Diabetologia 8, 326–333 (1972)

The Ultrastructure of the Human Pancreatic Islets

II. The Islets of Neonates

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Received: February 4, 1972, accepted: June 8, 1972

Summary. The ultrastructure of the islets of Langerhans was studied in seven human neonates. Out of the five cell types, described by the same authors in the islets of adults, four were also found in the islets of neonates: B cells, A cells, type III cells and type IV cells. Type III cells were far more numerous in the newborn in contrast with type V cells which were not found. In comparison with the islets of adults those of neonates showed a considerably higher number of pale granules in the B cells and a complete absence of fat vacuoles in all four cell types.

L'ultrastructure des îlots de Langerhans de pancréas humains. II. Les îlots de nouveau-nés

Résumé. L'ultrastructure des îlots de Langerhans a été étudiée sur sept pancréas de nouveau-nés. Quatre des cinq types cellulaires que les auteurs ont décrits dans les îlots d'adultes ont été retrouvés chez le nouveau-né: cellules B, cellules A, cellules du type III et cellules du type IV. Les cellules du type V font défaut chez le nouveau-né qui, par contre présente une plus grande abondance de cellules du type III. D'autres différences notables avec l'adulte sont présentes chez le nouveau-né: une proportion nettement plus grande de grains pâles dans les cellules B et l'absence de vacuoles graisseuses dans les cellules insulaires de tous types.

Die Ultrastruktur der Inseln des menschlichen Pankreas. II. Die Inseln von Neugeborenen

Zusammenfassung. Die Ultrastruktur der Langerhans'schen Inseln wurde in den Pankreata von 7 Neugeborenen untersucht. Vier der fünf Zelltypen, welche die Autoren in des Inseln Erwachsener beschrieben hatten, konnten auch beim Neugeborenen gefunden werden: B-Zellen, A-Zellen, Typ III- und Typ IV-Zellen. Die Typ V-Zellen fehlen beim Neugeborenen. Dieser hat dagegen mehr Zellen vom Typ III. Noch andere wichtige Unterschiede lassen sich beim Neugeborenen finden: eine deutlich größere Menge blasser Granula in den B-Zellen, sowie das völlige Fehlen von Fett-Vacuolen in den Inselzellen aller Typen.

Key words: Human newborn-pancreas, islets of Langerhans, ultrastructure, endocrine cells.

Introduction

The presence in the islets of the mammalian pancreas of insulin-producing B cells and glucagon-secreting A cells is no longer a matter of dispute, but the existence and the functional significance of other types of islet-cells are still controversial. Studies with the light microscope, using granule stains or silver impregnation, have led to a confused situation which electron microscopy has not yet been able to clarify.

In the pancreases of adults, which have been used for most of the ultramicroscopical studies of islets in man, the problem is complicated by the fact that islet cells other than A or B cells are rare and easily escape detection. In the islets of human neonates, such cells are more numerous than in adults and are characteristically concentrated in the peripheral mantle of non-B cells, two factors which aid in their identification.

Up to now, very few electron microscopic studies of the endocrine pancreas of the human foetus have

Table 1

Case No.	age (weeks)	weight (gram)	sex	Clinical diagnosis child	mother
1	35	2.800	M	rhesus incompatibility	normal
2	30	1.280	M	T.t.syndrome	normal
3	30	980	M	T.t.syndrome	normal
4	35	1.400	F	anencephaly	normal
5	32	2.200	F	prematurity	normal
6	26	2.330	F	rhesus incompatibility	slight diabetes (20 U insulin/day)
7	35	2.630	M	prematurity	normal

T.t.syndrome = *Transfuseur-transfusé* syndrome

appeared in the literature (Hellman, 1965/66, Björkman *et al.*, 1966, Wellman *et al.*, 1971). It therefore appeared worthwhile to compare the ultrastructure of the pancreatic islets of human neonates with those of adults.

Material and Methods

The material for this study was obtained within a few minutes of death from seven babies. Table 1 gives the clinical data of these cases.

One part of the specimen of pancreatic tissue was fixed in Bouin's fluid, embedded in paraffin, sectioned at

