J. Anat. (1974), **117**, 3, pp. 619–634
With 11 *figures*
Printed in Great Britain

The developing human fetal pancreas: an ultrastructural and histochemical study with special reference to exocrine cells

MATTI LAITIO, ROBERT LEV AND DONALD ORLIC

Department of Pathology, University of Turku, Finland, and the Departments of Pathology and Anatomy, New York Medical College, Valhalla, New York

(*Accepted* 14 *March* 1974)

INTRODUCTION

Although the adult human pancreas (Ekholm & Edlund, 1959; Kern & Ferner, 1971; Lacy, 1962; Like, 1967) and the fetal islet tissue (Björkman *et al.* 1966; Hellman, 1965/1966; Wellmann, Volk & Brancato, 1971; Like & Orci, 1972) have been the subject of numerous electron microscopic studies, little is known of the ultrastructure of the developing human exocrine pancreas (Like & Orci, 1972).

The purpose of the current study was to investigate zymogen granule production during the middle trimester of fetal life, which is the period of acinar cell maturation (Liu & Potter, 1962), and to compare the ultrastructural characteristics of the granules and of the organelles involved in their production, i.e. the rough endoplasmic reticulum and the Golgi apparatus, with the corresponding adult pancreatic acinar organelles. Histochemical procedures were employed to complement the electron microscopic findings.

MATERIALS AND METHODS

For electron microscopy fragments of pancreas were obtained from 7 fetuses ranging from 9 to 20 fetal weeks (Table 1) within 1–2 minutes following hysterotomy. In fetuses up to 14 weeks estimates of fertilization age were based primarily on crown–rump lengths; after that greater emphasis was placed on crown-heel lengths and body weights. The specimens were placed in 3% glutaraldehyde in 0·1 M cacodylate buffer (pH 7·4) for 2 hours, post-fixed in phosphate-buffered 1% osmium tetroxide (pH 7·4) for 90 minutes, dehydrated in a graded series of alcohol solutions and propylene oxide and embedded in Epon (Luft, 1961). Sections 0·5–1·0 μm thick were stained with toluidine blue (pH 7·2) or examined in the unstained state using phase microscopy. Thin sections were stained with lead citrate (Reynolds, 1963) and uranyl acetate (Watson, 1958) and examined with a Siemens IA electron microscope.

For histoenzymatic studies adjacent segments of fresh pancreas from 3 fetuses between 15 and 20 weeks (numbers 5–7, Table 1) were quick frozen and cryostat-sectioned at 6–8 μm. Enzymes studied included: (i) alkaline phosphatase which demonstrates the ductal surface coat (Frexinos, 1968), (ii) acid phosphatase, a lysosomal marker, (iii) leucine aminopeptidase (= naphthylamidase) and non-specific

Table 1. *Data on 7 fetuses examined with the electron microscope*

Fetus	Crown–rump length (cm)	Crown–heel length (cm)	Weight (g)	Estimated fertilization age (weeks)
1	4·0	5·0	5	9
2	8·0	11·7	32	12
3	10·0	15·0	66	13·5
4	10·5	16·0	80	14
5	11·5	17·5	96	15
6	12·0	18·5	125	16
7	17·5	27·5	250	20

esterase, which are present in developing acinar cells (Burstone & Folk, 1956; Chessick, 1953) and (iv) thiamine pyrophosphatase, a Golgi marker. The methods used have been detailed previously (Lev, Siegel & Bartman, 1972).

For other histochemical studies, paraffin sections of pancreases from 12 fetuses between 12 and 35 weeks, obtained during an earlier study, were stained with hematoxylin and eosin for the demonstration of general morphology, with chromotrope 2R or the dihydroxy-dinaphthyl-disulfide method for protein (Lev & Gerard, 1967) and with the periodic acid-Schiff reaction following diastase digestion (D-PAS) (Lillie, 1965) for neutral glycoproteins. These stains were used with the object of determining the time of appearance of exocrine acini and of their zymogen granules.

RESULTS

Light microscopy

Epon sections. At 9 weeks the pancreas consisted of tubules and solid nests of undifferentiated epithelial cells surrounded by abundant loose connective tissue (Fig. 1*a*). The tubules were lined by tall columnar cells containing large oval nuclei. In some areas solid epithelial outgrowths were found; no cytological differences were observed between the epithelial cells in the tubules and their outgrowths. Mitoses were seen not infrequently in the epithelial cells. No zymogen granules were identified at this stage.

At 12–14 weeks numerous outgrowths from the tubules were seen (Fig. 1*b*), forming early lobules. At this stage differentiated endocrine cells were more numerous than differentiated exocrine cells, although both were rare. Both types of cell were randomly distributed in the peripheral regions of the early outgrowths and were also found singly among the epithelial cells lining the tubules.

During the 14–20 weeks stage lobular configuration became more apparent. The interstitial connective tissue became progressively less conspicuous with age. Well-formed exocrine acini began to appear at 14–16 fetal weeks and matured rapidly thereafter. The acinar cells exhibited a progressive increase in the number of zymogen granules and in cytoplasmic basophilia with toluidine blue (Fig. 1*c*). The latter feature is believed to reflect the presence of ribonucleic acid and is presumably related to the increase in rough endoplasmic reticulum found in electron micrographs

