Pancreatic Endocrine Cell Fractions in Erythroblastosis Fetalis

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SUMMARY

Pancreatic sections from 21 cases of rhesus disease and 20 control newborn infants of 30–40-wk gestational age were stained by the immunoperoxidase method for insulin, glucagon, somatostatin, and pancreatic polypeptide (PP). The fractional area occupied by each cell type was estimated, taking note of whether the gland contained PP-rich (ventral lobe) or PP-poor islets (dorsal lobe). In the PP-rich part of the pancreas, the volume fraction of all four endocrine cell types was significantly greater in the rhesus cases than in the controls. No difference was found between the two groups in the PP-poor part of the gland. The results show that abnormal development of the PP-rich part of the pancreas occurs in erythroblastosis fetalis. The localization of the changes to one part of the pancreas may explain some of the earlier conflicting reports on this topic. DIABETES *32*:313–315, April 1983.

The endocrine pancreas of the erythroblastotic infant has been described variously as showing islet hyperplasia or as normal.[1] Most erythroblastotic infants suffer from rhesus incompatibility and are known to be at increased risk of hypoglycemia due to hyperinsulinemia,[2] which might stem from B-cell hyperplasia. Recent developments in histochemical staining techniques permit the identification of four islet cell types[3,4] and have shown differences between the ventral and dorsal lobes, which contain pancreatic polypeptide (PP)-rich and -poor islets, respectively.[5,6] We have demonstrated an increase of the glucagon and PP cell fractions in the pancreas from infants of diabetic mothers when care is taken to classify the islets into PP-rich or -poor,[7] and the present study was undertaken to see if use of the same methods would reveal abnormalities in erythroblastotic pancreas.

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Received for publication 28 June 1982 and in revised form 12 November 1982.

MATERIALS AND METHODS

One formalin fixed paraffin-embedded pancreatic block from each of 21 infants with erythroblastosis fetalis due to rhesus incompatibility was obtained from the Histopathology Departments of Sheffield Children's Hospital and the Jessop Hospital for Women, Sheffield, and the Academisch Ziekenhuis Sint-Rafael, Leuven, Belgium. The gestational age of the erythroblastotic infants ranged from 30 to 40 wk and the age at death varied from less than 1 h to 4 days. One pancreatic block from each of 20 control infants of similar gestational and postnatal age was obtained from Sheffield. Infants used as controls had died from a variety of diseases, but cases with conditions known to affect the endocrine pancreas were excluded. The postmortems of the erythroblastotic and control infants were performed between November 1961 and February 1981. The retrospective nature of the study made it impossible to classify or match the erythroblastotic and control infants except for gestational and postnatal age.

Serial sections (6 μm) were cut and adjacent sections were stained for insulin, glucagon, PP, and somatostatin by the immunoperoxidase method.[8] Endogenous peroxidase and erythrocyte pseudoperoxidase activity was inhibited by the method of Streefkerk[9] using a methanol/hydrogen peroxide (11:6 vol/vol) mixture. The following primary antisera were used: 1:1000 guinea pig anti-porcine insulin (Miles Laboratories Ltd., Slough, England), 1:1000 rabbit anti-porcine glucagon (a gift from Dr. C. M. D. Ross, Weston Park Hospital, Sheffield), 1:10,000 rabbit anti-bovine pancreatic polypeptide (a gift from Dr. R. E. Chance, Eli Lilly Laboratories, Indianapolis, Indiana), and 1:1000 rabbit anti-synthetic somatostatin (a gift from Professor W. Gepts, Brussels, Belgium). The reaction period for the above antisera was 24 h at 4°C. The secondary and tertiary antisera were 1:20 swine anti-rabbit IgG (Dako Immunoglobulins, Copenhagen, Den-

mark) and 1:100 horseradish peroxidase/rabbit anti-horseradish peroxidase complex (Dako Immunoglobulins, Copenhagen, Denmark). The reaction period for both was 40 min at room temperature. Sections were washed between different antisera with Tris saline buffer (pH 7.4, 0.05 M) and nonspecific background staining was reduced by pretreating the sections with 1% normal swine serum in Tris saline buffer. Visualization of the peroxidase was carried out according to the method of Graham and Karnovsky,[10] using 125 mg of 3,4,3',4' tetra-amino biphenyl hydrochloride (BDH Chemicals Ltd., Poole, England) in 340 ml of citric-acetate buffer (pH 5.0, 0.05 M) containing 200 μl of 100 vol hydrogen peroxide.

