasional lysosome (Fig. 9) indicates that some degree of functional activity is present (Mollenhauer & Whaley, 1963).

SUMMARY

The fine structure of the porcine hepatocyte resembles, in most respects, that of other mammalian species. In particular, the endoplasmic reticulum is morphologic-

ally closely similar to that of man, as are the microbodies in so far as they lack distinct nucleoids.

However, there are large cytoplasmic lacunae which appear to be peculiar to the pig hepatocyte, although their significance is unknown. 'Faceted' microbodies are sometimes observed; these structures do not appear to have been described previously in normal hepatocytes and also seem to be characteristic of the pig. The Kupffer cells present a distinctive appearance in having abundant cytoplasm which frequently occupies a large part of the sinusoidal lumen. They also possess large lysosomal bodies containing monoparticulate glycogen in addition to partially lysed cellular debris. Thick interlobular septa were observed; these are well known from light-microscopical studies to be characteristic of the pig liver.

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