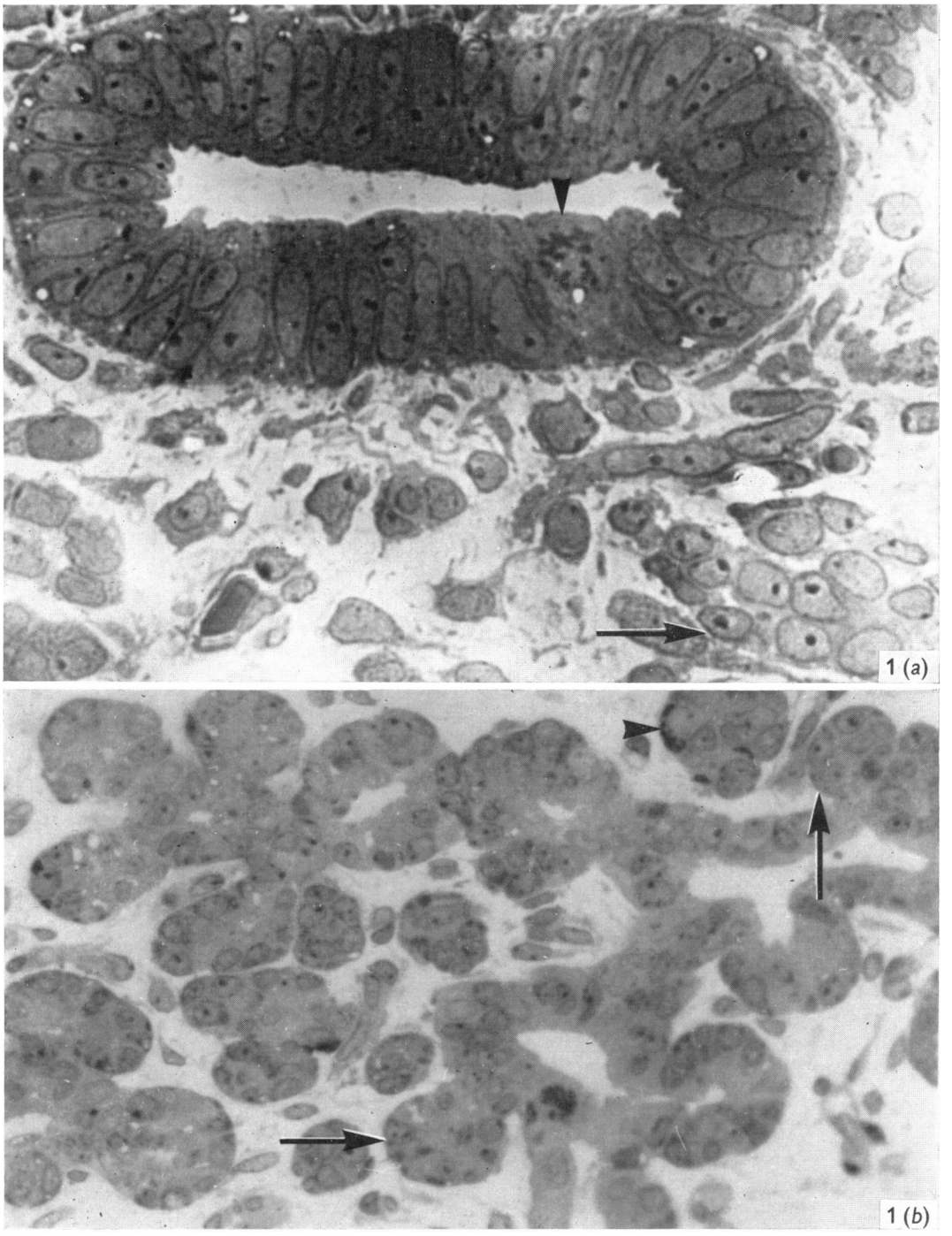


Fig. 1. Light micrographs of Epon-embedded 0·5 μm sections of developing human fetal pancreas. Sections have been stained with toluidine blue. (*a*) Nine fetal weeks. At the top is a small duct lined by tall columnar epithelium containing elongated nuclei and a mitotic figure (arrowhead). Solid islands of similar cells are seen in the loose connective tissue (arrow). × 250. (*b*) Twelve weeks. Pushing out from the small ducts are numerous clumps of epithelial cells (arrows). Some of these are recognized as endocrine cells (arrowhead). No zymogen granules are seen in this field. × 600. (*c*) Twenty weeks. There is extensive periductal proliferation. Note the clumps of darkly staining exocrine cells (EX) and scattered endocrine cells (ED). Numerous lightly staining duct cells and centroacinar cells are also to be seen. L, duct lumen; C, capillary. × 375.

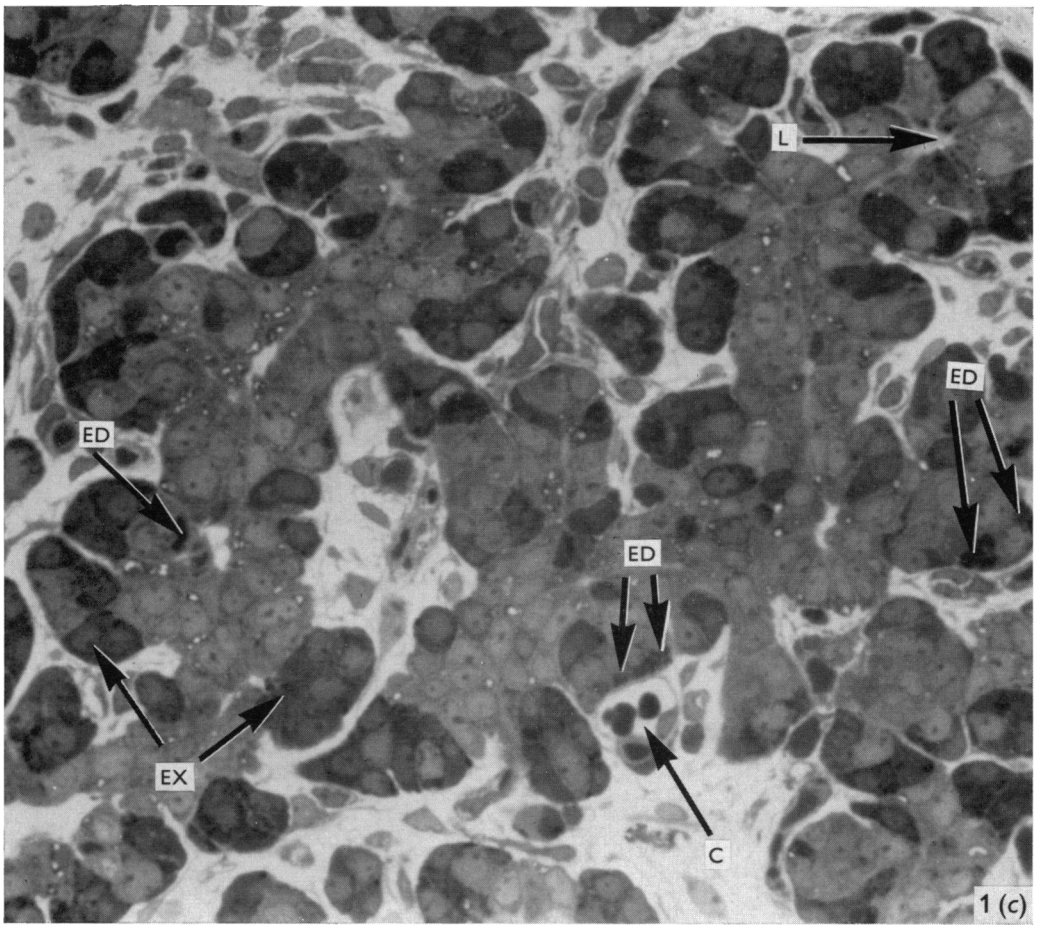


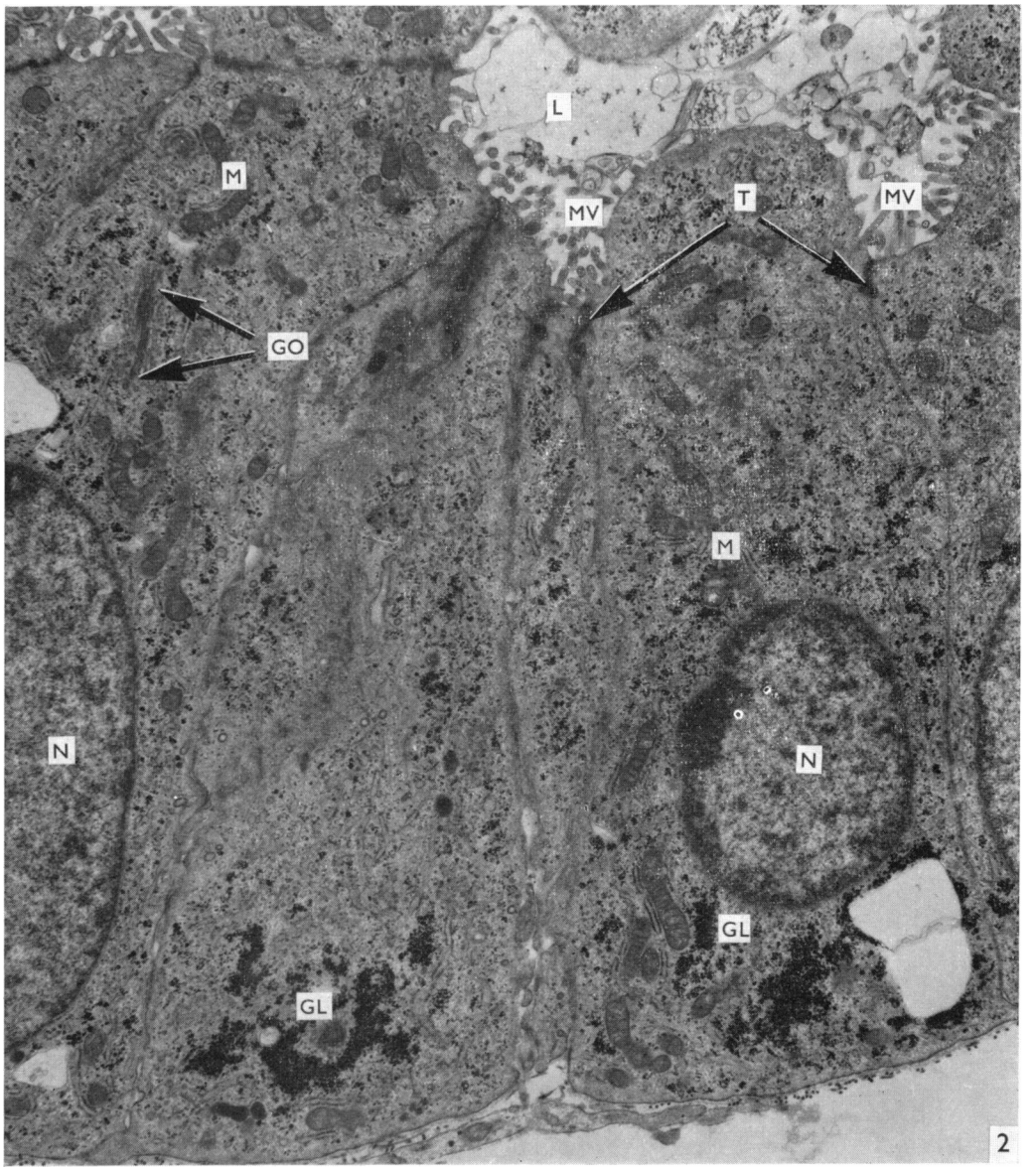
Fig. 1 *c*. For legend see p. 621.

