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Significance of Argyrophil Parenchymal Cells in the Pancreatic Islets in Persistent Neonatal Hypoglycemia with Hyperinsulinism of Familial Type

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INTRODUCTION

By modifying the Davenport alcoholic silver nitrate procedure, Bo Hellman, together with Claes Hellerström, discovered in 1960 that the A-cells of the pancreatic islet parenchyma could be subdivided into the A_1- and A_2-cells (7). This discovery was a major advance in our knowledge of the histophysiology of the endocrine pancreas. With the advent of also other argyrophil procedures (5), as well as that of modern immunohistochemical (IHC) techniques (12), it is now known that the islet A_1- and A_2-cells produce somatostatin and glucagon, respectively, and that the argyrophil islet parenchymal cells form part of the large gastro-entero-pancreatic (GEP) neuroendocrine system, appearing already early in evolution (3, 13).

Despite the availability of modern IHC techniques, the argyrophil procedures have not lost their importance when the pathogenesis of various kinds of diseases of the endocrine pancreas has to be investigated by a complete clinico-pathologic examination. A typical example is the disease persistent neonatal hypoglycemia with hyperinsulinism (PNHH) where, already before the advent of IHC in the structural analysis of the pancreatic islets, the Hellerström-Hellman and Grimelius (5) procedures contributed to give some new aspects of the pathogenesis of the disease. Thus, it could be shown by a quantitative correlation of the results of the Hellerström-Hellman and Grimelius procedures that there was a relative decrease of the A_1-cells and a marked increase in the parenchymal cells visualized by the Grimelius technique (11). In subsequent studies by IHC it has been shown independently from several laboratories (1, 4, 6, 8) that in PNHH the volume density of the insulin cells is increased whereas that of the somatostatin and glucagon cells is decreased. Some discrepancy thus seems to exist between the results of argyrophil staining techniques and those of IHC/morphometric procedures as regards the glucagon cells.

The present report gives some morphometric observations on the underlying

structural changes in the endocrine pancreas of an infant, belonging to one of two families with PNHH. The structural analysis offers an additional illustration of the role of the argyrophil cells in the pathogenesis of PNHH.

CASE REPORT

A recent previous report (4) gave a detailed account of the case histories, the clinical picture with results of the laboratory investigations, and the therapeutic trials in three infants with PNHH, belonging to these two families. In one of the families two newborns had previously died shortly after birth with a clinical and post-mortem picture compatible with PNHH. The present case belongs to the second family ("case 5" of the previous report; 4), where a two-year elder brother at the age of 6 months had been subtotally pancreatectomized for PNHH. The previous work was essentially concerned with the results of an IHC and morphometric analysis of the islet parenchyma of the specimens from subtotal pancreatectomies performed in a girl from the first family and in the elder brother of the present patient. The specimens were analyzed together with autopsy specimens from age-matched "controls" (4).

Since that report was given, a supplementary study has been made of the hereditary background of the five children with manifest or suspected PNHH. Pedigrees were constructed, based on interviews of the parents and some other relatives (Figs. 1 and 2). The carbohydrate metabolism and the endocrinological status of the four parents were also clinically examined; no signs of abnormality were discovered.

During pregnancy, the mother of the present case had pre-eclampsia and was delivered by cesarian section already after 35 weeks of gestation. The boy ("JB") was large for the gestational age (birth weight: 2,985 g; length: 49 cm). Already one hour after birth a marked hypoglycemia (0.7mmol/l) was detected and repeated plasma insulin assays showed hyperinsulinism. The plasma glucagon level was also elevated. As treatment with large intravenous doses of carbohydrates (1 g/kg/h), diazoxide, and zinc-protamine-glucagon mainly resulted in an extreme body weight increase, metabolic acidosis, and urinary excretion of pyruvate and lactate, a subtotal pancreatectomy was performed when the child was 13 months old.

At operation, the pancreas was found to be grossly normal, and no tumor-like nodules were observed. The postoperative course was uneventful. By means of diazoxide and longacting glucagon administration, supplemented with carbohydrate feedings, the blood glucose and plasma insulin values could be controlled, so that at the age of 2½ years the child now has a normal weight increase.

METHODS

The resected specimen was immediately cut up into thin slices. Some of them were fixed in Bouin's fluid, others in 10% formalin. After conventional fixation and paraffin embedding, serial sections, 5 µm thick, were cut, and adjacent sections were stained with hematoxylin and eosin, van Gieson's stain, aldehydefuchsin, the Grimelius silver nitrate procedure, the Hellerström-Hellman silver technique, and "immunostained" for the four islet hormone cells, both by the indirect immunofluorescence procedure and by the peroxidase-anti-peroxidase (PAP) method (12), as described in previous reports from our laboratories (4, 9, 10). The antisera used and their working dilutions, as well as the kinds of IHC controls (12) applied, have also been previously specified, as have the morphometric procedures, including the use of pancreatic specimens from age-matched controls(4, 9, 10).