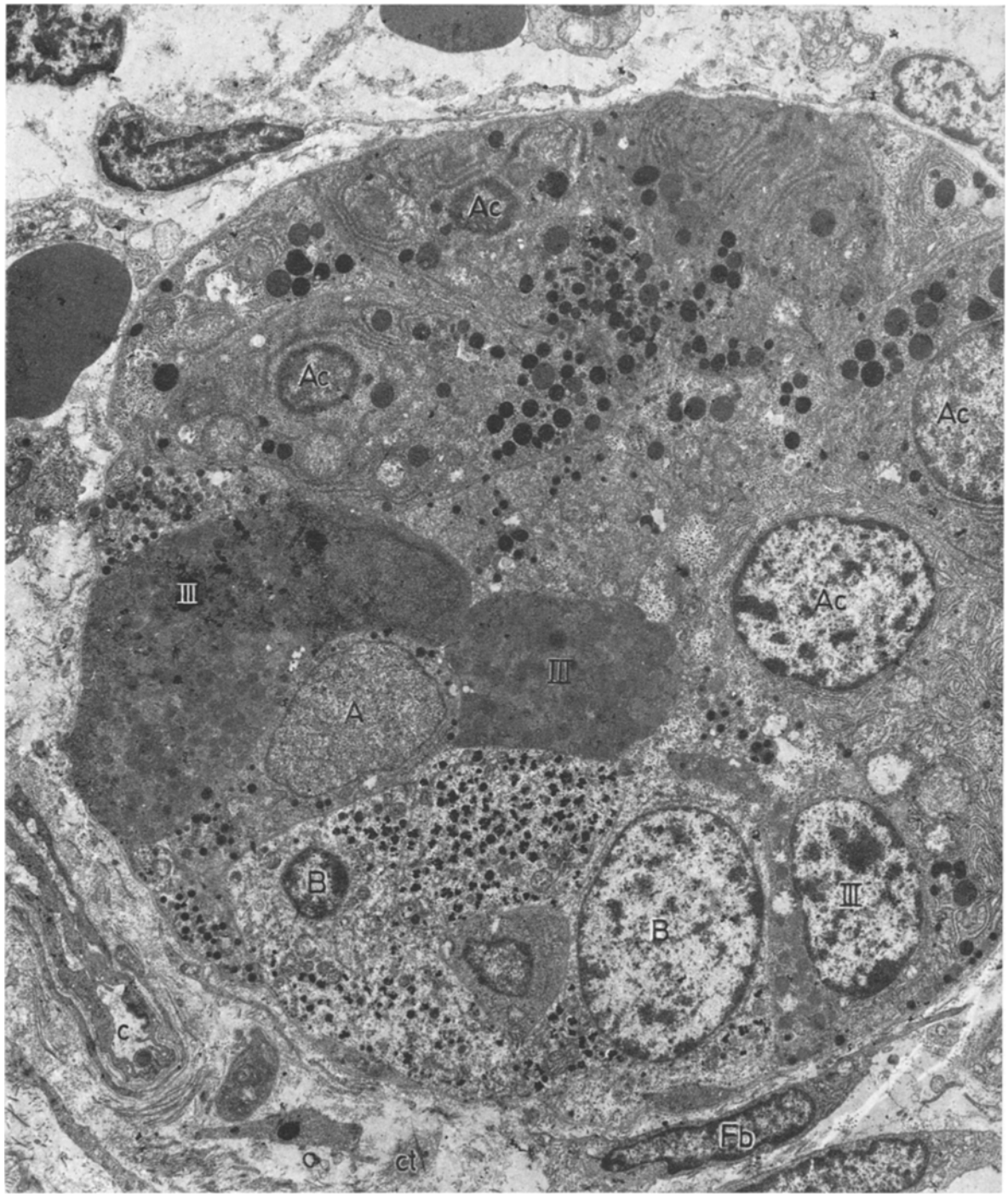

Fig. 1. A small bud of pancreatic tissue composed of acinar cells (Ac) and different types of endocrine cells; B cells (B), A cells (A) and type III cells (III). The pancreatic bud is surrounded by connective tissue (ct) containing fibroblasts (Fb) and capillaries (c). (Glutaraldehyde-Osmium × 5100)

5 μ and stained with the chromium hematoxylin-phloxine method of Gomori (1941) or the aldehyde thionin-trichrome method of Paget (1959). The other part, reserved for electron microscopy, was primarily fixed for 2 h in a 2.5% solution of purified glutaraldehyde (Anderson 1967) in 0.075 M cacodylate buffer at pH 7.4 and postfixed for 1½ h in a 1% solution of osmium tetroxide in 0.1 M of the same buffer at pH 7.4.

Fixation and dehydration were carried out at 4°C. The specimens were embedded in Durcupan. Pancreatic islets were located in semi-thin sections stained by an alcoholic solution of safranin. Ultra-thin sections were contrasted with permanganate (Reedy, 1965) followed by Reynold's solution (1963). The sections were examined with a Zeiss EM9A electron microscope.

normalities were seen in the pancreas of the anencephalic newborn (case 4).

Electron microscopy: The delineation of the endocrine and exocrine parts of the pancreatic tissue was not always clearcut. In addition to well developed islets, small epithelial buds, composed of both exocrine and endocrine cells and surrounded by connective tissue, were frequently seen (Fig. 1). Neither in their ultrastructure, nor in the characteristics of their granules, did the endocrine cells of these minute islets differ from those of well developed islets.