of comparable tissue (see below). By 20 weeks well formed islets were also identified, although isolated endocrine cells persisted between the acinar cells.

Paraffin sections. Primitive exocrine acini were noted at the end of the third fetal month and matured rapidly during the succeeding 2 months. Although scattered zymogen granules could be identified in some acini by the end of the fourth month with the various protein and D-PAS stains, significant numbers of granules were not found until the fifth fetal month. The granules were very susceptible to autolysis and were difficult to demonstrate when the postmortem interval exceeded 2 hours.

Electron microscopy

Tubular cells. In the 9 week fetal pancreas no differences were noted between the epithelial cells lining the tubules and those in the solid islands. The tubular cells were tall columnar and their morphology was relatively constant in tubules of different diameters (Fig. 2). Their microvilli were numerous and slender and their cores



Figs. 2–11 represent electron micrographs of pancreas from 9 to 20 week old fetuses. Sections have been stained with uranyl acetate and lead citrate. Abbreviations: L, lumen; MV, microvilli; LM, lateral cell membrane; Z, mature zymogen granules; GO, Golgi apparatus; RER, rough endoplasmic reticulum; M, mitochondria; GL, glycogen; N, nucleus.

Fig. 2. Nine weeks. Section through a primitive duct and its lumen. Microvilli are numerous and fairly well developed. The cells display prominent tight junctional complexes (T) and straight lateral membranes which occasionally interdigitate. The nuclei are located in the basal regions of the cells. Glycogen aggregates are most prominent in the basal cytoplasm. Mitchondria are found throughout the cell but predominate in the apex; they are often in close association with the rough endoplasmic reticulum. The Golgi apparatus is evident in one of the cells. × 8250.

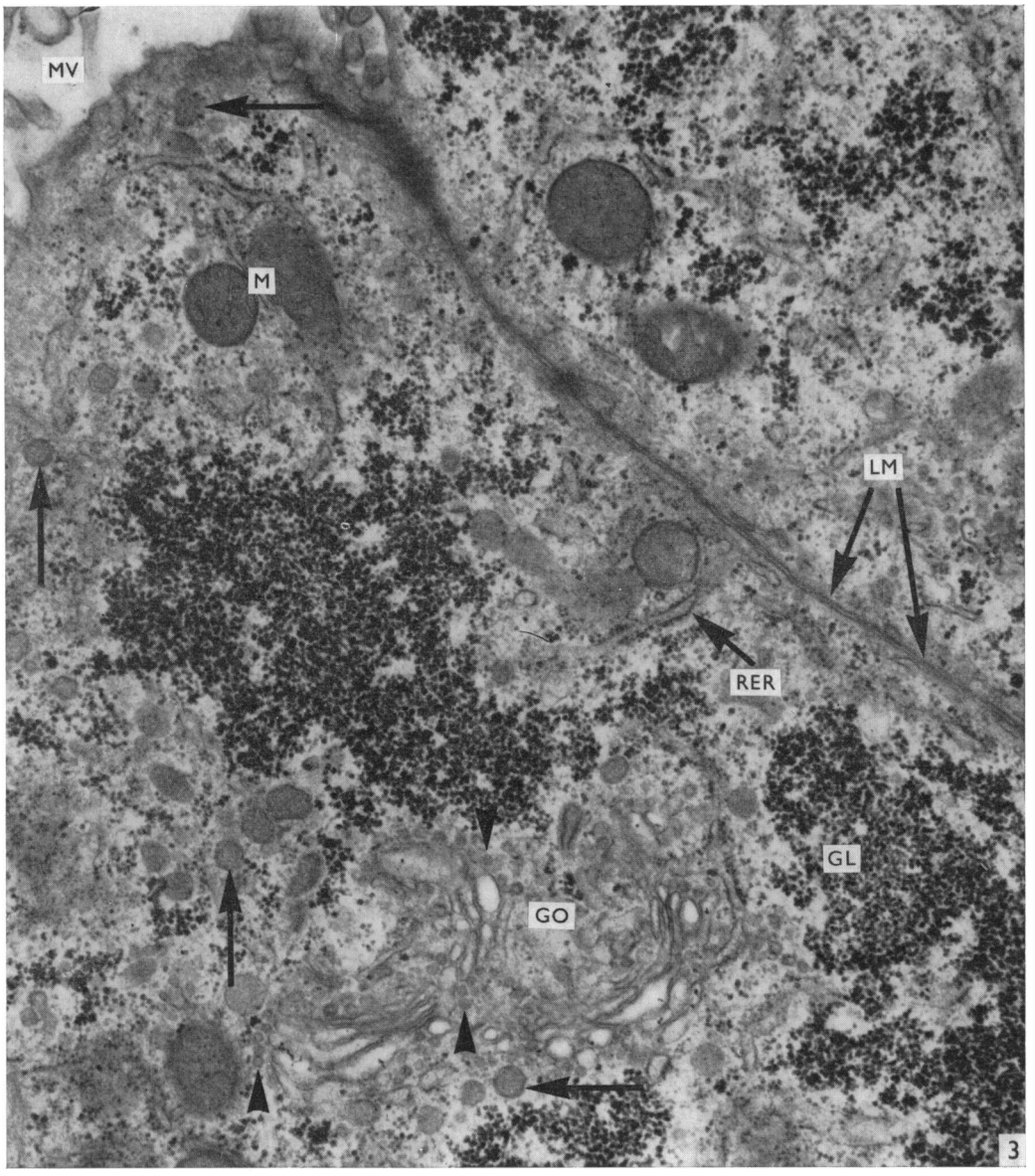


Fig. 3. Twelve weeks. Two adjacent cells from a group of proliferating periductal cells. One of these displays a prominent Golgi apparatus whose vesicles contain electron-opaque material (arrowheads). Slightly larger, but similar, secretory granules (arrows) are also seen throughout the apical cytoplasm of the same cell which is thought to be a developing acinar cell. × 30000.

