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The Cytological Composition of the Foetal Endocrine Pancreas in Normal and Pathological Conditions

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Summary. The islets of Langerhans of the neonatal pancreas are composed of B cells and non-B cells. The non-B cells are a heterogeneous group and the composition appears to vary according to the staining method used. Silver impregnation methods sometimes impregnate a small number of B cells. No complete identity exists between argyrophilic cells (Hellerström-Hellman modifications of the Davenport technique) and D cells. In babies from mothers with reduced carbohydrate tolerance the islets contain an increased proportion of B cells and Ag+ cells. Such an increase is not found in babies with erythroblastosis or alpha-thalassaemia. An active hypothalamo-hypophyseal system seems to be obligatory for complete maturation of the foetal islet tissue. In babies of diabetic mothers hyperplasia of the B cell appears only when a complete hypothalamo-hypophyseal system is present.

La composition cytologique du pancréas endocrine foetal dans les conditions normales et pathologiques

Résumé. Les îlots de Langerhans du pancréas néonatal sont composés de cellules B et de cellules non-B. Les cellules non-B forment un groupe hétérogène, dont la composition varie selon la technique de coloration. L'imprégnation argentique marque un petit nombre de cellules B. Il n'y a pas d'équivalence entre les cellules argyrophiles (modifications de Hellerström et Hellman de la technique de Davenport) et les cellules D. Il existe une augmentation du rapport cellules B et cellules argentaffines dans les îlots de Langerhans d'enfants dont la mère présente un métabolisme glucidique perturbé. Ce phénomène n'a pas pu être mis en évidence chez les enfants présentant de l'érythroblastose ou de l'alpha thalassémie. On peut admettre la nécessité d'un système hypothalamo-hypophysaire actif pour la maturation complète des îlots foétaux. On constate chez les enfants de mères diabétiques une hyperplasie des cellules B lorsqu'un système hypothalamo-hypophysaire complet est présent.

Die cytologische Zusammensetzung des foetalen endokrinen Pankreas unter normalen und pathologischen Bedingungen

Zusammenfassung. Die Langerhans'schen Inseln des neonatalen Pankreas bestehen aus B Zellen und nicht-B Zellen. Die nicht-B Zellen bilden eine heterogene Gruppe, deren Zusammensetzung je nach der gebrauchten Färbungsmethode ändert. Silberimprägnierungsmethoden imprägnieren eine kleine Zahl B Zellen. Es besteht keine vollständige Identität zwischen argyrophilen (nach Davenport-Hellerström-Hellman) Zellen und D Zelle. Bei den Neugeborenen von Müttern mit reduzierter Kohlenhydrattoleranz enthalten die Inseln eine größere Proportion B Zellen und Ag+ Zellen. Eine solche Zunahme fehlt bei Neugeborenen mit Erythroblastose oder Alpha Thalassämie. Ein aktives hypothalamo-hypophysäres System ist notwendig um eine vollständige Reifung des foetalen-Insel-Gewebes zu erreichen. Bei Neugeborenen diabetischer Mütter kommt Hyperplasie der B Zellen nur dann vor, wenn ein vollständiges Hypothalamo-hypophysäres System anwesend ist.

Key words: Islet cells, B cells, argyrophilic cells, D cells, infants of diabetic mothers, anencephalics, anti-D-iso-immunization, pregnant diabetic, hypothalamo-hypophyseal system, perinatal mortality, diabetic screening.

Introduction

The cytological composition of the islets of Langerhans in man is still uncertain. Besides A and B cells, other types of cells (A^1, A^2, D etc.) have been described [3, 4, 8, 13, 14, 18, 35, 36]. The identity of these unusual cell types and their functional significance has not been definitively settled. Evidence has been presented by the Swedish School that A^2 cells secrete glucagon. The function of the A^1 cells remains unknown. Some authors [7, 12, 14] are of the opinion that these cells are identical to the D cells described by Bloom, but others [3, 19] are reluctant to admit this identity. Recent evidence suggests that the A^1 cells secrete gastrin [29]. Furthermore, little work has been performed on the variation of the cytological composition of foetal islets in different pathological conditions.

Macropolynesia, due mainly to an increased number of B cells, is a classical feature of the pancreas in infants of diabetic mothers. Macropolynesia has also been found in babies affected by anti-D-erythroblastosis and by haemolysis due to alpha-thalassaemia [6, 9, 11, 25, 30, 31, 34, 37, 38, 39, 41, 42], but it has not been proven that in these latter conditions it is also due to an hyperplasia of the B cells [2, 5].

The purpose of this paper is to report our findings with different staining techniques on the cytological composition of the islets of the human newborns and its variations in different pathological conditions.

Material and Methods

The material for this study consists of the endocrine pancreases from 107 foetuses and newborn infants delivered after the 20th week of gestation.

The cases were classified according to the oral glucose tolerance test (G.T.T.) of the mother, and the condition of the offspring.