RESULTS AND DISCUSSION

As shown in Figs. 1 and 2, both pedigrees of the five recently reported (4) patients with suspected or manifest PNHH showed some cases of juvenile or maturity-onset diabetes mellitus in distant relatives to the PNHH children. The case history of the neonatally dead infant on the present patient's mother's side (Fig. 2) was found to be rather suggestive of PNHH (no autopsy material for histopathological analysis was available from that infant).

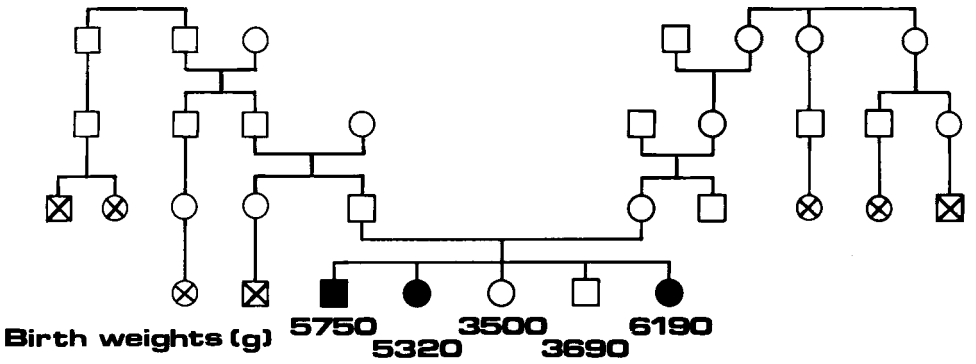

Fig.1: Pedigree of a kindred of five children where three died from suspected or manifest PNHH (Cases 1, 2, and 3 of a previous report; 4).
Although the parents and grand parents were healthy, there seems to be a slightly increased incidence of diabetes mellitus, both on the father's and on the mother's side.

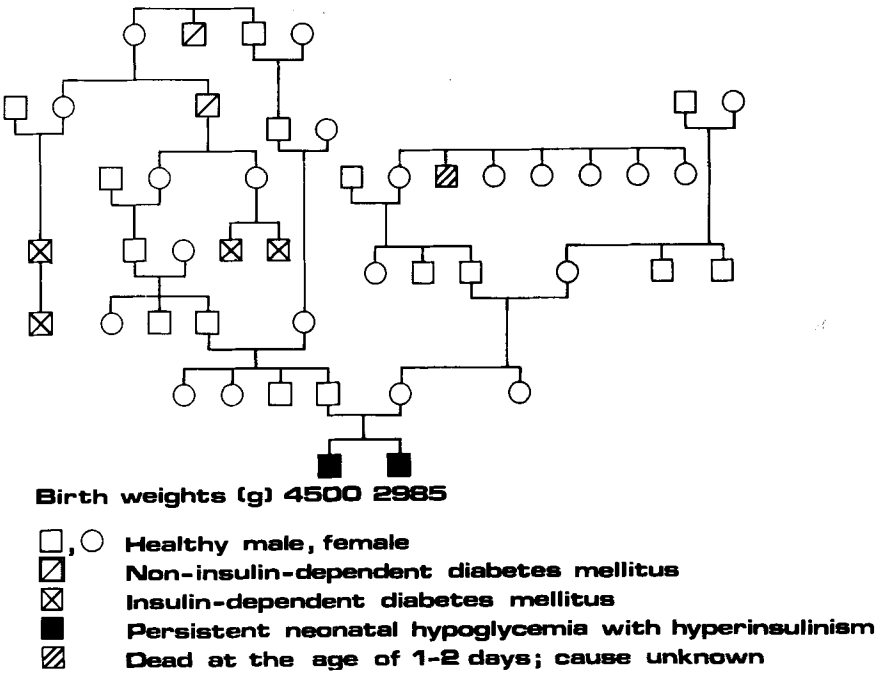

Fig.2: Pedigree of the present case and his elder brother (Cases 5 and 4, respectively, of a previous report; 4).
The parents and grand parents did not show any signs of endocrine or metabolic disease. On the father's side there seems to be a slightly increased incidence of diabetes mellitus. The neonatally dead infant on the mother's side may have been a case of PNHH (to judge from the case history).

It has been proposed that not only the islet cell lesions in hypoglycemia due to erythroblastosis and diabetes mellitus, but also those in idiopathic PNHH, may represent some kind of inheritable islet cell dysmaturation syndrome (4, 6). The present pedigrees give only some faint support for such a hypothesis.