contained longitudinal filaments which extended into the subjacent apical cytoplasm. Junctional complexes were prominent. The lateral membranes were nearly straight and did not exhibit interdigitations. The nuclei showed occasional indentations of the nuclear membrane, and generally contained prominent nucleoli. The Golgi complex was not particularly well developed (Fig. 2). No secretory granules were

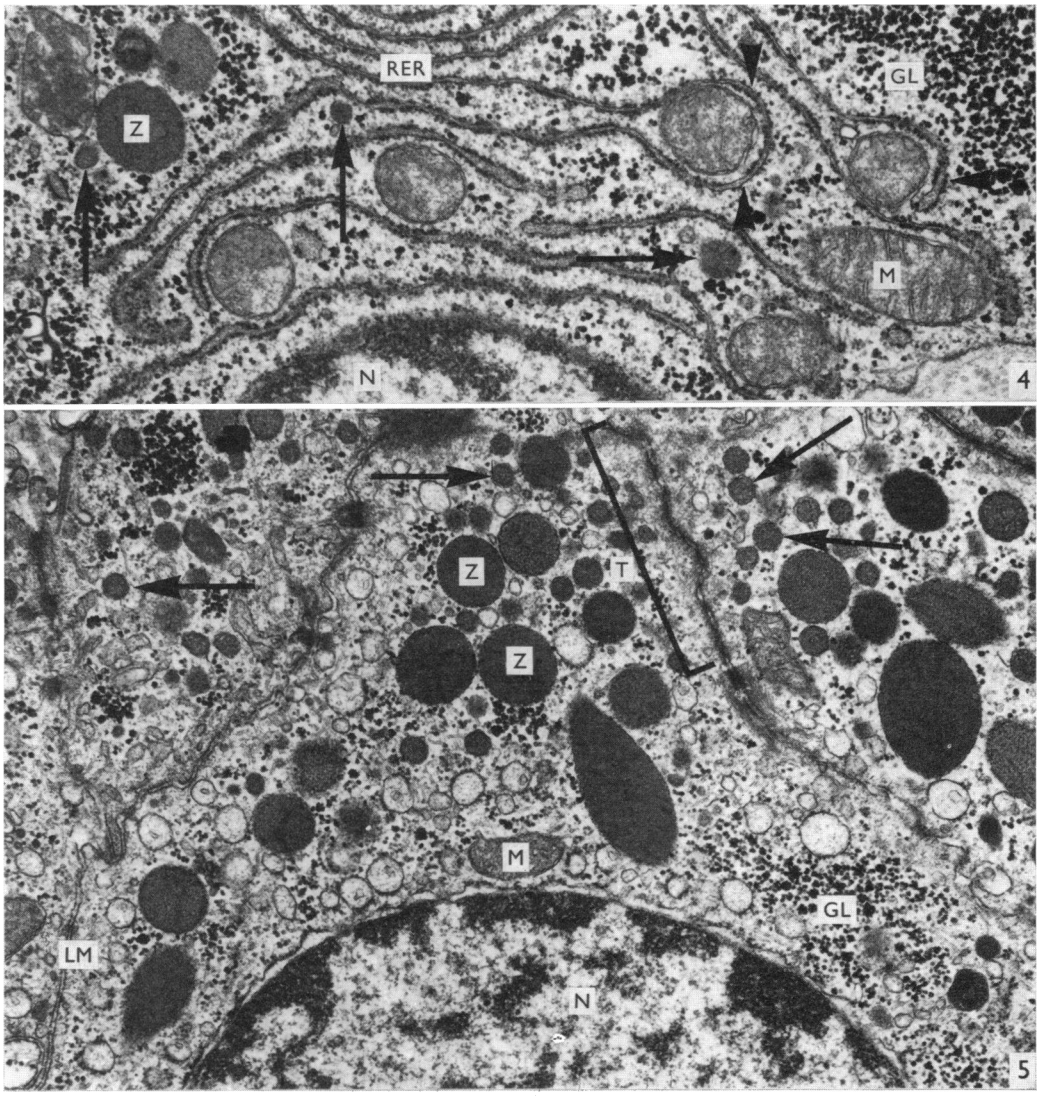
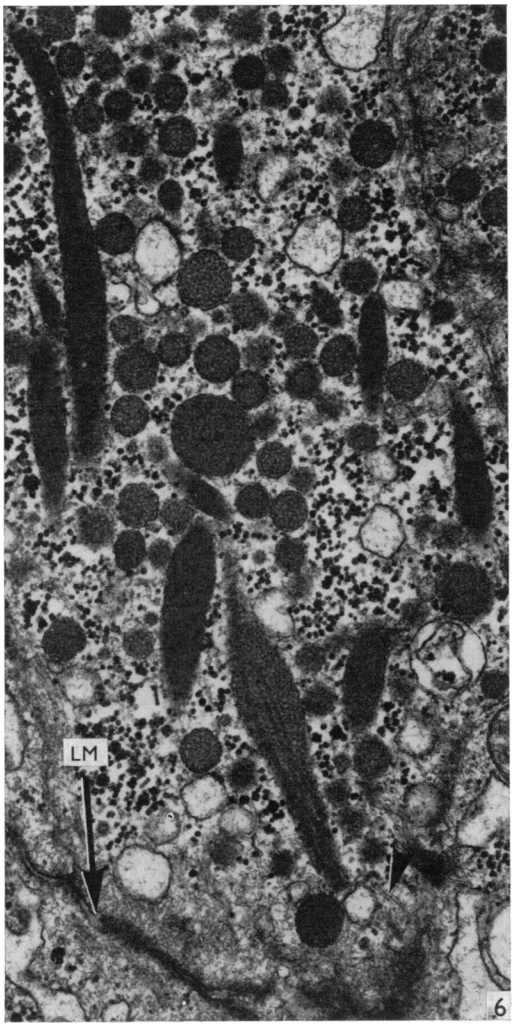


Fig. 4. Fourteen weeks. Developing acinar cell. One mature zymogen granule is seen and in addition there are numerous small secretory granules (arrows). Numerous mitochondria surrounded by rough endoplasmic reticulum (arrowheads) are also present. × 21 000.

Fig. 5. Fifteen weeks. Developing acinar cells. There are scattered spherical mature zymogen granules and several spindle-shaped granules. In addition there are many smaller granules (arrows) which are generally less electron-dense than the mature granules. A well-developed junctional complex (T) should be noted. × 20 250.

identified. Mitochondria were found throughout the cell and were often surrounded by profiles of rough endoplasmic reticulum; this feature was more prominent in older fetuses (Figs. 4 and 9 inset). Small numbers of free ribosomes were scattered throughout the cytoplasm, as were glycogen aggregates. The latter were especially prominent towards the base of the cells (Fig. 2).