As in the pancreatic islets of human adults,

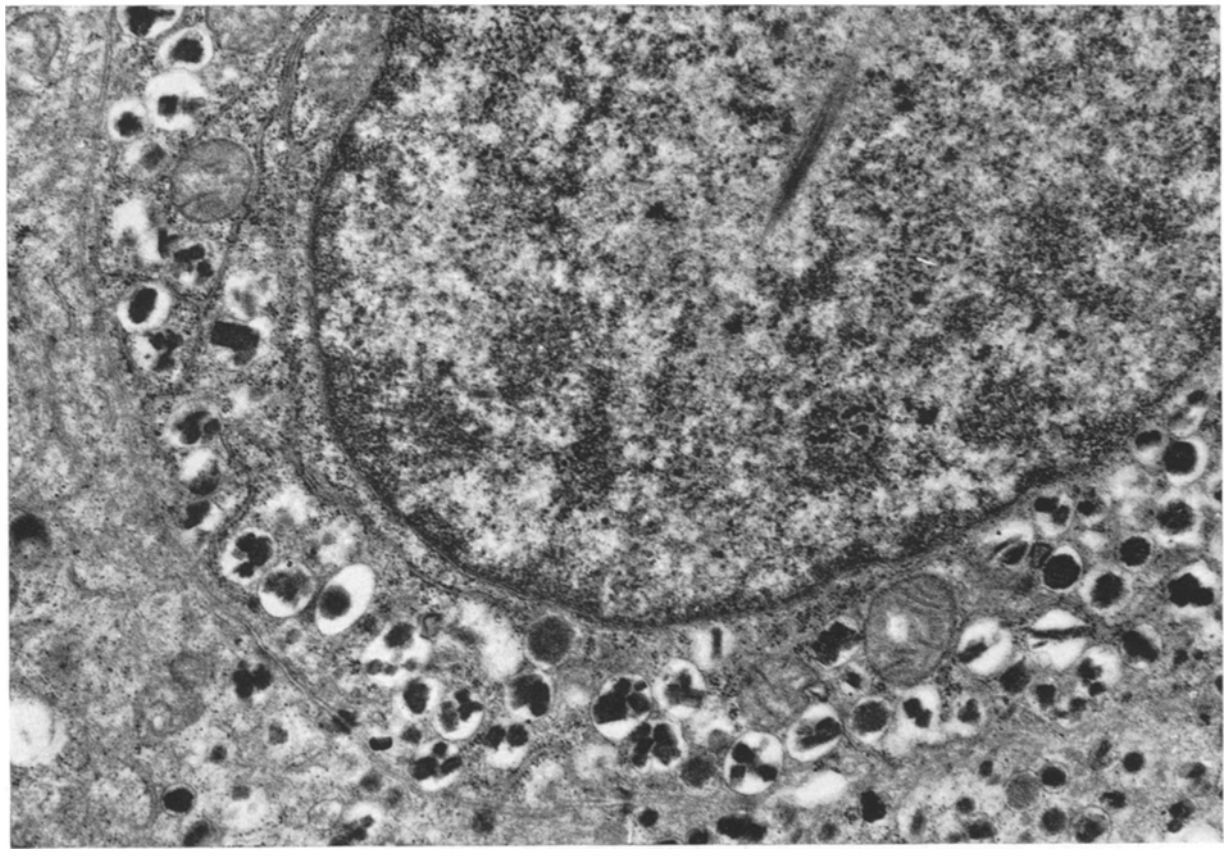

Fig. 2. Parts of two B cells. The nucleus of one B cell contains a fibrillar inclusion; its cytoplasm shows a well developed endoplasmic reticulum, forming parallel arrays of flattened sacs limited by rough membranes. (Glutaraldehyde-Osmium × 21000)

Results

Light microscopy: In all seven cases the general appearance of the pancreas conformed to the classical description of the human foetal pancreas (Bargmann, 1939; Ferner, 1952). A slight degranulation was noted in the islets of case 3, a very underdeveloped infant. A certain degree of islet hyperplasia was noted in cases 1 and 6. This was not unexpected since these babies were affected with erythroblastosis foetalis; moreover the mother of case nr. 6 was slightly diabetic. No ab-

(Deconinck *et al.*, 1971) we were able to identify four types of islet cells on the basis of their ultrastructure and the characteristics of their granules. In the order of their decreasing frequencies they were: B cells, A cells and two other types of cells, which we have provisionally designated as type III and type IV cells.

1. The B cells: These cells were easily identified by their characteristic granules that consisted of a very polymorphous, electron dense core enclosed within a wide round sac limited by a single smooth membrane. Their overall diameter ranged between 350 and 500 mμ.

