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Case Report

Eosinophilic Pancreatitis in the Newborn Infant of a Diabetic Mother

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Summary. The authors have studied the pancreas of a premature female infant born to a diabetic mother. The findings included a peri-insular eosinophilic leucocyte infiltration, macropolinesia and a marked increase in B cells. In the exocrime parenchyma small B cells aggregates were also observed. B cells contained voluminous hypercromatic muclei and degranulated cytoplasm. Morphometric data demonstrated an increase in islet tissue. These morphological findings are indicative of excessive insulin secretion. The presence of eosinophilic leucocytes in pancreatic tissue and the pathogenic mechanism involved are discussed.

Key words: Newborn – Diabetic mother – Macropolinesia – B cell degranulation – Eosinophilic insulitis.

Introduction

Marked hypertrophy and hyperplasia of the islets of the Langerhans have been described in newborn infants of diabetic mothers (van Beek, 1939; Warren and Le Compte, 1952; Cardell, 1953; McKay et al., 1953; Woolf and Jackson, 1957; Driscoll et al., 1960; D'Agostino and Bahn, 1963; Mancini et al., 1963; Silverman, 1963; Hultquist, 1964; van Assche, 1968; Molsted-Pedersen and Tygstrup, 1968; Payan et al., 1968; Borchard and Müntefering, 1969; Benevolo and Torre, 1971; Freytag and Klöppel, 1973). A less frequently observed histological picture characterized by peri-insular eosinophilic leucocyte infiltration, which is present for only a few hours after birth, has been termed "acute eosinophilic insulitis" (Freytag and Klöppel, 1973).

The present report describes the histological and immunohistochemical aspects of this rare entity and speculates on its probable pathogenesis.

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Case Report

A female infant was born at the 34th week of gestation to a 28-year-old diabetic mother who had received insulin therapy for approximately three years. Antibodies against insulin were not investigated in the mother. At birth, the infant weighed 3800 g (above the 90th centile on the Lubchenco scale), and showed increased thickness of the subcutaneous fat and muscular hypotonicity. A few hours after birth, the patient developed spontaneous tremors of the limbs, and suffered a cyanotic attack which lead to progressive respiratory distress. Repeated checks of serum glucose (glucose-oxidase method) and insulin levels showed oscillating values, ranging between 15 and 22 mg/100 ml for glucose and 40–50 μU/ml for insulin.

At *autopsy* bilateral pulmonary atelectasis complicating hyaline membrane disease was revealed.

Fragments of *pancreas* were fixed in 10% formalin, Bouin and Zenker solutions and embedded in paraffin. Routine staining was performed on sections 5 μm in thickness (H&E, PAS, Azan-Mallory). The Grimelius method was employed for A_2 cells (Grimelius, 1968), the Davenport method modified by Hellerstrom and Hellman for A_1 cells (Hellerstrom and Hellman, 1960) and a modified aldehyde fuchsin stain (TAF) (Bussolati and Bassa, 1974) for B cells. On tissue sections fixed in Bouin solution, indirect immunofluorescence was performed using anti-insulin and anti-glucagon serums, courteously provided by Prof. G. Bussolati (Institute of Pathological Anatomy II, University of Turin). For the second step rabbit anti-immunoglobulin antibodies raised in the goat and labelled with fluorescein isothiocyanate (FITC) (Behring Institute) were employed. Control studies were performed by 1. omitting the first step, 2. omitting the second step or 3. by using non-immune serum. In addition direct immunofluorescence was employed with anti-Ig G serum coupled to FITC (Behring Institute) again using appropriate controls. Observations were carried out using Zeiss Microscope equipped for ultraviolet light with a Osram HB 200 mercury lamp, exicitation filters BG 12 and BG 38 and interference filters n.44 and n.53. Morphometric investigations (Van Assche, 1968) of the pancreas were also performed to calculate the percentage of islets tissue and the ratio of A_2 to B cells; measuring the maximum and minimum diameter of 50 islets, the average surface area was calculated as the area of an ellipse multiplying the semiaxes by π. The number of islets within an area of 0.5 cm^2 of pancreas was also calculated. The percentage of islet tissue was calculated according to the formula: 2 N × average surface area. The percentage of islet cell-types was calculated by counting 2000 cells.