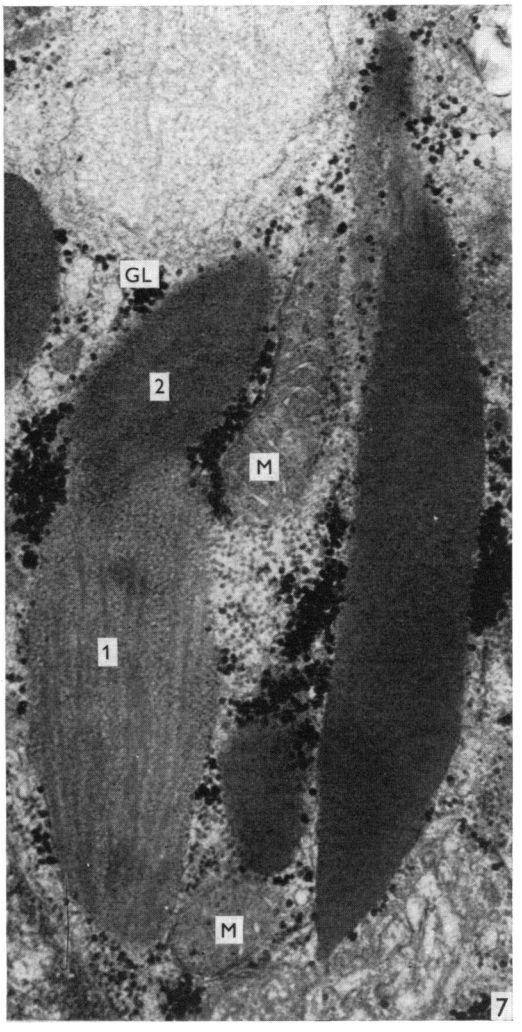


Fig. 6. Fifteen weeks. Developing acinar cell. There is a striking variation in the size, shape and electron density of the secretory granules. The small spherical granules predominate here. A microtubule (arrowhead) is noted. ×20250.

Fig. 7. Twenty weeks. Acinar cell. Higher magnification of the spindle-shaped granules, which contain granular material and longitudinal fibrils. There are no limiting membranes between or surrounding granules nos. 1 and 2. ×35000.

Acinar cells. At 12 fetal weeks a few young acinar cells were identified. These contained small zymogen-like granules in their apical cytoplasm which measured 0·3–0·6 μm in diameter and contained slightly electron-dense material (Fig. 3). Classical, mature zymogen granules were not seen at this time. The Golgi apparatus was more prominent than in the adjacent tubular cells. Granules smaller than, but morphologically similar to, the apically-located zymogen-like granules described above were noted in the vicinity of the Golgi cisternae (Fig. 3); these appeared to represent Golgi vacuoles filled with secretory material.

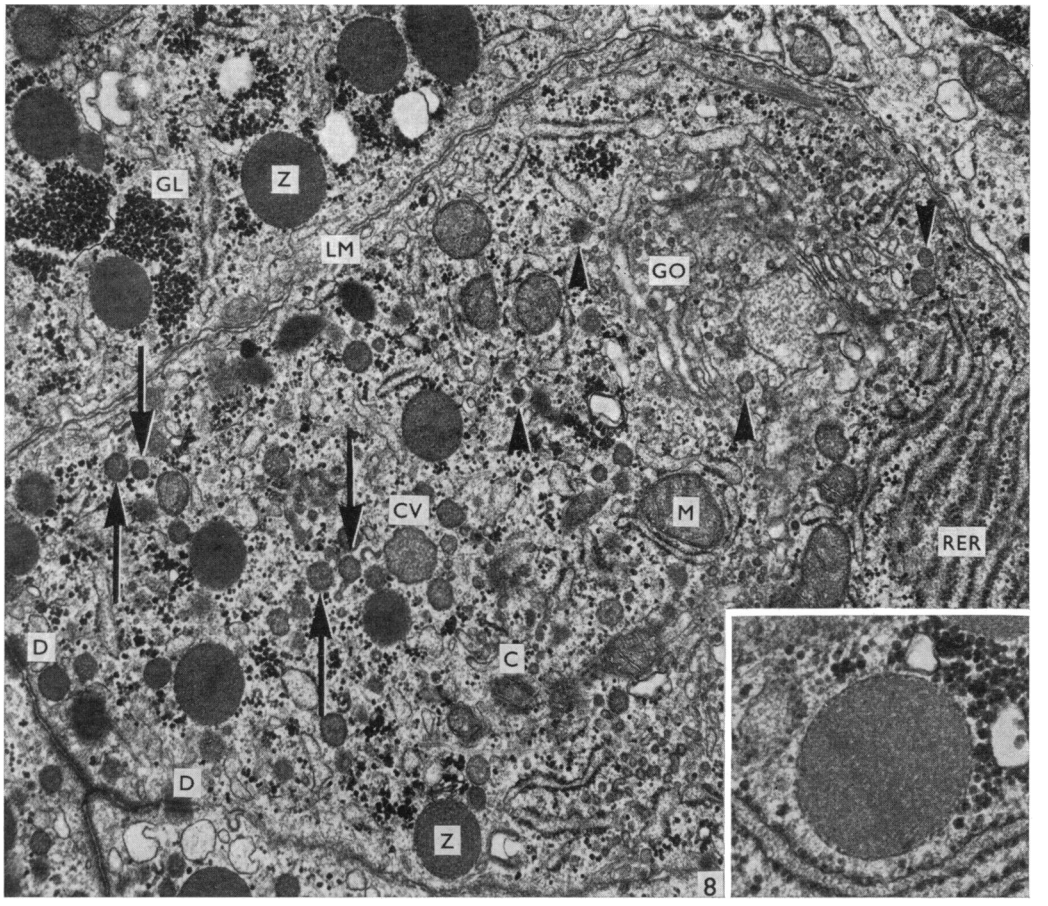


Fig. 8. Sixteen weeks. This section through the apical region of several adjacent acinar cells shows a prominent Golgi apparatus, a well-developed endoplasmic reticulum, scattered mature zymogen granules of different sizes, smaller granules (arrows) and a condensing vacuole (CV). Some of the enlarged Golgi vesicles contain granular material (arrowheads). A centriole (C) and several desmosomes (D) are also identified. × 20250.

Inset. Sixteen weeks. Acinar cell. Higher magnification showing a typical membrane-bound zymogen granule. × 36000.

During the 12–19 fetal week period there was a gradual increase in the number of mature zymogen granules in the developing acinar cells (Figs. 4–10). These granules were located between the Golgi cisternae and the apical surface membrane. Discharge of granules into the acinar lumen was never observed. The granules were round, membrane-bound, measured approximately 1 μm in diameter, and contained homogeneous, moderately electron-dense material (Figs. 4, 5, 8 and 9). Condensing vacuoles (Fig. 8) were also found. The small zymogen-like granules described above in the 12 week specimen were found throughout this period but their numbers showed a progressive decrease with fetal age. No transitions were found between these small granules and the mature zymogen granules. Another type of secretory granule was observed in acinar cells of the younger (12–16 fetal week) specimens. These granules

