EFFECTS OF SUB-TOTAL GASTRO-INTESTINAL PANCREATECTOMY OF THE RAT FOETUS

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SUMMARY

Sub-total pancreatectomy in utero was performed in 18-day-old rat foetuses. Pancreatectomized, sham-operated and control foetuses were collected 3 days later and body weight, glucose and insulin levels in blood, and glycogen content and glucose-6-phosphatase (G-6-Pase) activity of the liver were determined. Pancreatectomized foetuses showed only very small pancreatic remnants (< 1 mg) and accordingly their insulin levels were much lower (four to five times) than those of sham-operated or control foetuses; their blood glucose levels were slightly increased and liver glycogen content and G-6-Pase activity were slightly reduced; their body weights were also reduced.

These results are discussed in relation to other relevant data in the literature. They afford direct experimental evidence of the endogenous origin of insulin in the foetal blood. It is suggested that during the last days of intra-uterine life insulin merely completes the action of the glucocorticoids on glycogen storage in rat foetal liver and probably contributes to foetal body growth. Its relative ineffectiveness on the foetal blood glucose level is not explained. As pancreatectomized foetuses develop sub-normal liver G-6-Pase activity, glucagon is probably not responsible for the increase in this activity occurring during normal development before birth.

INTRODUCTION

During the last days of pregnancy, the foetal rat pancreas is the site of rapid physiological maturation as judged by changes in the ultrastructural appearance (see Perrier, 1970; Pictet, Clark, Williams & Rutter, 1972) and insulin content (see references in Sodoyez-Goffaux, Sodoyez & Foa, 1971). Simultaneously, insulin levels in foetal blood increase sharply (Félix, Jacquot & Sutter, 1969; Cohen & Turner, 1972; Girard, Cuenod, Marliès, Kervran, Rieutort & Assan, 1973) and rise much above that of the mother, especially in starving mothers. From this, and other, indirect experimental evidence, it has been concluded that foetal insulin in the circulation originates in the foetal pancreas.

The physiological role of this foetal insulin is still poorly understood, especially with reference to glucose and liver glycogen metabolism. It has been proven (Jacquot, 1959; Jacquot & Kretchmer, 1964; Plas & Jacquot, 1966; Plas, Chapeville & Jacquot, 1973) that both in vivo and in vitro, glucocorticoids are necessary for the development of the biosynthetic pathway of glycogen storage in the foetal liver. Manns & Brockman (1969) suggested that insulin might also regulate this function in vivo. In vitro, the results of Eisen, Goldfine & Glinsmann (1973) and also Plas et al. (1973) showed that if glucocorticoids are essential for the ‘induction’ of the biosynthetic enzyme glycogen synthetase...
(EC 2.4.1.11), then insulin completes their action by converting the enzyme to its I form, and thus permits a larger accumulation of glycogen.

We have found that it is practically impossible to deprive rat foetuses of insulin by alloxan treatment and that the effects of streptozotocin are unpredictable (death of the foetus, reduced insulin levels, or no effect at all may result). Therefore we have tried to perform pancreatectomy in utero, despite its obvious difficulty, in order to evaluate its effects on the development of the foetus.

MATERIALS AND METHODS

Animals

Wistar rats (CF strain of the C.N.R.S.) received water and commercial rat food (U.A.R.) ad libitum. They were housed in a constant temperature room at 22 °C and exposed to a 12 h light:12 h darkness cycle. Male rats were present in the cages of the females between 18.00 and 08.00 h, and coitus was assessed by examination of vaginal smears. Day 0 of gestation represents the 24 h period following coitus, which was assumed to have taken place on average at 01.00 h. Surgery or sampling of material was performed at 11.00 h, i.e. at \( n \) days + 10 ± 7 h. For the sake of simplicity, this is referred to as \( n \) days post coitum (p.c.).

Surgery

Eighteen-day pregnant rats were anaesthetized with bromochlorotrifluoroethane (Fluothane; ICI-Pharma). After laparotomy, one uterine horn was exteriorized gently in order to avoid its contraction and to preserve its relative transparency. The foetal pancreas is white and can be easily distinguished through both uterine and foetal abdominal walls. An opening was made in the uterine wall, according to the procedure of Jost (1947), in front of the foetal pancreas. The abdominal wall of the foetus was then incised and the stomach, pancreas and most of the intestines were smoothly pulled out and excised, care being taken not to injure the liver, kidneys, adrenals and umbilical cord. Haemostasis was performed by electrocoagulation and the foetal abdominal wall was not sutured. Some foetuses were sham-operated. The uterine cavity was finally closed as described by Jost (1947).

Sampling

The pregnant rats were killed by decapitation at 21 days p.c., and the foetuses were rapidly collected. Félix (1968) found that at 21 days p.c. foetal blood glucose levels increase after artificial delivery, therefore the foetal blood was collected from an axillary artery within 1 min after maternal death and either immediately deproteinized (for glucose estimation) or allowed to clot and then centrifuged (for insulin estimation). There was enough blood from each foetus to measure the individual glycaemias, but for insulin measurements the sera sometimes had to be pooled: therefore in Table 1 the number of insulin determinations is less than the number of glucose determinations, but the mean values apply to the same whole group of animals in both cases.