Results

The pancreas was structurally normal with many acini strongly PAS-positive. The islets ware increased in volume and number (macropolinesia) (Fig. 1). A marked infiltration of eosinophilic granulocytes and macrophages were observed surrounding islets and extending out into perivascular and periductular connective tissue. Using the Azan-Mallory stain a fine network of loosely arranged collagenous fibres was observed between eosinophilic leucocytes, blood vessels and ducts. Many B cells with enlarged hyperchromatic nuclei, often containing large nucleoli, were observed. Cells staining positively with the TAF stain were present; most of them were almost degranulated, but a few contained granules either arranged homogeneously or accumulated at one pole of the cell. Small groups of 2–3 TAF positive cells were also observed in the excrine parenchyma. With the Grimelius method and with the Davenport method, A_2 and A_1 cells were numerically normal and sparsely granulated. In the majority of endocrine cells a diffuse positivity in the cytoplasm was observed using indirect immunofluorescence with serum containing anti-insulin antibodies (Fig. 2); only a few cells had a greater net positivity at one pole of the cytoplasm. Furthermore it was possible to detect isolated cells in the exocrine parenchyma with a more

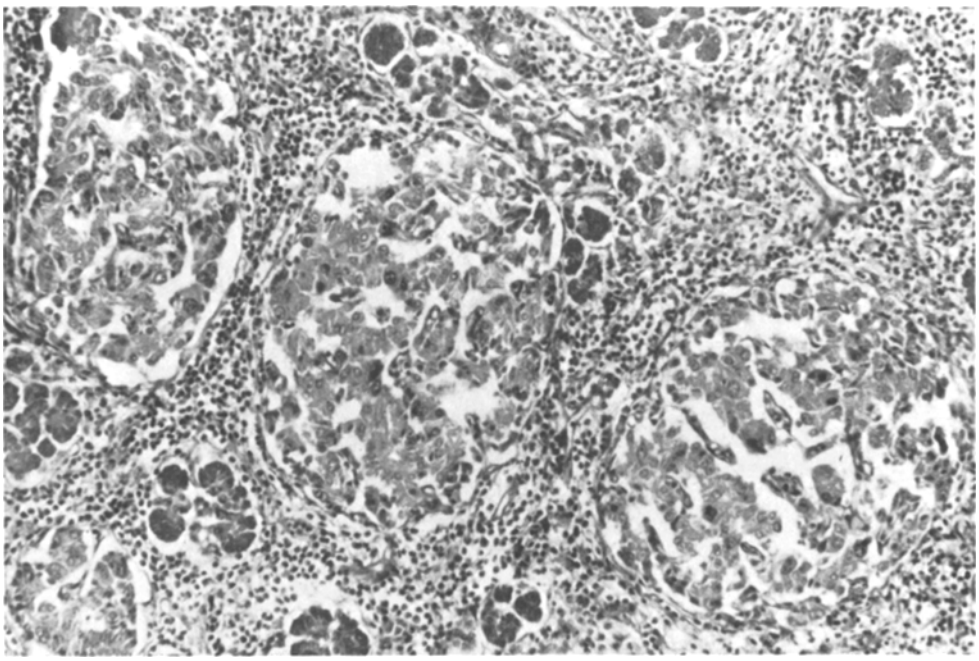

Fig. 1. Massive eosinophil infiltration surrounding hypertrophic islets of Langerhans (PAS × 140)