Control staining was performed by preabsorbing the primary antisera with their respective antigens or by omission of the primary antisera. Both procedures resulted in negative staining. Preabsorption of the primary antisera with antigens other than those against which they were raised did not prevent or diminish positive staining.

One section from each case was also stained with Masson's trichrome or hematoxylin.

Morphometric analysis. Using a Wild binocular microscope at a magnification of ×25, the PP-rich region of the pancreatic polypeptide stained section from each case was outlined with a mapping pen. By placing the insulin, glucagon, or somatostatin section on top of the pancreatic polypeptide section, the PP-rich areas on these sections were also outlined. Measurements were performed separately in PP-rich and PP-poor regions of all pancreas.

The fractional surface area of one pancreatic section per case occupied by the immunostained cytoplasm of insulin, glucagon, somatostatin, or PP cells was measured using an automatic image analyzer (Quantimet 720) attached to a Nikon SKE series microscope with a ×10 objective and interfaced with a Hewlett Packard 9815A desk top calculator. The area of tissue measured in each section was 38.5 mm^2 or less if the tissue area was smaller. Large areas of interlobular connective tissue, blood vessels or processing artefacts were not included in the measurement area. An estimate of the cellular density in the PP-rich and PP-poor regions of erythroblastotic pancreas was made by taking the average of 20 counts of the number of nuclei in a 40 μm^2 area.

Differences between control and rhesus pancreas were tested by using Student's *t* test.

RESULTS

Not all the sections were suitable for morphometric analysis, due to occasional high levels of nonspecific staining. All the pancreatic sections from the control sections had PP-poor regions but due to random tissue sectioning only 17 had PP-rich areas. Similarly all 21 rhesus pancreatic sections had PP-poor regions while 9 had PP-rich areas. The numbers of sections analyzed for each cell type are shown in Table 1 and in all comparisons there was no significant difference in gestational age between the control and erythroblastotic cases. In the PP-rich part of the pancreas, the volume fraction of each of the four endocrine cell types was significantly greater in the erythroblastotic pancreas than in the controls, whereas no significant difference was found between the groups in the PP-poor part of the gland. Seven of the 9 erythroblastotic cases were suitable for measurement of cellular density. The mean ± SD number of cells/40 μm^2 in the PP-poor region was 16.8 ± 2.9 which did not differ significantly from that in the PP-rich region: 17.2 ± 3.1.

DISCUSSION

The present study has demonstrated an abnormality in the morphology of the islets of Langerhans in the pancreas of rhesus affected infants which is confined to the PP-rich part of the gland. Prior to the advent of specific stains for each of the endocrine cell types, it was not possible to discriminate histologically between the ventral and dorsal lobes and therefore those investigators who studied the dorsal lobe, which is larger and more commonly sampled, would be expected to find no difference between rhesus and control tissues, whereas those who studied the ventral lobe would observe macropolynesia. This might explain why some investigations have reported an increase in islet numbers and diameter[11–16] while others have reported an islet diameter within normal limits.[17] These results preface our observation that all four endocrine cell types participate in the macropolynesia of the rhesus pancreas. The retrospective nature of this investigation imposed constraints on the conclusions which may be drawn from the results. The blocks of tissue from both groups of infants had been collected over a number of years by different pathologists. Hence there was no standardization in the part of the gland examined histologically. PP-rich lobules were found in only 9 out of 21 rhesus organs, compared with 17 out of 20 controls. Whether this reflects a variation due to the non-selective sampling procedure or whether it indicates that the PP-rich lobe is smaller (and therefore less likely to be sampled in a random block) in rhesus organs is not known. Furthermore, an apparent increase of endocrine cell volume fraction could occur if the effect of erythroblastosis were to inhibit development of the exocrine tissue in the PP-rich lobe, but this was ruled out by

TABLE 1
Volume fraction of insulin, glucagon, pancreatic polypeptide, and somatostatin cells in the pancreas of rhesus-immunized and control infants

	PP-poor		PP-rich	
	Rhesus	Control	Rhesus	Control
Insulin	2.39 ± 0.23 (21)	2.54 ± 0.16 (17)	2.04 ± 0.30 (9)	1.27 ± 0.09 (14)†
Glucagon	1.00 ± 0.09 (20)	0.82 ± 0.08 (18)	0.66 ± 0.20 (8)	0.09 ± 0.02 (15)†
Somatostatin	0.84 ± 0.09 (21)	1.08 ± 0.19 (8)	1.15 ± 0.29 (7)	0.45 ± 0.09 (7)*
PP	0.09 ± 0.05 (21)	0.01 ± 0.02 (17)	3.34 ± 0.55 (9)	1.93 ± 0.33 (14)*