A tendency of these granules to accumulate towards the vascular pole of the cell was frequently observed.

The nuclei of the B cells were unremarkable exept for the occasional presence of fibrillary inclusions (Fig. 2), a feature which was found in no other islet cell type. A small number of myelinic figures were found, often in close spatial relationship with mitochondria (Fig. 3). Dense bodies were very rare. Profiles of rough endoplasmic reticulum were scattered throughout the entire cytoplasm, but were more in evidence in the sparsely granulated areas of cytoplasm.

2. The A cells: The granules of these cells were also often concentrated towards the capillary poles. They had an excentric, very electron-dense core separated, by a semi-lunar and less dense peripheral part, from a tightly fitting smooth membranous sac. The diameter of these granules ranged from 150 to 300 mμ in some cells, and from 350 to 450 mμ in others. In a small number of A cells, the granules were less uniform in size and small granules coexisted with large ones. The A cells were generally located in the peripheral mantle of the islets and near the capillaries.

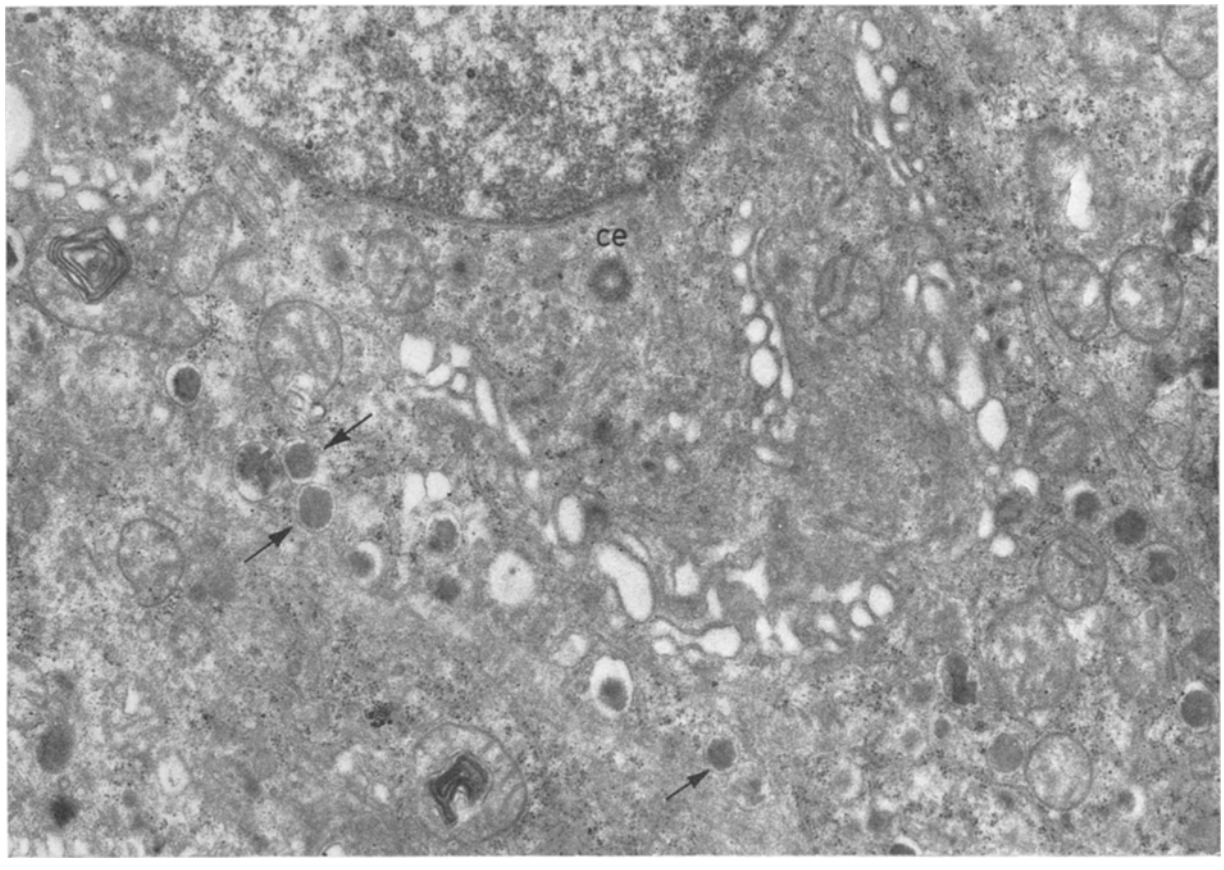

Fig. 3. Another B cell displaying a prominent Golgi complex containing a centriole (ce). Two mitochondria contain a myelinic figure. Besides typical granules, this cell shows some atypical granules of more homogeneous appearance and of lower electron density (↗). (Glutaraldehyde-Osmium × 21000)

Free ribosomes were seen in small amounts. One or two centrioles, sometimes lying in the vicinity of the Golgi area, could often be found. The Golgi apparatus was of a variable size (Fig. 3).