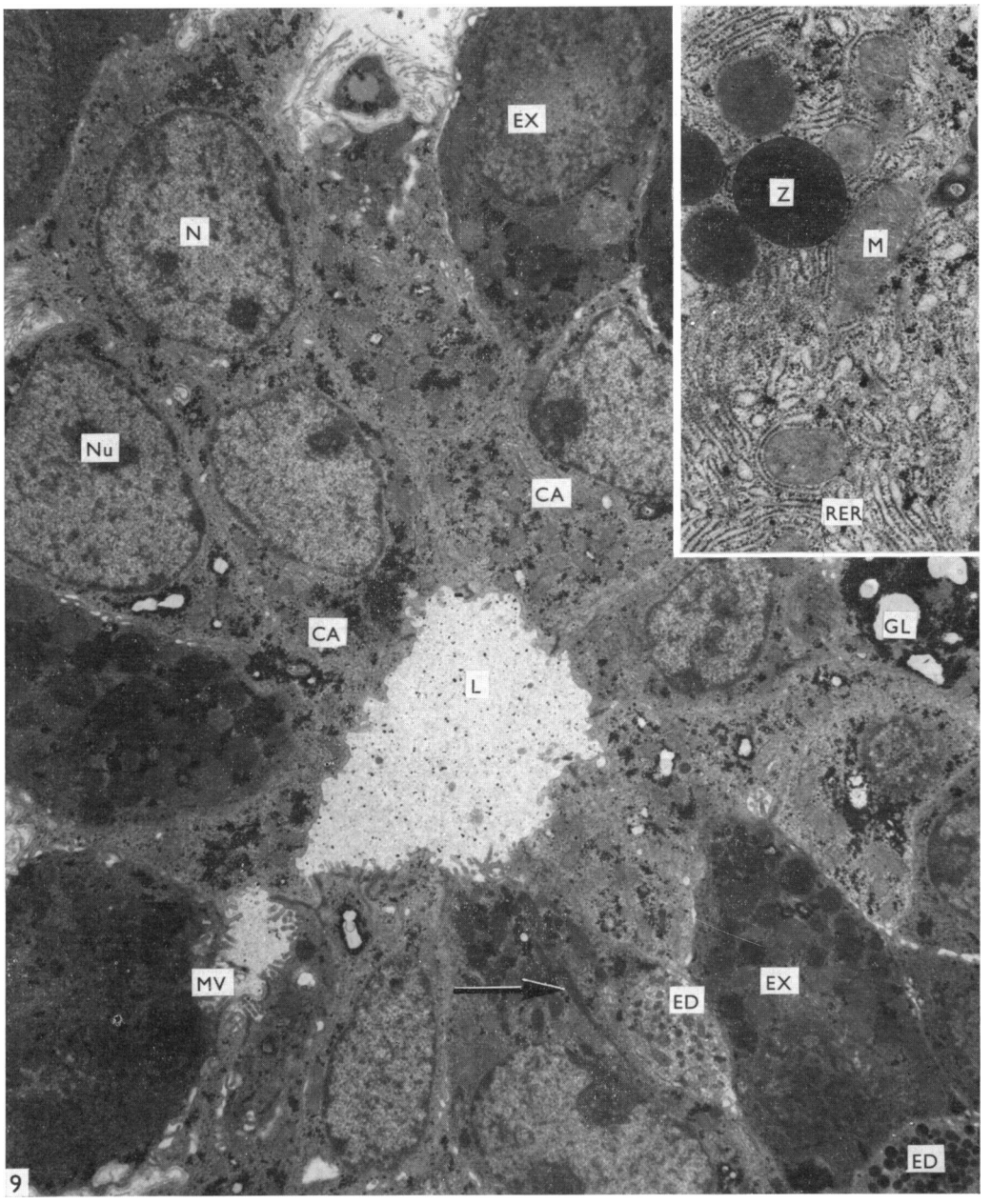


Fig. 9. Twenty weeks. This low power photograph shows several acinar (EX) and centroacinar (CA) cells lining the lumen of an acinus. The centroacinar cells possess fewer organelles and a less electron-dense cytoplasm than the acinar cells. Note the elongated secretory granules in one acinar cell (arrow). Several endocrine cells (ED) are also seen. ×4500.

Inset. The lamellar formation of rough endoplasmic reticulum is apparent here. ×13750.

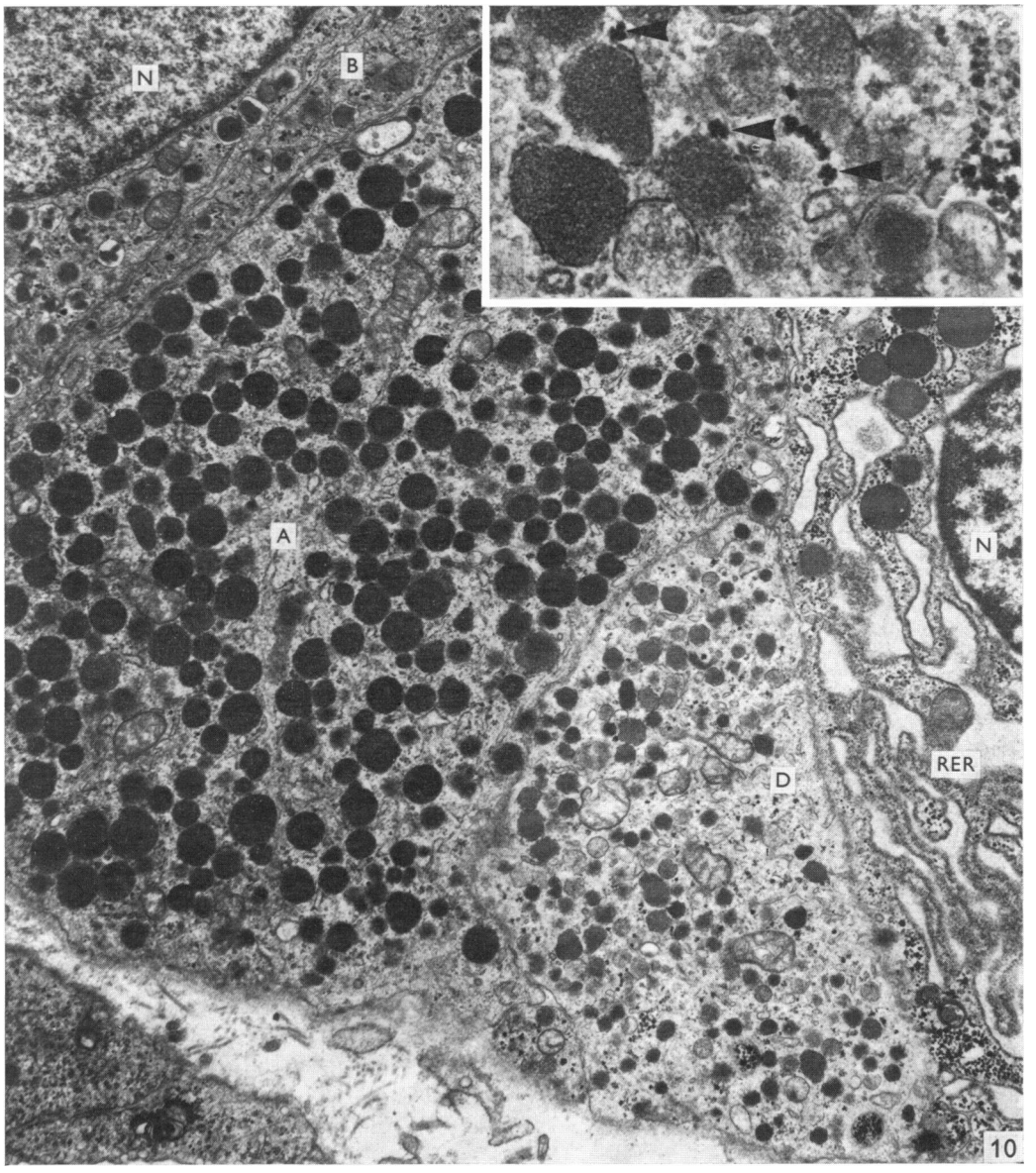


Fig. 10. Sixteen weeks. This electron micrograph is from a peripheral periductal area (similar to that shown in Fig. 1*b*). It shows portions of A, B and D endocrine cells and an acinar cell containing zymogen granules and dilated channels of rough endoplasmic reticulum. × 14625.

Inset. Higher magnification of D cell showing several granules and glycogen aggregates (arrowheads). × 56250.