At the microscopical examination there were no kinds of tumor-like nodules (focal hyperplasias, microadenomas) in the resected specimen from the subtotal pancreatectomy. Instead, the islet parenchyma showed all the typical features of "nesidioblastosis" (4), *i.e.* a dissemination of the islet hormone cells in the acinar parenchyma; a ductulo-insular proliferation; polyploidy, hyperplasia and hypertrophy of the aldehydefuchsinophil cells; and an increase in the number of chromophobe "agranular" precursor islet cells (11). The numbers of the Grimelius argyrophil islet cells seemed to be slightly increased, or at least within normal limits, whereas only few argyrophil islet parenchymal cells were detected by the Hellerström-Hellman procedure.

The IHC analysis confirmed a wide-spread dissemination of insulin cells in the acinar parenchyma (4). It also revealed that the number of PP cells was

negligible which is natural as the specimens resected essentially consisted of the cauda and corpus regions of the pancreas. Comparing Grimelius-stained and immunostained adjacent serial sections, it was observed that there seemed to be a discrepancy between the Grimelius argyrophil cells and the glucagon immunoreactive cells. All the argyrophil cells did not seem to be glucagon immunoreactive. As to the Hellerström-Hellman argyrophil cells and the somatostatin immunoreactive islet parenchymal cells, there was, however, excellent correspondence.

The two slightly different morphometric procedures used gave essentially the same results as to the volume densities of the islet parenchymal cells and as to the total islet parenchyma relative to the total amount of epithelial tissue in the resected specimen. The data from the most comprehensive of these morphometric analyses are given in Table 1, where also the results of analogous analyses in three "normal controls" from a previous study (10) are included.

Table 1: Results of a morphometric analysis of the endocrine pancreas in specimens of the corpus-cauda region obtained at a subtotal pancreatectomy in a 13-month-old child with PNHH.
The data were obtained on serial sections, immunostained for the four islet hormones by the PAP procedure. They are compared with the mean values from an analogous study of fresh autopsy specimens from three 6-month-old infants dying from cardiac or cerebrovascular malformations and bronchopneumonia ("normal controls")(10).

Case	Volume density (in per cent)				
	Total endocrine parenchyma *)	Relative proportion of islet parenchymal cells			
		Insulin	Glucagon	Somatostatin	PP
J.B.	7.4	75.7	8.5	12.5	3.2
(Means of three "normal controls")	6.5	62.1	15.1	21.3	1.6

*) As visualized by IHC, using antisera against insulin, glucagon, somatostatin, and PP only (9, 10).

With due reference to the source of error inherent in the fact that the "normal controls" were half a year younger than the PNHH patient, it might, nevertheless, be concluded that the pattern of quantitative changes in the endocrine pancreas of the present patient was the same as in the islet parenchyma of the two previously analyzed infants of the two families with PNHH(4).

Thus, in the resected specimen there was a rather moderate increase in the volume density of the total amount of endocrine parenchyma and in the relative proportion of insulin cells, whereas that of both the somatostatin and glucagon cells was markedly decreased. (The PP-cells were too few to allow any valid conclusions).

In these respects also the results of the present study support the previously launched idea of PNHH as an islet cell dysmaturation syndrome (4, 6) where the deficient somatostatin control of insulin release, coupled with a glucagon decrease and an increased activity of the insulin cells, represent the main factors in the pathogenesis of the dramatic symptoms.

As indicated in the footnote to Table 1, it should be stressed that the actual volume density of the total islet parenchyma might be larger than that found by the IHC techniques used. This is supported by the fact that in an earlier study of three cases of persistent neonatal hypoglycemia where the proportion of the islet parenchyma was assessed in sections stained by Grimelius procedures (11), the values obtained were higher than those found by the IHC technique in the pancreatic specimens of the present child and in those of the previously reported infants of these two families (4).

An even more striking difference was, however, the large proportions of Grimelius argyrophil cells observed in the earlier, non-IHC investigation (11).The implications of the discrepancy between the increased Grimelius argyrophil islet cells and the decreased glucagon immunoreactive cells in PNHH will be the subject of a more comprehensive critical analysis in the near future. The reports that ultrastructurally "agranular" cells can constitute as much as 25 to 30 per cent of the islet cells in pancreatic specimens from infants with PNHH (11) and that at least some of these cells can be argyrophil (2), indicate that correlated light microscopic, IHC, morphometric, and ultrastructural investigations are urgently needed to get an insight into the role of the argyrophil islet parenchymal cells in the pathogenesis of this mysterious disease.

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