Besides these typical B cells, a small number of B cells with atypical granules were also seen: their cores were always round, less electron dense and more closely fitted in their sacs. No quantitative or qualitative differences in their granules could be discerned between the B cells of the seven cases used for this study.

In case 3, viral particles, apparently of the herpes group, were found in nuclei and cytoplasm of some A cells; (this finding will be fully described in a separate paper).

3. The type III cells: These were mostly found in the peripheral mantles of the islet, but nearer to the central core of B cells. Their nuclei were unremarkable. The mitochondria resembled those of the B cells. A small amount of rough endoplasmic reticulum composed of flattened sacs could be found throughout the entire cytoplasm. The Golgi complex, often prominent,

was located in the granular cellpole near the nucleus (Fig. 4). Their flattened sacs were sometimes separated by narrow bands of electron-dense cytoplasm. The secretory granules displayed a marked vascular polarity; they were large, having an overall diameter of 450 to 800 mμ. Their cores exhibited a considerable variation in electron density and were closely fitted in a smooth membrane. The largest granules, generally of low electron density, were peripherally placed while the smaller granules were more often located in the vicinity of the Golgi complex and usually were electron-dense.

Discussion

The pancreatic islets of human neonates are of the so-called mantle type, which means that they are composed of a central core of B cells surrounded by a peripheral mantle of non-B cells. Whereas in normal adults, the B cells represent, on the average, 75% of the islet cell population (Gepts, 1957), they amount to approximately 45% in the islets of babies (Van Assche, 1968). In an extensive study with the light microscope one of us (Van Assche, 1970) has shown that the cytological picture of the islet mantle varies with the