Group I: normal maternal G.T.T. during pregnancy or postpartum: 40 cases

Group II: slightly reduced carbohydrate tolerance: 9 cases

Group III: diabetes: 10 cases

Group IV: erythroblastosis (rhesus iso-immunization): 10 cases

Group V: hydrops foetalis due to alpha-thalassaemia: 5 cases

Group VI: anencephalics without functional hypothalamo-hypophyseal (H.H.) system
a) normal maternal G.T.T.: 9 cases
b) abnormal maternal G.T.T.: 8 cases
c) maternal G.T.T. not performed: 9 cases

Group VII: anencephalics with functional hypothalamo-hypophyseal (H.H.) system
a) normal maternal G.T.T.: 3 cases
b) abnormal maternal G.T.T.: 4 cases

Glucose tolerance tests were performed on the mother giving a standard load of 100 g glucose. Blood sugars were estimated on capillary blood before and 45, 90, 135 and 180 min after the oral intake of 100 g glucose, by a method adapted from Nelson-Somogyi [32]. The glucose tolerance test was considered as normal if no more than one of the following values was exceeded:

1) fasting glucose level	100 mg%;
2) at 45 min	180 mg%;
3) at 90 min	130 mg%;
4) at 135 min	120 mg%;
5) at 180 min	100 mg%.

Slightly reduced carbohydrate tolerance was accepted if two or more values exceeded the above levels, and diabetes with two or more values exceeding the following levels:

1) fasting level	120 mg%;
2) at 45 min	210 mg%;
3) at 90 min	170 mg%;
4) at 135 min	150 mg%;
5) at 180 min	135 mg%. (Fig. 1)

All cases of erythroblastosis (anti-D iso-immunization) showed gross foeto-placental oedema. Except in one case the maternal oral glucose tolerance test was normal. In seven infants one or more intrauterine transfusions had been performed. Pancreatic tissue from infants with hydrops foetalis due to alpha thalassaemia was generously provided by Prof. L.E. Lie-Injo from Kuala Lumpur, Malaysia.

Anencephalics were classified in two groups, according to the absence (group VI) or presence (group VII) of a functional hypothalamo-hypophyseal (H.H.) system. Our criteria for the presence of a functional hypothalamo-hypophyseal system were:

1) macroscopical presence of a hypothalamo-hypophyseal structure;
2) demonstration of a foetal adrenal cortex by microscopical examination;
3) subnormal total oestrogen levels in maternal urine.

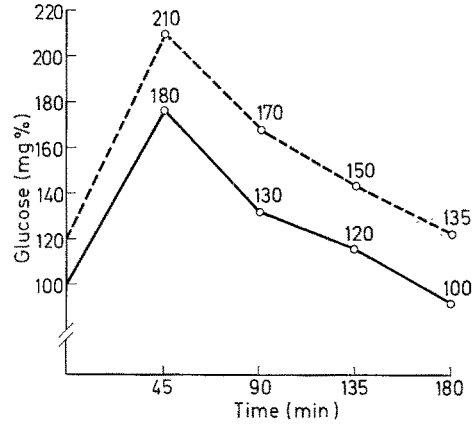


Fig. 1. Upper limits of normal glucose tolerance, and slightly reduced carbohydrate tolerance

In each of these groups, cases were subclassified according to the results of the oral glucose tolerance test (G.T.T.) in the mother. No distinction was made between slightly or frankly reduced carbohydrate tolerance.

The pancreas was removed within two hours of death and a piece of the tail was fixed in Bouin's solution for two days. Sections at 3 or 5 μ were cut from paraffin-embedded tissue.

The following staining methods were used:
Gomori's chrome-alum haematoxylin-phloxine method [16];
Ivic's Victoria-blue acid-fuchsin method [24];
a modified aldehyde-thionin-trichrome method [33];
silver impregnation techniques
a) Hellerström-Hellman [18];
b) Holmes [22].

To evaluate the percentage of the different cell types 2000 cells were counted with the nucleus as counting base. All the cells of each islet were systematically counted.

For the comparison of the different cell types 3 methods were used:
In 10 cases alternate sections (3 μ thickness) were prepared and coloured by the aldehyde-thionin-trichrome method, by the silver impregnation technique (Hellerström-Hellman) and by Ivic's method.
In 10 cases slides were coloured by aldehyde-thionin-trichrome or by Ivic's method after removal of silver by the oxidation process.
In 10 cases slides were counterstained by the aldehyde-thionin-trichrome method after silver impregnation (Holmes technique).

Results

I. The different cell types

a) *B cells.* As shown in Table 1, we did not find significant differences in the percentage of B cells among islet cells as demonstrated by three staining techniques (Gomori's chrome-alum phloxine method; Ivic's Victoria-blue acid-fuchsin; and aldehyde-thionin-trichrome).

Table 1. *Percentage of B cells with different staining techniques*

Number	Gomori chrome alum	Ivic	Aldehyde thionin trichrome
29	38	40	41
37	31	28	30
47	38	38	39
50	35	34	37
52	45	46	44
53	36	37	37
43	40	41	43
118	62	63	65
26	73	71	70
32	49	50	48
33	57	57	55
56	63	66	65
57	40	38	41
84	58	59	56
74	47	50	52
42	40	43	45
114	22	23	21
121	39	40	41
151	35	35	33
119	58	56	55
Mean ± S.D.	45.3 ± 12.7	45.7 ± 12.9	45.9 ± 12.4

The mean percentage of B cells in the normal group is 40% ± 7.5 (M. ± S.D.). B cell hyperplasia was defined as a percentage higher than 40% + 15 i.e. 55%.