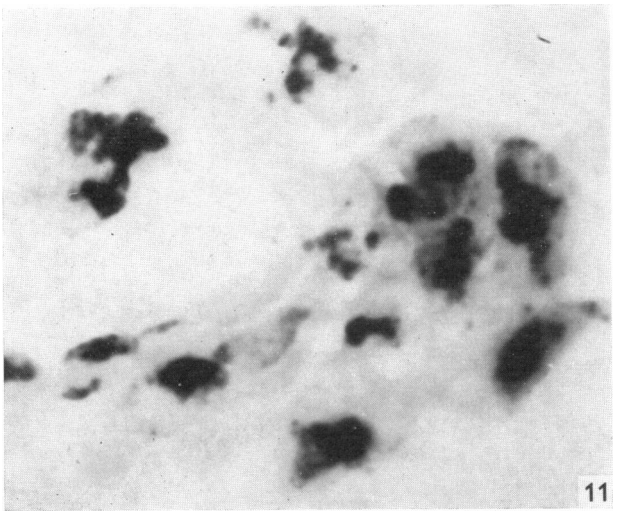


Fig. 11. Sixteen weeks. Dense reaction product is noted in isolated epithelial cells and in a clump of epithelial cells constituting an exocrine acinus. The non-reactive cell background appears grey. Incubated for non-specific esterase for 30 minutes. × 375.

were elongated and spindle-shaped. They measured up to 8 μm in length and contained longitudinal fibrils (Figs. 6 and 7). In some instances apparent fusion of these elongated granules was observed (Fig. 7). During the maturation of acinar cells, as mature zymogen granules became increasingly numerous, these elongated forms became scarcer but some were still present at 20 weeks (Fig. 9).

During acinar cell maturation there was a decrease in the number of free ribosomes and a concomitant increase in the amount of rough endoplasmic reticulum. The latter displayed areas of lamellar formation (Fig. 9 inset) in the base and lateral parts of the cell as in the adult pancreas. These lamellae often partly encircled mitochondria (Figs. 4 and 9 inset). Fewer mitochondria were found in the apex in these mature acinar cells than at earlier stages. The microvilli showed a slight, progressive increase in length with age and became more regularly arranged. The junctional complexes were somewhat less prominent than in the primitive undifferentiated tubular epithelial cells. Moderate amounts of glycogen were found in the acinar cells, especially around the apically-located zymogen granules (Figs. 8 and 9). Lysosomes were inconspicuous at all stages of development of acinar cells.

Centroacinar cells. These were more evident in the older specimens (Fig. 9) when well-formed acini were identifiable. Their microvilli were irregularly spaced and less well developed than those of the acinar cells. The lateral membranes were quite straight and presented no interdigitations. Golgi apparatus, endoplasmic reticulum and mitochondria were less well developed in the centroacinar cells than in the zymogen cells. Moderate amounts of glycogen were scattered throughout the cytoplasm.

Endocrine cells. Initially, in the 12–16 week stage endocrine cells appeared either singly or in small groups among tubular cells and their outgrowths. When discrete islets became numerous during the fifth month, most endocrine cells were found in

them. Identification of the 3 different endocrine cells types (Fig. 10) was made on the basis of the morphology of their secretory granules, the features of which have been described in great detail previously (Björkman *et al.* 1966; Like & Orci, 1972). In all three types of endocrine cells aggregates of glycogen were identified (Fig. 10 and inset), contradicting the findings of another group of workers (Like & Orci, 1972) although the amount of glycogen was less than that found in acinar or duct cells.

Histoenzymatic results. Control tissues (human intestine, rat liver) exhibited strong reactions for the various enzymes tested. When substrate was omitted from the incubation medium no reactions were observed in these tissues or in the pancreatic specimens.

Pancreatic duct epithelial surfaces did not show significant staining with alkaline phosphatase at any fetal age (although intestinal cells were strongly positive), even when the incubation period was extended from 20 minutes to 1 hour. The vascular endothelium in fetal pancreatic stroma, however, did stain: this could be eliminated by adding phenylanaline (0·05 M) inhibitor to the incubation medium.

Non-specific esterase, an acinar cell marker, was found in acinar cell cytoplasm in all age groups (Fig. 11); this reaction was eliminated by the addition of the organophosphate inhibitor E-600 (10^{-7} M) to the medium. Particulate reaction deposits of leucine aminopeptidase (naphthylamidase), another acinar cell marker, were also noted in these acinar epithelial cells. The latter reaction was slightly more intense in the 20 week specimen.

Thiamine pyrophosphatase was indicated by moderately intense, punctate deposits in the supranuclear cytoplasm of acinar cells. No increase was noted in staining intensity with increasing fetal age.

The acid phosphatase reaction product appeared as ill-defined reddish granules, mostly in the apical half of the acinar cells, and in stromal mononuclear cells. All staining was eliminated by adding NaF (0·01 M) inhibitor to the medium. Epithelial staining intensity did not vary with fetal age.

DISCUSSION

According to our observations and those of others (Liu & Potter, 1962), primitive pancreatic exocrine acini first appear during the third fetal month and mature rapidly thereafter. The results of electron microscopy and of the various protein stains applied in the current study indicate that significant numbers of mature zymogen granules appear in the fifth fetal month, which is in agreement with the histological and biochemical studies of previous authors (Keene & Hewer, 1929; Lieberman, 1966). Werner (1948), on the other hand, found no granules and only very low proteolytic activity before the seventh month, but this may have been due to rapid post-mortem autolysis as suggested by Koldovsky (1969): this view is supported by our observation that granule preservation is very poor if the post-mortem interval exceeds 2 hours.

Although proteolytic activity has been demonstrated in the fetal pancreas *in vitro*, it is not known if pancreatic enzymes contribute to *in utero* digestion of proteins or other substances in swallowed amniotic fluid. In one experiment designed to investi-

gate this, milk, starch, and to a lesser extent, meat introduced into the rat fetal stomach underwent some digestion *in vivo* (Hartmann & Wells, 1948). Studies of this type, but of a more quantitative nature, should be extended to primates. In addition, it has been established that the fetal intestine can absorb substances injected into the amniotic fluid such as protein (Lev & Orlic, 1972, 1973; Orlic & Lev, 1973) and iron (Orlic, Lev & Rosenthal, 1974).

Of special interest was the predominance of atypical zymogen granules at the onset of granule production in the fourth fetal month. Small granules similar to those observed in the present study have been found in the adult guinea-pig pancreas after prolonged stimulation *in vitro* with carbamylocholine and pancreozymin (Jamieson & Palade, 1971) and following X-ray treatment (Volk, Wellmann & Lewitan, 1966). It is possible that in all these instances the small granules represent precursors of mature zymogen granules, although there was no direct morphological evidence of such a transition in our material. In addition, elongated fusiform granules measuring up to 8 μm in length were identified in the present study; such granules have not been described earlier. In our specimens there was a suggestion of fusion of these granules, indicating that their growth may occur in this manner.

It has been well documented that the rough endoplasmic reticulum and the Golgi apparatus are both involved in the early stage of zymogen granule synthesis in the adult (Caro, 1961; Caro & Palade, 1964). In our fetal studies the rough endoplasmic reticulum, as visualized ultrastructurally, became prominent during the same period, namely the fourth fetal month, that (small) zymogen granules first became numerous. Likewise, the Golgi apparatus became increasingly conspicuous at this time, as indicated by electron microscopy and the histoenzymatic reaction for thiamine pyrophosphatase. The Golgi vesicles, moreover, contained granular material of about the same electron density as that of the small zymogen granules in the adjacent cytoplasm, suggesting that the granules do in fact originate in the Golgi region, as is the case in the adult pancreas (Caro, 1961; Caro & Palade, 1964).