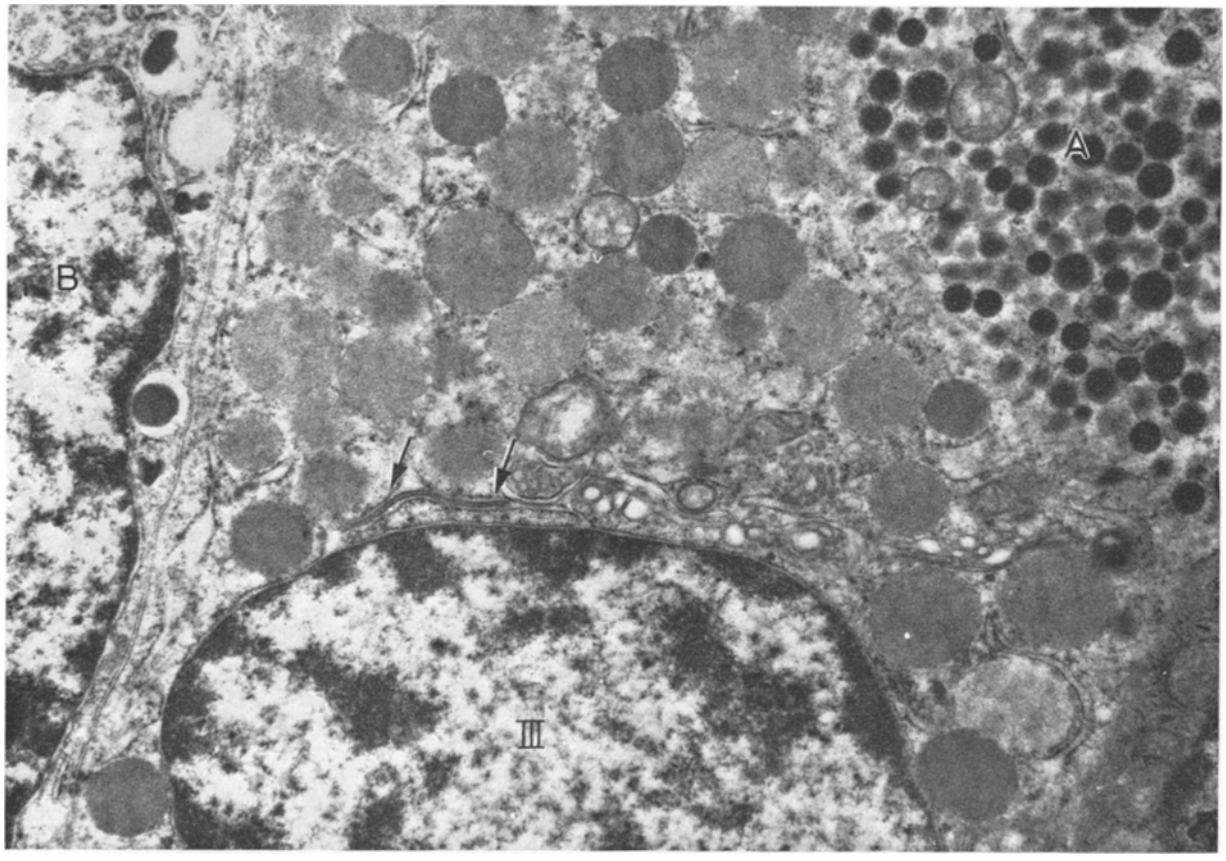

Fig. 4. A type III cell (III) surrounded by an A (A) and a B cell (B). The type III cell is characterized by large granules of variable electron densities and dark bands of cytoplasm separating Golgi cisternae (↗). (Glutaraldehyde-Osmium × 16800)

4. The type IV cells: Cells of this type were rare and, in contradistinction with those of type III, were often located in the vicinity of A cells.

The general structure of their nuclei and cytoplasm resembled that of the A cells from which they could be differentiated by their secretory granules, those of the type IV cells exhibiting a homogeneous, moderately electron-dense core, surrounded by an often closely fitting smooth membrane (Fig. 5). Their overall size ranged between 150 and 350 mμ in diameter. Cells of type IV were sometimes seen in close apposition to a nerve fibre (Fig. 6).

method of staining, and that no strict correspondence can be established between the cell types disclosed by different staining techniques. Since these techniques are devoid of histochemical basis, they cannot be relied upon for a correct identification or localisation of islet cell types.

The results of the present ultramicroscopic study of the neonatal endocrine pancreas confirm our findings obtained in a similar study of the islets of adults. As in the latter, we have been able to identify four types of islet cells: B cells, A cells, and a third and fourth type of cell.

Except for the respective proportion of the cell types, we did not find major differences between islet cells of adults and of neonates. It is more difficult to establish a strict correspondance between our classification and that from other studies on the ultrastructure of the foetal pancreas. Hellman (1965/66) and Björkman *et al.* (1966), in very young foetuses, described four types of islet cells : B cells, a_1 and a_2 cells, and agranular cells. Their description of B cells fits closely with ours and it can also be assumed that their a_2 cells correspond to our A cells. However, we have been unable to identify the equivalent of their α_1 cell, nor did we find agranular cells in our material. Wellman *et al.* (1971), who also ignore agranular cells, recognized three types of cells in foetal islets : A, B and D cells. They expressed the opinion that the latter are identical with the D cells, first described with the light microscope by Bloom (1931). From their description of this cell type, we feel that it could well correspond to our cell-type III.

In comparison with the B cells of adults (Deconinck *et al.*, 1971) those of neonates show a few minor differences. As in adults, two types of granules may be found in their B cells, polymorphous ones and homogeneous ones, that are larger, paler and non crystalline. Granules of the latter type are much more numerous in neonates than in adults. Pale B cell granules have already been described in the human (Kawanishi *et al.*, 1966; Like, 1967; Greider *et al.*, 1970; Wellmann *et al.*, 1971; Deconinck *et al.*, 1971).

It has been suggested that the insulin stored in the pale granules has not yet reached its mature form (Greider *et al.*, 1970) or is rapidly turned over (Wellmann *et al.*, 1971). If these explanations were correct, one should expect to find a larger proportion of pale granules in cases in which biological evidence for a rapid turnover of insulin exists, e.g. in babies affected with erythroblastosis or born of a diabetic mother. However, in our cases 1 and 6, which represent examples of such conditions, we have not been able to observe a higher proportion of pale granules. Therefore, their significance remains obscure.

Another difference between the B cells of adults and neonates, is the absence in the latter of fat globules. This difference is not restricted to the B cells; it also applies to the other types of islet cells. The significance of these differences is not clear. These lipid inclusions should not be confused with the myelinic figures, which