As shown in Table 2, we found hyperplasia of the B cells in three groups (newborns from mothers with a slightly or frankly reduced carbohydrate tolerance, groups II and III; anencephalics from mothers with a reduced carbohydrate tolerance, provided that in these foetuses a functional hypothalamo-hypophyseal system was present, group VII b). The differences between these groups and the normal group are statistically significant ($p < 0.001$).

In the erythroblastotic group and in infants affected by alpha-thalassaemia we found a normal percentage of B cells.

b) *Phloxine-positive cells.* The B cells are situated in the centre of the foetal islets and are stained blue with Gomori's chrome-alum haematoxylin. With the same method all the other cells (non- B cells) are stained red (phloxine +) and are situated at the periphery ("mantle"). Therefore their proportion is complementary to that of the B cells (Fig. 2).

c) *Acid-fuchsin-positive cells (AF+) and acid-fuchsin-negative cells (AF−).* In sections stained by Ivic's method we found two types of non-B cells in the mantle area: first, cells heavily stained with acid fuchsin (AF+); and secondly, cells unstained or only slightly stained (AF−) (Fig. 3). The mean percentage of AF+ and AF− cells is invariable in all groups studied. This is shown in Table 3.

Table 2. *Percentage of B cells in the different groups. Slides stained with Ivic's technique*

Group		Percentage of B cells M. ± S.D.	B cell hyperplasia percent of cases
I	Normal maternal G.T.T. n=40	40 ± 7.5	5
II	Slightly reduced carbohydrate tolerance n=9	54.8 ± 12.3	44
III	Diabetes n=10	63.8 ± 8.9	90
IV	Erythroblastosis n=10	40 ± 8	10
V	α-Thalassamia n=5	39.8 ± 3	0
VI	Anencephalics without functional H.H. system		
	a) Normal G.T.T. n=9	40 ± 6	0
	b) Abnormal G.T.T. n=8	38 ± 9.7	0
	c) Without G.T.T. n=9	37 ± 9.8	0
VII	Anencephalics with functional H.H. system		
	a) Normal G.T.T. n=3	40, 40, 44	0
	b) Abnormal G.T.T. n=4	54, 70, 59, 56	75

d) *Green cells (D?) and red cells.* In slides coloured with aldehyde-thionin-trichrome we found two types of cells in the mantle zone: green cells (D cells) and red cells (Fig. 4). As shown in Table 4, the percentage of D cells was about the same in the different groups.

e) *Silver-positive cells* (argyrophilic cells). With the Hellerström and Hellman silver-impregnation technique, we found black-coloured cells (Ag+) and light-brown coloured cells (Ag−) in the mantle area (Fig. 5).

In 12 babies of mothers with a normal glucose tolerance test a comparison was made between phloxine-positive cells (non-B cells) and Ag+ cells. The mean value of phloxine-positive cells for these cases was 62% (Table 5). In silver-impregnated preparations only 40.5% of the islet cells were silver positive. This means that roughly only 65% of the phloxine-positive cells are argyrophilic.

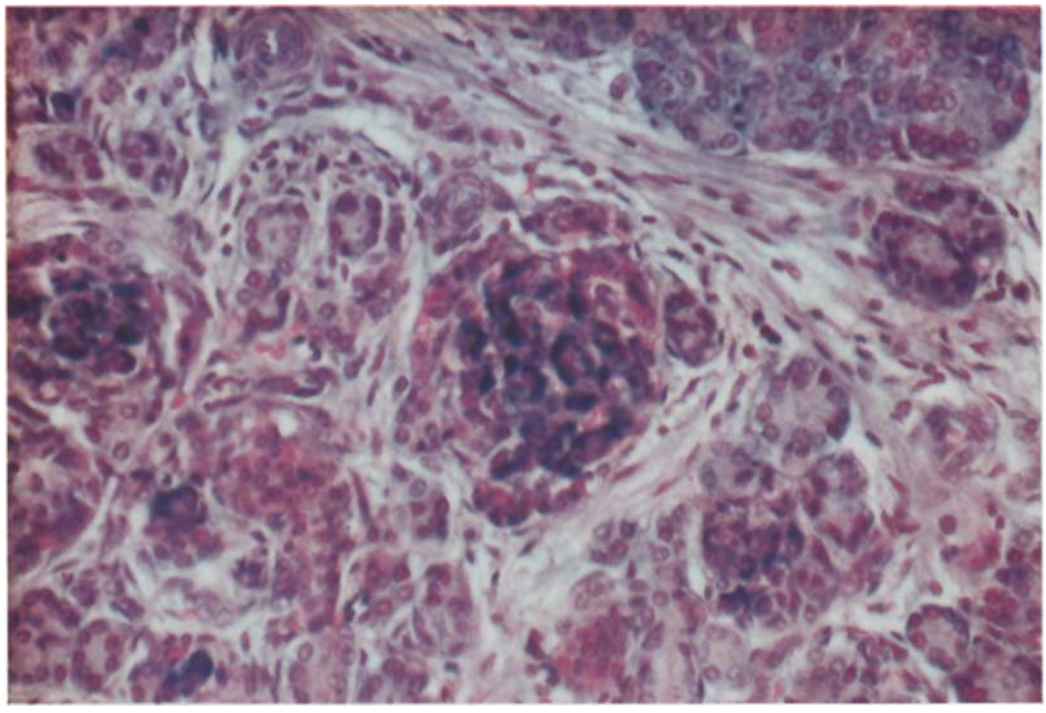


Fig. 2. The foetal islets are composed of a central core of B cells (blue cells) and a peripheral area of non-B cells (red cells)
Gomori-stained section