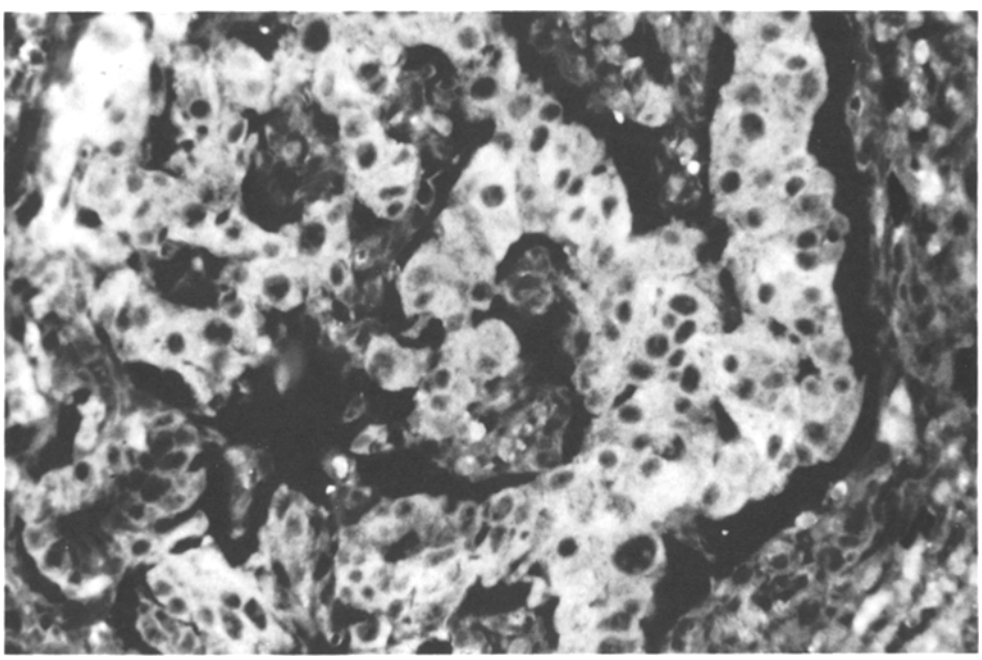

Fig. 2. Indirect immunofluorescence with anti-insulin serum, shows a varying degree of cytoplasmatic positivity of the B cells (FITC × 350)

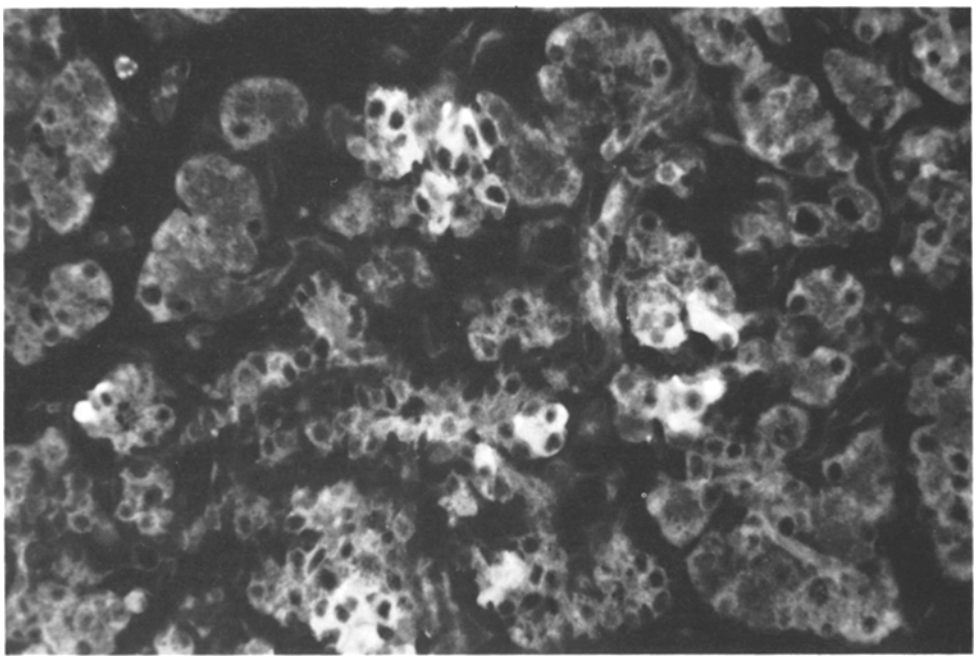

Fig. 3. Small groups of strongly positive B cells in exocrine parenchyma (anti-insulin indirect immunofluorescence, FITC × 350)