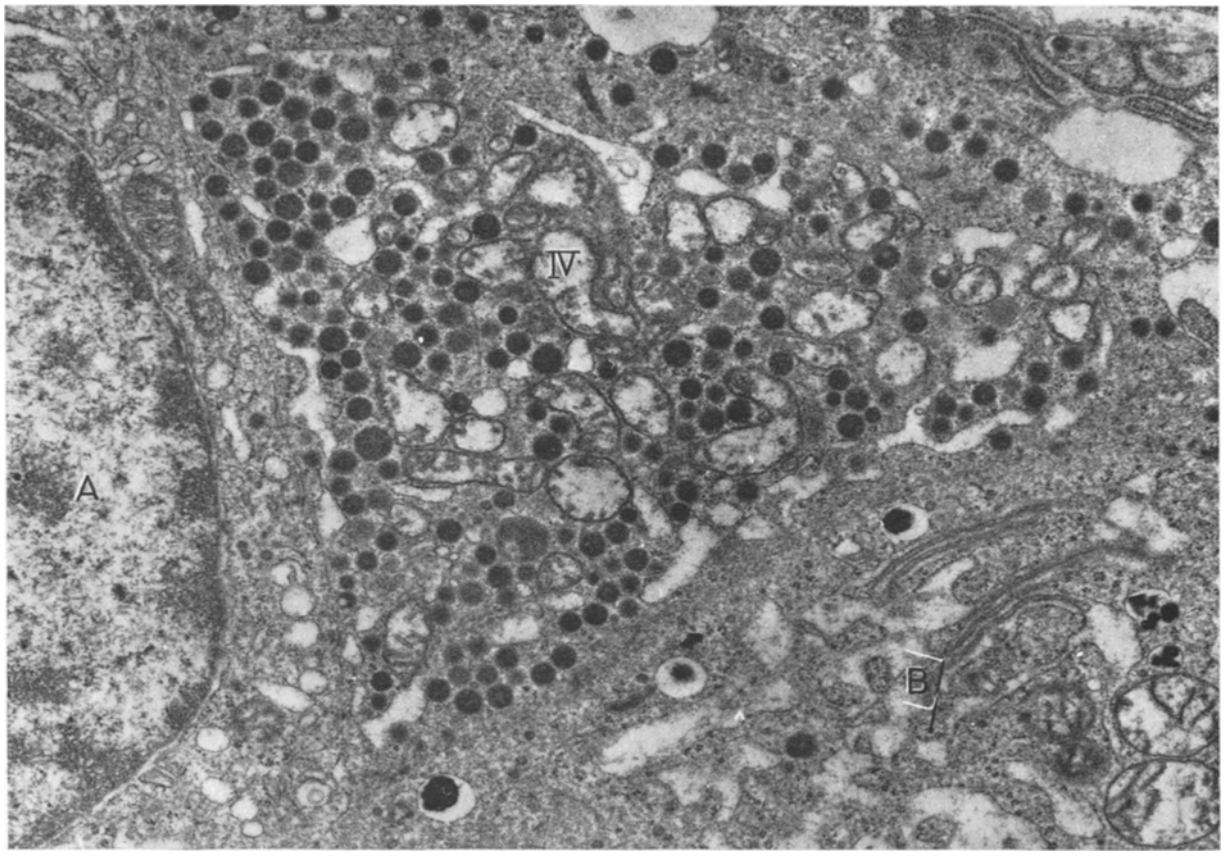

Fig. 5. Type IV cell (IV) surrounded by an A (A) and a B cell (B). This cell type is defined by homogeneous rounded granules of approximately the same size as A granules. (Glutaraldehyde-Osmium × 21 000)

can be found in all islet cells, in babies as well as in adults.

The ultrastructure of neonatal B cells does not provide any explanation for the poor reactivity of these cells towards glucose stimulation. The number of their granules does not appear to be inferior to that in adults. This agrees with measurements of insulin content, which have shown that neonatal B cells contain approximately the same amount of insulin as those of adults (Gepts *et al.*, 1970). The reason for the poor insulin response of neonatal islets must probably be sought in biochemical factors.

Greider *et al.* (1970) and by Wellmann *et al.* (1971). Wellmann *et al.* (1971) believe that these cells are identical with the D cells of Bloom (1931). In our studies of the islets of adults, we were impressed by the similarity of these cells with those that are responsible for the secretion of gastrin in the pyloro-duodenal mucosa. Indeed, Lomsky *et al.* (1969) and more recently Greider and McGuigan (1971) claimed to have demonstrated, by immunofluorescence methods, that gastrin-producing cells are present in the islets of Langerhans. However this finding could not be confirmed either by Creutzfeldt *et al.* (1971) or by our-