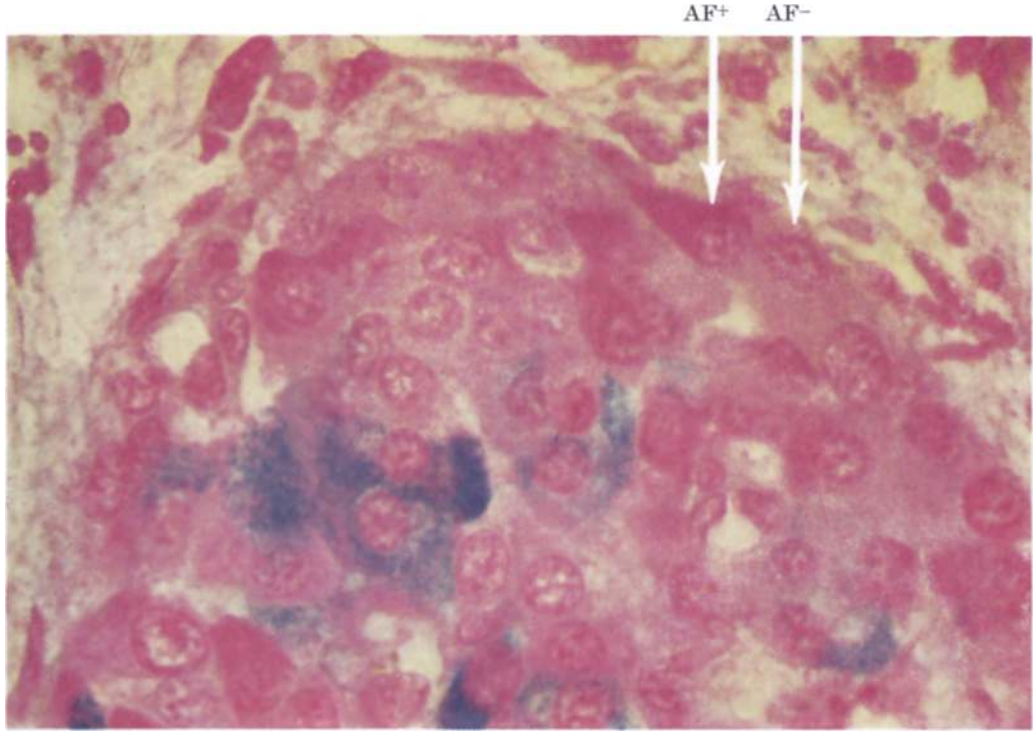


Fig. 3. Two types of non-B cells are present in the mantle area: first, cells very well coloured with acid fuchsin (AF+ red); and secondly, cells slightly coloured or even not (AF−)
Section stained by Ivic's method

Fig. 4. Two types of non-B cells are seen in the mantle area: green cells (D cells ?) and red cells
Aldehyde thionin trichrome stained section

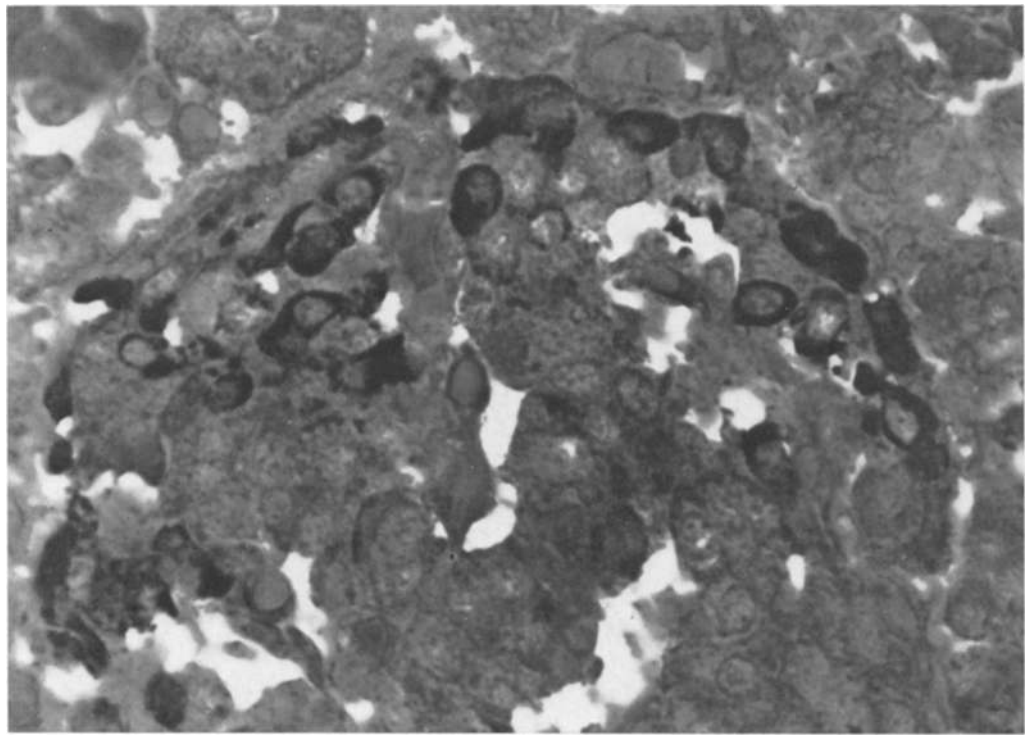


Fig. 5. Most of the black cells (Ag+) are situated in the peripheral area. In the central core most of the cells are brown (Ag−)
Slide stained with Hellerström-Hellman silver impregnation technique