*$P < 0.05$, †$P < 0.01$ compared with rhesus group.
Numbers of observations are shown in parentheses.

our finding that the cellular density of both regions was the same. The discovery that the change in erythroblastotic pancreas is limited to the PP-rich part of the gland helps to resolve some of the earlier discrepancies in the literature.

Why erythroblastosis should affect the PP-rich lobe of the pancreas only is a mystery that merits further study in an animal model.

ACKNOWLEDGMENTS

We acknowledge with thanks financial support from the Hawley Trust, the Wellcome Trust, and the Medical Research Council.

REFERENCES

[1] Hultquist, G. T., and Olding, L. B.: Endocrine pathology of infants of diabetic mothers. Acta Endocrinol. 1981; *97* Suppl.: 24.

[2] Raivio, K. O., and Osterlund, K.: Hypoglycemia and hyperinsulinemia associated with erythroblastosis fetalis. Pediatrics 1969; *43*:41–58.

[3] Larsson, L. I., Sundler, F., and Hakanson, R.: Immunohistochemical localization of human pancreatic polypeptide (hPP) to a population of islet cells. Cell Tissue Res. 1975; *156*:167–71.

[4] Baetens, D., DeMey, J., and Gepts, W.: Immunohistochemical and ultrastructural identification of the pancreatic polypeptide producing cell (PP cell) in the human pancreas. Cell Tissue Res. 1977; *185*:239–46.

[5] Orci, L., Baetens, D., Ravazzola, M., Stefan, Y., and Malaisse-Lagae, F.: Pancreatic polypeptide and glucagon: nonrandom distribution in pancreatic islets. Life Sci. 1976; *19*:1811–16.

[6] Orci, L., Malaisse-Lagae, F., Baetens, D., and Perrelet, A.: Pancreatic polypeptide rich regions in human pancreas. Lancet 1978; *2*:1200–1201.

[7] Milner, R. D. G., Wirdnam, P. K., and Tsanakas, J.: Quantitative morphology of B,A,D, and PP cells in infants of diabetic mothers. Diabetes 1981; *30*:271–74.

[8] Sternberger, L. A., Hardy, P. H., Cuculis, J. S., and Meyer, H. G.: The unlabelled antibody enzyme method of immunohistochemistry. J. Histochem. Cytochem. 1970; *18*:315–33.

[9] Streefkerk, J. G.: Inhibition of erythrocyte pseudoperoxidase activity by treatment with hydrogen peroxide following methanol. J. Histochem. Cytochem. 1972; *20*:829–31.

[10] Graham, R. C., and Karnovsky, M. J.: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of the mouse kidney: ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. 1966; *14*:291–302.

[11] Potter, E. L., Seckel, H. P. G., and Stryker, W. A.: Hypertrophy and hyperplasia of the islets of Langerhans of the fetus and of the newborn infant. Arch. Pathol. 1941; *31*:467–82.

[12] Gilmour, J. R.: Erythroblastosis foetalis. Arch. Dis. Child 1944; *19*: 1–25.

[13] Brahler, H. J., and Dallenbach-Hellweg, G.: Die Langerhansschen inselnbei fetaler Erythroblastose. Virchows Arch. Pathol. Anat. 1963; *336*: 544–49.

[14] Van Assche, F. A.: A morphological study of the Langerhans' islets of the fetal pancreas in late pregnancy. Biol. Neonate 1968; *12*:331–42.

[15] Van Assche, F. A., Gepts, W., de Gasparo, M., and Renaer, M.: The endocrine pancreas in erythroblastosis fetalis. Biol. Neonate 1970; *15*:176–85.

[16] Nicolopoulos, D., Hadjigeorgiou, E., Kaslaris, E., and Moschos, A.: The size of the Langerhans' islets in infants with severe jaundice of varying etiology. Biol. Neonate 1973; *22*:348–54.

[17] Zollinger, H. V.: Pathologische Anatomia und Pathogenese des familiaren Morbus haemolyticus neonatorum. Helv. Paediatr. Acta Suppl. 1946; *2*:127–60.