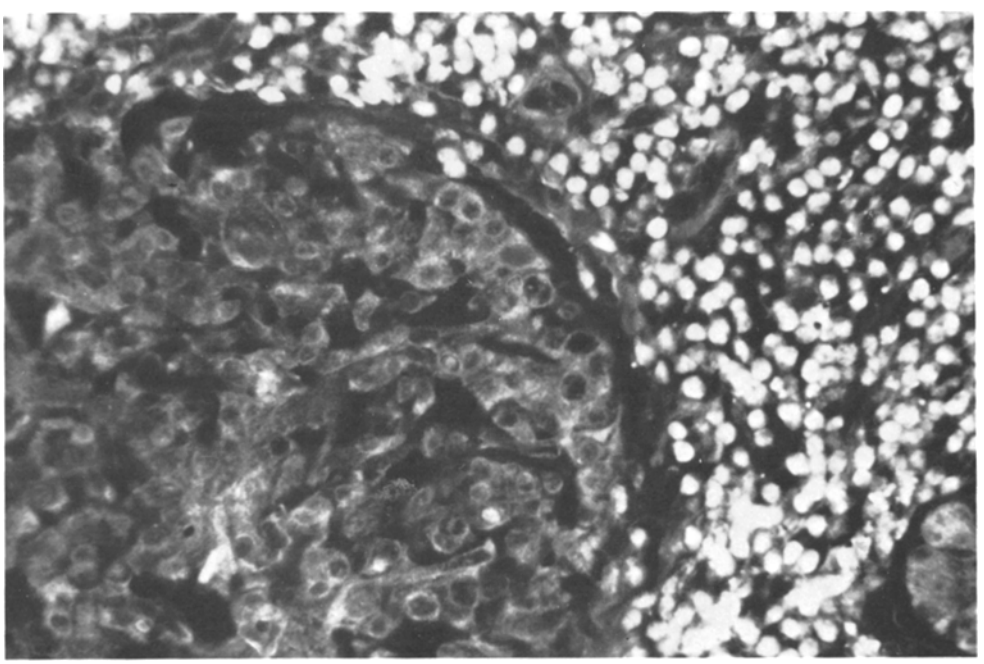

Fig. 4. Direct immunofluorescence with FITC-conjugated anti IgG serum; IgG is deposited in the cytoplasm of the inflammatory cells (FITC × 230)

Table 1. Quantitative estimation of the proportion of islet tissue and islet cells in a newborn of diabetic mother and in 10 controls (post mortem material). SD = Standard deviation

	Age (gestation period in weeks)	Islet tissue (% of total pancreatic parenchyma)	% B cells	% A_2 cells
Case examined	34	5.36	58	41
Controls ($n=10$)	33.6	1.21	39.1	60.9
mean value	(32–37)	(0.98–2.16)	(35–45)	(54–66)
	SD 1.776	SD 0.423	SD 3.213	SD 4.095

intense positivity than that observed in corresponding islet tissue (Fig. 3). Immunofluorescent findings for glucagon showed cytoplasmic positivity, peripherally oriented in the islets.

Direct immunofluorescence with anti IgG antibodies showed an increased number of cells with cytoplasmatic positivity and nuclear negativity in the peri-insular inflammatory infiltration (Fig. 4). A proportion yellow-orange of these cells also showed a primary granular fluorescence distinguished from secondary immunofluorescence using FITC.

Morphometric data are reported in Table 1.