Table 3. *Percentage of AF+ and AF− cells in the different groups*

Group		Percentage AF+ cells M.±S.D.	Percentage AF− cells M.±S.D.
I	Normal maternal G.T.T. n=34	73.5±8.8	26.5±8.8
II	Slightly reduced carbohydrate tolerance n=7	73 ±6.6	27 ±6.6
III	Diabetes n=9	83 ±3.2	17 ±3.2
IV	Erythroblastosis n=6	76 ±7.9	24 ±7.9
VI	Anencephalics without functional H.H. system		
	a) Normal G.T.T. n=7	73 ±6.6	27 ±6.6
	b) Abnormal G.T.T. n=5	77 ±5.8	23 ±5.8
	c) Without G.T.T. n=5	78 ±6.8	22 ±6.8
VII	Anencephalics with functional H.H. system		
	a) Normal G.T.T. n=2	65, 71	35, 29
	b) Abnormal G.T.T. n=3	76, 64, 78	24, 36, 22

Table 4. *Percentage of green (D?) cells and red cells in the different groups*

Group		Percentage green cells M.±S.D.	Percentage red cells M.±S.D.
I	Normal maternal G.T.T. n=6	48±4.9	52±4.9
II	Slightly reduced carbohydrate tolerance n=2	42, 51	58, 49
III	Diabetes n=4	41, 44, 42, 43	59, 56, 58, 57
IV	Erythroblastosis n=3	40, 31, 36	60, 69, 64
VI	Anencephalics without functional H.H. system n=10	44±5.6	56±5.6
VII	Anencephalics with functional H.H. system n=1	42	58

A statistically significant increase ($p<0.05$) in the proportion of Ag+ cells was found in infants of diabetic mothers (group III). In comparison with the infants from normoglycaemic mothers (group I) and erythroblastotic babies (group IV), an increased proportion of Ag+ cells was observed in infants from mothers with slightly reduced carbohydrate tolerance (group II) and in anencephalics of a diabetic mother, provided that the foetal hypophysis was active (group VII b). The increase is not statistically significant, due to the small number of cases and also to the large variation of individual values.

Table 5. *Comparison between Ag+ and Phloxine+ cells*

Group		Percentage Phloxine+ cells M.±S.D.	Percentage Ag+ cells[a] M.±S.D.	Percentage Ag+ Phloxine+ cells M.±S.D.
I	Normal maternal G.T.T. n=12	62± 5.8	40.5±8.6	65±13.0
II	Slightly reduced carbohydrate tolerance n=4	47± 5.6	36.5±6.2	77±27.8
III	Diabetes n=6	35±10.3	31 ±8.4	88±33.1
IV	Erythroblastosis n=6	55±8.1	31 ±9.5	56±13.3
VI	Anencephalics without functional H.H. system			
	a) Normal G.T.T. n=3	62± 5.0	45 ±1.7	72± 4.3
	b) Abnormal G.T.T. n=6	66±10.8	41 ±4.0	62± 9.9
	c) Without G.T.T. n=4	63±11.1	43 ±4.6	68±10.7
VII	Anencephalics with functional H.H. system			
	a) Normal G.T.T. n=2	58± 2.8	37 ±6.3	64± 7.7
	b) Abnormal G.T.T. n=3	38± 7.3	29 ±1.0	77±19.6

[a] Hellerström-Hellman

II. Comparison between the different cell types

a) Quantitative methods. A comparison between the proportion of different non-B cells as demonstrated with different staining techniques, shows that AF− cells are less frequent than green (D?) cells, that green cells are less frequent than Ag+ cells, and that Ag+ cells are less frequent than phloxine-positive cells (Table 6). This is also true for the individual cases in all groups, except for one case in group II and for

three cases of group III, where more Ag+ cells than phloxine-positive cells are found. The fact that with the different staining techniques the percentage of the respective types of non-B cells varies, whereas on the other hand no parallel variations of these cell types occur in different clinical conditions, argues strongly against a relationship between staining characteristics and functional activity.

b) Qualitative methods. — Alternate sections.

In alternate sections (3 μ thickness) we found argyrophilic cells in some islets, which in the alternate sections contain granules of the B cell type (Figs. 6—7).

— Slides re-stained by Ivic's method after removal of the silver (Hellerström-Hellman method):

We were unable to obtain satisfactory results in slides re-stained by Ivic's method or by the aldehyde-thionin-trichrome technique after removal of silver.

— Slides counterstained with aldehyde-thionin-trichrome technique after Holmes technique:

In such slides we found, besides cells strongly impregnated with silver and unimpregnated B cells, a small number of unimpregnated green (D) cells (Fig. 8) and also a small number of B cells with black silver granules (Fig. 9).