It is not clear why the cell surface of the developing pancreatic duct system, in contrast to that of the adult (Frexinos, 1968), failed to exhibit significant alkaline phosphatase activity. It is certainly not due to absence or structural immaturity of microvilli, since these are well developed by the fifth fetal month. The absence of alkaline phosphatase may reflect functional immaturity of the duct system, but this is only a speculation.

Our observations regarding the origin of both acinar and islet cells from proliferating duct epithelium are in agreement with earlier authors (Bencosme, 1955; Hellman, 1965/1966; Like & Orci, 1972; Liu & Potter, 1962). In addition, in agreement with others working with human fetal pancreas (Like & Orci, 1972; Liu & Potter, 1962; Conklin, 1962), no transitions were found between exocrine and endocrine cells.

SUMMARY

The ultrastructural features of developing human fetal exocrine pancreas were examined in 7 specimens between 9 and 20 fetal weeks in age. Histochemical procedures were performed on 3 of these specimens and on 12 additional pancreases between 12 and 35 fetal weeks. No acinar cells or zymogen granules were found in

the 9 week specimen. By 12 weeks primitive exocrine acini were identified and these matured rapidly in the next 2 months. At the 12–15 week stage ultrastructural examination revealed a predominance of small zymogen granules and elongated granules in the acinar cells. These became progressively less numerous in the 16–20 week period when classical mature zymogen granules became increasingly prominent. The Golgi apparatus and rough endoplasmic reticulum were inconspicuous at 9 weeks but developed rapidly in the acinar cells from 12 weeks onwards in parallel with zymogen granule production. The demonstration of numerous zymogen granules by the fifth fetal month correlates well with the biochemical demonstration of proteolytic activity in pancreatic extracts during this same period.

We are grateful to all those who helped in obtaining fetal material and to Miss Marguarita Schmid and Miss Lillian Bedell for technical assistance. Dr J. A. G. Rhodin kindly reviewed the manuscript. The work was supported by Grant HD-01666-06, U.S.P.H.S.

REFERENCES

BENCOSME, S. A. (1955). The histogenesis and cytology of the pancreatic islets in the rabbit. *American Journal of Anatomy* **96**, 103–151.

BJÖRKMAN, N., HELLERSTRÖM, C., HELLMAN, B. & PETERSSON, B. (1966). The cell types in the endocrine pancreas of the human fetus. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **72**, 425–445.

BURSTONE, M. S. & FOLK, J. E. (1956). Histochemical demonstration of aminopeptidase. *Journal of Histochemistry and Cytochemistry* **4**, 217–226.

CARO, L. G. (1961). Electron microscopic radioautography of thin sections: the Golgi zone as a site of protein concentration in pancreatic acinar cells. *Journal of Biophysical and Biochemical Cytology* **10**, 37–45.

CARO, L. G. & PALADE, G. E. (1964). Protein synthesis, storage and discharge in the pancreatic exocrine cell. An autoradiographic study. *Journal of Cell Biology* **20**, 473–495.

CHESSICK, R. D. (1953). Histochemical study of the distribution of esterases. *Journal of Histochemistry and Cytochemistry* **1**, 471–485.

CONKLIN, J. L. (1962). Cytogenesis of the human fetal pancreas. *American Journal of Anatomy* **111**, 181–193.

EKHOLM, R. & EDLUND, Y. (1959). Ultrastructure of the human exocrine pancreas. *Journal of Ultrastructure Research* **2**, 453–481.

FREXINOS, J. (1968). Étude ultrastructurale et histoenzymologique du pancréas exocrine. Thesis, Imprimerie Fournie, Toulouse.

HARTMANN, J. F. & WELLS, L. J. (1948). Fate of food introduced directly into the fetal stomach. *Proceedings of the Society for Experimental Biology and Medicine* **68**, 327–330.

HELLMAN, B. (1965/1966). The development of the mammalian endocrine pancreas. *Biologia neonatorum* **9**, 263–278.

JAMIESON, J. D. & PALADE, G. E. (1971). Synthesis, intracellular transport, and discharge of secretory proteins in stimulated pancreatic exocrine cells. *Journal of Cell Biology* **50**, 135–158.

KEENE, M. F. L. & HEWER, E. E. (1929). Digestive enzymes of the human foetus. *Lancet* i, 767–769.

KERN, H. F. & FERNER, H. (1971). Die Feinstruktur des exokrinen Pankreasgewebes vom Menschen. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **113**, 322–343.

KOLDOVSKY, O. (1969). *Development of the Functions of Small Intestine in Mammals and Man*. New York: Karger.

LACY, P. E. (1962). Electron microscopy of the islets of Langerhans. *Diabetes* ii, 509–513.

LEV, R. & GERARD, A. (1967). The histochemical demonstration of protein in epithelial mucins. *Journal of the Royal Microscopical Society* **87**, 361–373.

LEV, R. & ORLIC, D. (1972). Protein absorption by the intestine of the fetal rat *in utero*. *Science* **177**, 522–524.

LEV, R. & ORLIC, D. (1973). Uptake of protein in swallowed amniotic fluid by monkey fetal intestine *in utero*. *Gastroenterology* **65**, 60–68.

LEV, R., SIEGEL, H. I. & BARTMAN, J. (1972). Histochemical studies of developing human fetal small intestine. *Histochemie* **29**, 103–119.

LIEBERMAN, J. (1966). Proteolytic enzyme activity in fetal pancreas and meconium. Demonstration of plasminogen and trypsinogen activators in pancreatic tissue. *Gastroenterology* **50**, 183–190.

LIKE, A. A. (1967). The ultrastructure of the secretory cells of the islets of Langerhans in man. *Laboratory Investigation* **16**, 937–951.

LIKE, A. A & ORCI, L. (1972). Embryogenesis of the human pancreatic islets: a light and electron microscopic study. *Diabetes* **21** (Suppl. 2), 511–534.

LILLIE, R. D. (1965). *Histopathologic Technic and Practical Histochemistry*. 3rd edition. New York: McGraw-Hill.

LIU, H. M. & POTTER, E. (1962). Development of the human pancreas. *Archives of Pathology* **74**, 439–452.

LUFT, J. H. (1961). Improvement in epoxy resin embedding methods. *Journal of Biophysical and Biochemical Cytology* **9**, 409–414.

ORLIC, D. & LEV, R. (1973). Fetal rat intestinal absorption of horseradish peroxidase from swallowed amniotic fluid. *Journal of Cell Biology* **56**, 106–119.

ORLIC, D., LEV, R. & ROSENTHAL, W. S. (1974). Fetal rat utilization of ^{55}iron absorbed by fetal intestine from swallowed amniotic fluid. *Blood* **73**, 729–736.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

VOLK, B. W., WELLMANN, K. F. & LEWITAN, A. (1966). The effect of irradiation on the fine structure and enzymes of the dog pancreas. I. Short-term studies. *American Journal of Pathology* **48**, 721–753.

WATSON, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. *Journal of Biophysical and Biochemical Cytology* **4**, 475–479.

WELLMAN, K. F., VOLK, B. W. & BRANCATO, P. (1971). Ultrastructure and insulin content of the endocrine pancreas in the human fetus. *Laboratory Investigation* **25**, 97–103.

WERNER, B. (1948). Peptic and tryptic capacity of the digestive glands in newborns. A comparison between premature and fullterm infants. *Acta paediatrica* **35** (Suppl. 6), 1–80.