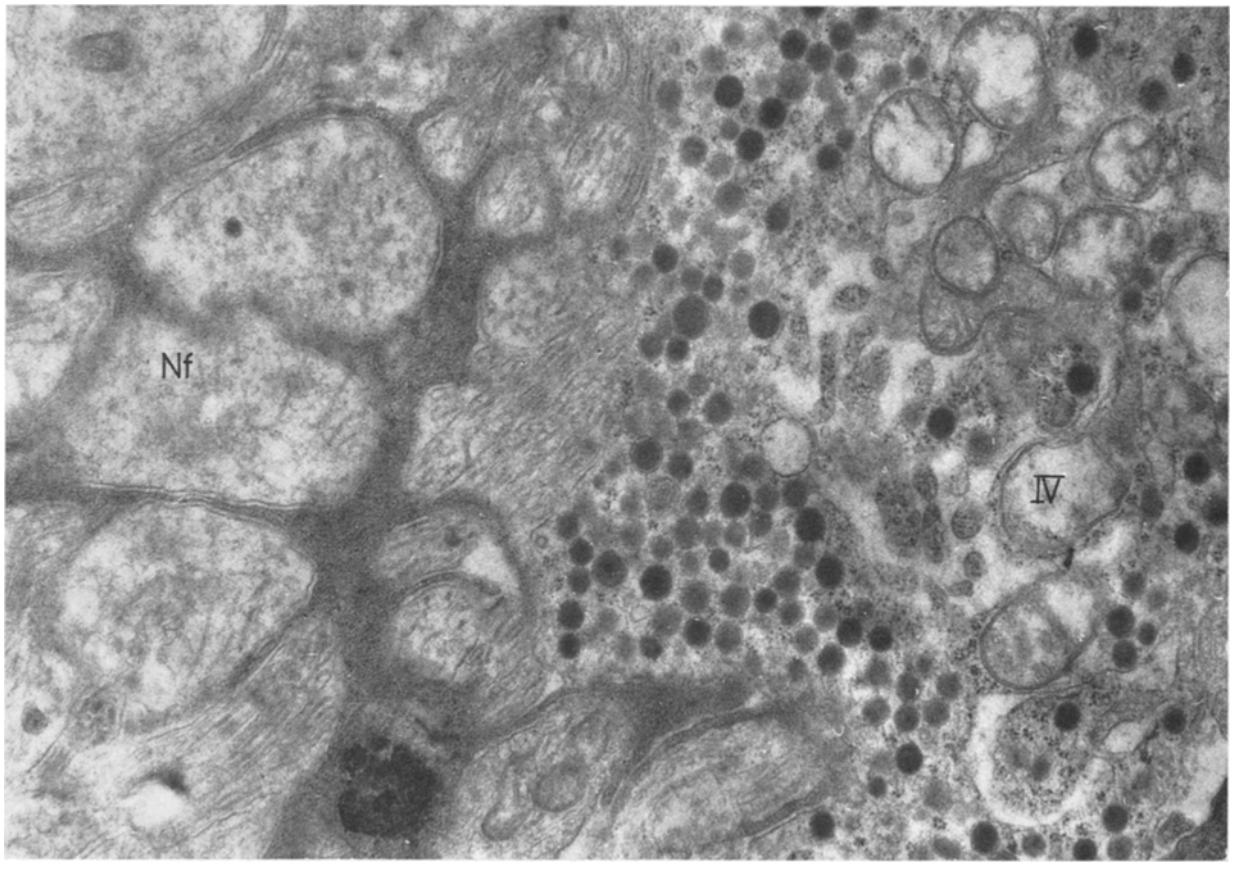

Fig. 6. A type IV cell (IV) in close apposition to a nerve fibre (Nf). (Glutaraldehyde-Osmium × 21 000)

In their recent paper Wellmann *et al.* (1971) described granules with a pale core and a dark crescentic halo. In our material we have never been able to find such granules. Since Wellmann *et al.* (1971) used only osmic acid as fixative, whereas we applied a double fixation with glutaraldehyde and osmic acid, we are inclined to attribute this discrepancy to technical reasons.

The type III cells in the islets of neonates are identical with those which we have described in the islets of adults (Deconinck *et al.*, 1971), but they are more numerous in the former. In adults, similar cells have been described by Shibasaki and Ito (1969), by

selves (unpublished observation). Clearly, morphological resemblance is, by itself, no sufficient evidence of identity. The functional significance of type III cell therefore remains to be established.

Cells of type IV are as rare in the islets of neonates as in those of adults. They differ from type III cells by the smaller size of their granules and their more homogeneous electron density. They differ from A cells by the absence of an electron-dense core. These cells resemble those that have been described by Orci *et al.* (1968), Forssmann *et al.* (1969) and by Shibasaki and Ito (1969); according to these authors, they are equivalent to the D cells of Bloom (1931). Solcia *et al.*

(1969), from a comparative study of the ultrastructure of the islet cells and the endocrine cells of the gastroduodenal mucosa, put forward the hypothesis that these cells might produce secretin. This hypothesis should await confirmation by histochemical methods. In our material, we have been impressed by the frequent vicinity of these cells to nerve fibres, but we are unable to suggest a physiological significance for this association.

Acknowledgements. This work was supported by grant nr. 1190 of the Fonds National de la Recherche Scientifique Médicale. Sincere thanks are due to Mrs. H. De Backer for technical assistance, to Mr. C. Cortier for his valuable help in the printing of the photographs and to Mrs. C. Ballon for expert preparation of the manuscript.

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