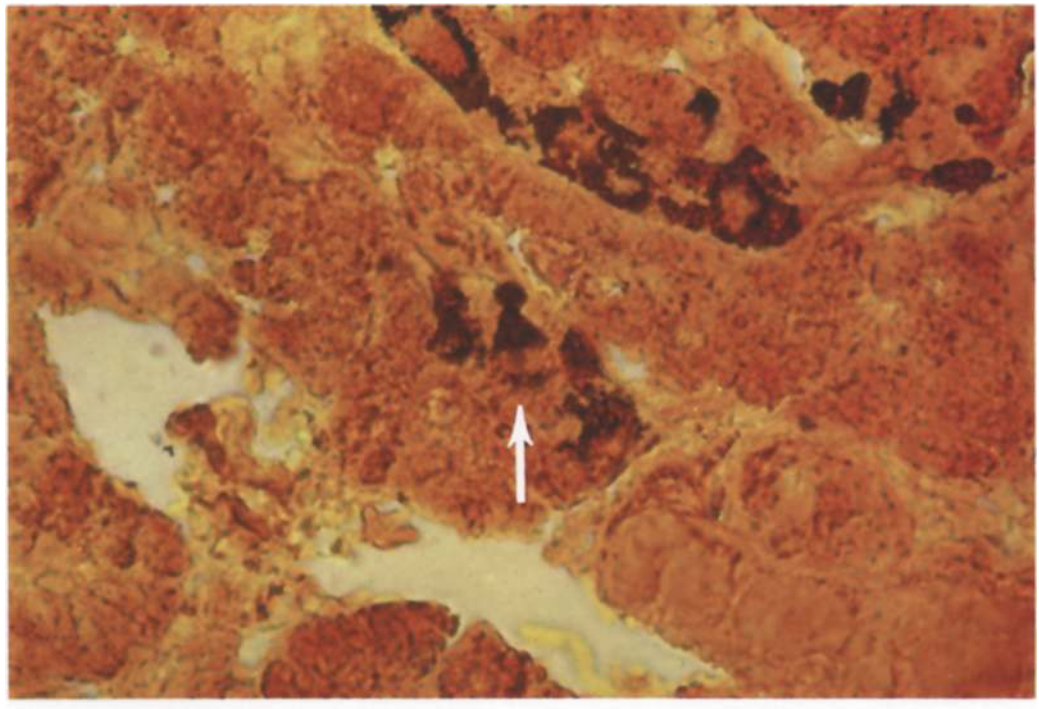
Discussion

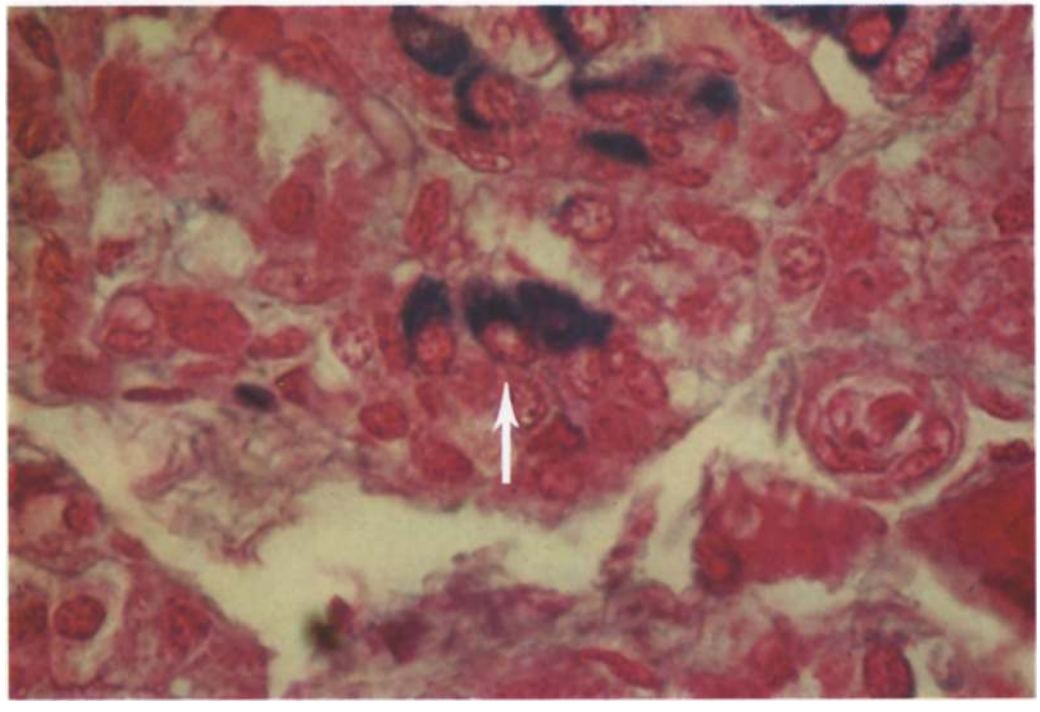
Our study of the cytological composition of the islets demonstrates that only one cell type, the B cell, can be constantly identified with the different staining techniques. The other islet cells represent an heterogeneous group, whose components vary according to the staining method used.