Discussion

In accordance with other observations in the newborn of diabetic mothers we have found in macroscopically normal pancreas, a notable increase in the volume and number of islets (macropolinesia). Moreover morphometric data showed the endocrine tissue to represent 5.36% of total pancreatic parenchyma, while controls gave values of 1.21% (SD 0.423). The islets consisted mostly of B cells with voluminous nuclei and cytoplasm which was for the great part degranulated; furthermore small groups of insulin-secreting cells were observed within the exocrine parenchyma, a phenomenon strongly suggestive of new islet cell formation (Logothetopoulos, 1972). In this study these morphological aspects, in the presence of elevated serum insulin values suggest that the newborn pancreas has undergone an intense synthesis and secretion of insulin.

The changes described in the pancreas of the newborn of a diabetic mother are attributed to maternal hyperglycaemia. Nevertheless it is possible to observe macropolinesia and peri-insular eosinophilic granulocyte infiltration in the pancreas of subjects born to prediabetic normoglycaemic mothers (Woolf and Jackson, 1957). It is believed therefore that other factors are involved in the picture described. Recently, the presence of IgG autoantibodies against insulin (Exon et al., 1974; McCuisl et al., 1974; Tamás et al., 1975) and pancreatic endocrine cell types (Bottazzo et al., 1974; Doniach and Bottazzo, 1977; Pouplard et al., 1977) has been reported in diabetics. In the newborn of a diabetic mother insulin antibodies cross the placental barrier and couple with fetal insulin thereby stimulating and increasing output of the hormone (Martin et al., 1975). Such

an antigen-antibody reaction occurs in the sinusoids of islets, establishing a diffusion gradient for insulin from B cells or extracellular spaces (Bürkle et al., 1971). Furthermore, efferent blood from the islet flows into a capillary network distributed in the surrounding exocrine parenchyma (Ferner, 1959) where probably the maximum concentration of insulin is reached (Mancini et al., 1963); it is suggested that in the presence of anti-insulin antibodies there is a more intense immunopathic reaction taking place.

In accordance with such a pathogenetic hypothesis, it is possible experimentally to obtain similar findings to those seen in the newborn of a diabetic mother (Lacy et al., 1963; Lacy and Wright, 1965; Logothetopoulos and Bell, 1966; Freytag et al., 1969; Klöppel et al., 1971; Klöppel et al., 1972). After repeated administration af anti-insulin antibodies, islet hyperplasia and neogenesis and inflammatory infiltration is observed (Klöppel et al., 1971; Klöppel et al., 1972). The degree of insulitis and hyperglycaemia deponds on serum levels of the antibody.

In the light of these findings it is possible to understand the partecipation of eosinophils which play a prominent role in the phagocytosis of antigen-antibody complexes (Archer and Hirsch, 1963; Sabesin, 1963; Litt, 1964; Cochran and Dixon, 1968; Ishikawa et al., 1974) and in the neutralization of toxic substance that develop in the course of immunological reactions (Vaughn, 1953; Fernex, 1962; Archer, 1963). Furthermore, it must be kept in mind that in perinatal period such a role assumes greater importance since eosinophilic leucocyte maturation percedes that of the lymphatic and immunological system (Dourov, 1967). In extramedullary tissues – especially in those where eosinophilopoiesis is most active like the liver, pancreas and thymus – the presence of eosinophils is a normal finding (Dourov, 1967). With anti-IgG immunofluorescence, we have encountered cytoplasmic positivity in the majority of cells involved in the inflammatory infiltration; such a diffuse positivity is differentiated from true eosinophilic granular autofluorescence. This finding suggests IgG, and presumably antigen-antibody complex, endocytosis by eosinophils.

Furthermore, non-immunological factors can cause eosinophils to congregate in tissues. Recent studies have shows that eosinophil granulocytes bind estrogenic steroids with high specificity (Tchernitchin, 1967, 1972; Tchernitchin and Chandross, 1973; Goetzl et al., 1975; Tzchernitchin et al., 1976a, b; Geuskens et al., 1977). This suggests the possibility that eosinophils within tissues can be influenced by the local hormonal environment (Goetzl et al., 1975). In this way, one could postulate that the eosinophils regulate the pancreatic concentration of insulin in some hyperinsulinemic conditions.

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