With Gomori's chrome-alum haematoxylin-phloxine technique only one type of non-B cells is found. With the Ivic technique two types of non-B cells can be demonstrated: the acid-fuchsin-negative (AF—) and the acid-fuchsin-positive (AF+) cells. The AF— ressemble the C cells, which Bensley [1] described in the guinea pig, after staining by the gentian-violet fuchsin method. With aldehyde-thionin-trichrome two types of non-B cells are seen: green cells and red cells. The green cells as well as the AF— cells are located in the peripheral area of the islet. The green cells probably correspond to those that have been described and called D cells by Bloom [4]. Conklin [8] suggests that the D cells are immature B cells. Moreover, he observed grey cells, which he considered to be cells stained both with light green (as D cells) and with aldehyde fuchsin (as B cells). In our opinion these grey cells could be degranulated B cells; we are not

Table 6. *Comparison between the different non-B cells in the studied groups*
Phloxine-positive cells are considered as 100%. Acid-fuchsin-negative cells are less frequent than green cells; green cells are less frequent than argyrophilic (Ag^+) cells; and Ag^+ cells are less frequent than phloxine-positive cells. However, this does not mean that AF^- cells are always green cells and that green cells are always Ag^+. The proportion of Ag^+ cells out of phloxine-positive cells is increased in groups II, III and VIIb.

		Ivic	Aldehyde thionin trichrome	Hellerström and Hellman (Davenport)	Chrome Alum Phloxine
Group		Percentage AF^- cells	Percentage green cells	Percentage Ag^+ $Phloxine^+$ cells	Percentage $Phloxine^+$ cells
I	Normal maternal G.T.T.	26.5	48	65	100
II	Slightly reduced carbohydrate tolerance	27	46.5	77	100
III	Diabetes	17	42.5	88	100
IV	Erythroblastosis	24	25.5	56	100
VI	Anencephalics without functional H.H. system				
	a) Normal G.T.T.	27		72	100
	b) Abnormal G.T.T.	23	44	62	100
	c) Without G.T.T.	22		68	100
VII	Anencephalics with functional H.H. system				
	a) Normal G.T.T.	32		64	100
	b) Abnormal G.T.T.	27	42	77	100





Figs. 6 and 7. Three argyrophilic cells contain at the alternate section granules of the B cell type
Two alternate sections stained with the silver impregnation technique to Hellerström-Hellman and with Ivic's method

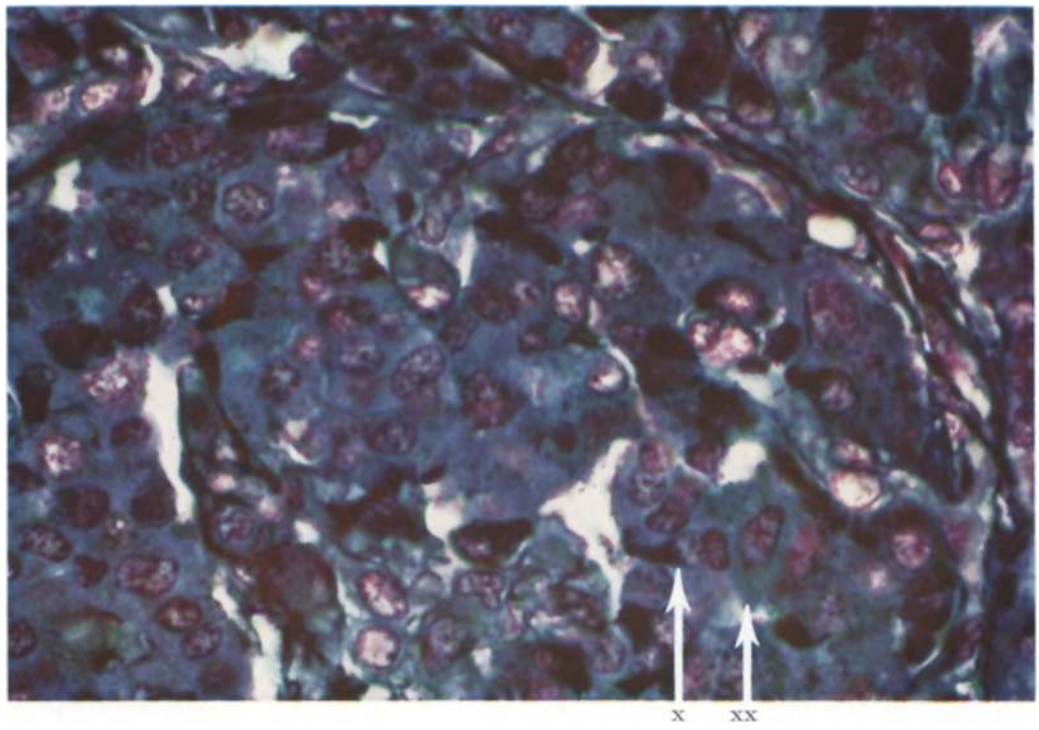


Fig. 8. We can see completely black cells (X), and light green coloured cells (XX)
Slide counterstained with aldehyde thionin trichrome after Holmes technique

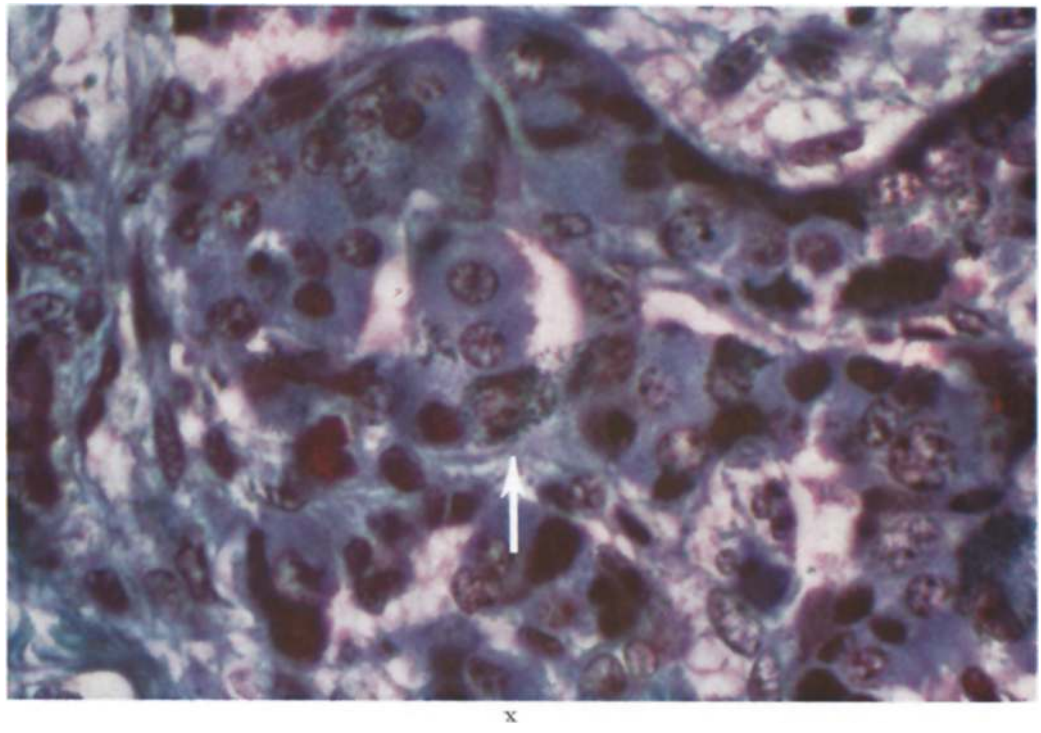


Fig. 9. A few cells contain blue and black granules in their cytoplasma (X)
Slide counterstained with aldehyde thionin trichrome after Holmes technique

convinced that they represent immature B cells. Like [28] doubts the existence of the D cell as a separate cell type.

Numerous silver impregnation techniques have been proposed for the study of the islets of Langerhans. It has been pointed out and confirmed by our study that these different techniques do not impregnate the same type of cells. With the Davenport technique and its modification by Hellerström and Hellman [18], less cells are blackened than with the Grimelius [17] or Holmes [22] technique. Moreover, in some cases we found more silver impregnated cells than non-B cells. This suggests that the silver-impregnation technique not only impregnates non-B cells, but also a variable number of B cells. This suggestion is confirmed by the study of slides impregnated with silver by the Holmes technique, and counterstained with aldehyde thionin trichrome. In such preparations cells that contain both blue and black granules can be found.

The Swedish group [3, 19, 20] in comparing preparations impregnated with silver by a modified Davenport method with the same preparations after removal of silver and re-staining with aldehyde fuchsin, state that only a part of the A cells are silver positive. They divide the A cells into two groups: A^1 cells (argyrophilic) and A^2 cells (non-argyrophilic). These authors have never found an argyrophilic cell containing granules of the B cell type. However, in our hands, granule staining after silver removal gave unreliable results.

We disagree with Fujita [14] and Epple [12], who stated that the D cell is identical with the silver-positive cell. Indeed, in silver-impregnated sections counterstained with aldehyde thionin we found green cells (D cells) unimpregnated with silver.

On the basis of the studies of our group with the light and electron microscope, our opinion is that four types of cells can be identified in human pancreatic islets: B cells, A cells, a third type of cell, which probably secretes gastrin, and a fourth type of cell, the function of which remains obscure [10]. In the islets of the newborn infants, the B cells constitute the central core, whereas the peripheral mantle is composed of non-B cells. In the adult, the cell types are intermingled all over the islets [10, 15]. Björkman *et al.* [3] and Hellman [19] found 4 types of cells in the endocrine pancreas of the human: A^1 cells, A^2 cells, B cells and agranular cells. De Coninck *et al.* [10], however, were unable to find agranular cells. They feel that the D cells of Bloom may constitute an heterogeneous group, composed of cells which probably secrete gastrin and another cell type of unknown functional significance.

B cell hyperplasia and an increased proportion of Ag + cells in the islets of the newborn are, in comparison with macropolynesia, more specific parameters for the detection of maternal diabetes, since these parameters are not found in anti-D erythroblastosis foetalis or in haemolysis due to alpha-thalassaemia. It is also interesting to point out that an increased number of such cells is found in different types of hyperglycaemia in animals and in man [14, 21, 26].

The hypothalamo-hypophyseal system is not involved in the basal maturation process of the foetal islets of Langerhans. However, hyperplasia of the islets of the B cells, such as found in offspring of diabetic mothers, can develop only when this system is present and functional. This conclusion can be derived from the fact that anencephalic babies from diabetic mothers fail to develop hyperplasia, when the hypothalamo-hypophyseal system has not been functional.

Whether this effect is a direct one or is mediated through other endocrine glands, cannot be demonstrated at the present time [40].

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