Livers were rapidly excised for determination of glycogen content and glucose-6-phosphatase (EC 3.1.3.9) (G-6-Pase) activity. Some livers were pooled, but the mean values apply to the whole group.

In pancreatectomized foetuses, the pancreatic remnants were prepared for histological examination and the integrity of the adrenals was checked, since these glands play a major role in the control of liver glycogen storage.
Gastro-intestinal pancreatectomy of rat

Assays

Glycogen
Livers were digested for 3 h at 100 °C in 60 % KOH solution; glycogen was precipitated by ethanol and purified. Glucose obtained after acid hydrolysis and neutralization was then measured according to the method of Hagedorn & Jensen (1923).

Blood glucose
Blood was deproteinized with an equimolar mixture of barium hydroxide and zinc sulphate. Glucose was measured in the supernatant by the glucose oxidase method (Huggett & Nixon, 1957).

Immunoreactive blood insulin (IRI)
Immunoreactive insulin in sera was measured generally by the double antibody procedure of Hales & Randle (1963) (SORIN commercial kits). Some determinations were also performed with the single antibody (dextran-coated charcoal) procedure according to Herbert, Lau, Gottlieb & Bleicher (1965), except for the conditions of incubation (4 days at 4 °C). In both assays, rat insulin (NOVO) was used as standard.

Glucose-6-phosphatase activity
Glucose-6-phosphatase activity was determined on 1/20 liver homogenates in 0·25 m-sucrose, stored frozen at −25 °C. The method was essentially that of Swanson (1955) (except for the use of acetate buffer, pH 5·5, 0·04 mol/l), inorganic phosphate being measured according to Fiske & Subbarow (1925). Results are expressed in µmol inorganic phosphate/g fresh tissue after 30 min of incubation at 37 °C.

RESULTS

Surgery
Three days after pancreatectomy (21 days p.c.) most foetuses were alive. Their abdominal walls had healed spontaneously. Most had very small pancreatic remnants (≤ 1 mg), i.e. less than 1/20 of the pancreatic weight of the litter-mate controls. Histological examination of these remnants revealed either a well-preserved organ structure with one or two islets, or a dissociation with some scattered B cells. Body weights of the pancreatectomized foetuses ranged between 3·1 and 4·1 g (3·55 ± 0·12 (s.e.m.) g), those of sham-operated foetuses ranged between 3·5 and 5·2 g (4·6 ± 0·15 g), and those of litter-mate non-operated controls ranged between 4 and 5·2 g (4·7 ± 0·12 g). The intact gastro-intestinal tract of control and sham-operated foetuses weighed approximately 0·4 g; pancreatectomized foetuses had only small remnants of this tract (essentially rectum and caecum).

Blood glucose
Mean blood glucose levels were higher in pancreatectomized than in sham-operated (P < 0·05) or non-operated (P < 0·02) foetuses (Table 1).

Blood insulin
Blood IRI, measured by the double antibody procedure, was much lower in pancreatectomized than in sham-operated (P < 0·001) or control (P < 0·001) foetuses (Table 1). Using the single antibody procedure, blood IRI was 93 ± 18 µu./ml in six control foetuses compared with 17 and 25 µu./ml in two pancreatectomized foetuses.
**Glycogen content and glucose-6-phosphatase activity in the liver**

Mean liver glycogen content was lower in pancreatectomized than in sham-operated ($P < 0.01$) or control ($P < 0.001$) foetuses (Table 1).

Mean G-6-Pase activity was slightly decreased in pancreatectomized foetuses, the decrease being significant ($P < 0.05$) compared with that of non-operated controls only.

**Table 1. Effects of foetal pancreatectomy on blood glucose and insulin, liver glucose-6-phosphatase activity and glycogen content. Blood insulin was measured by the double antibody-technique (mean ± S.E.M.; number of experiments in parentheses)**

<table>
<thead>
<tr>
<th>State of animals</th>
<th>Blood glucose (mg/100 ml)</th>
<th>Blood insulin (μU/ml)</th>
<th>Glucose-6-phosphatase activity (μmol $P_i$/g at 30 min)</th>
<th>Glycogen (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatectomized</td>
<td>51.8 ± 4.2 (12)</td>
<td>49.5 ± 4.3 (6)</td>
<td>69.8 ± 4.8 (12)</td>
<td>63.8 ± 6.1 (12)</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>42.6 ± 1.9 (14)</td>
<td>181 ± 23 (6)</td>
<td>83.8 ± 7.3 (9)</td>
<td>89.3 ± 4.1 (8)</td>
</tr>
<tr>
<td>Non-operated controls</td>
<td>42.3 ± 1.6 (24)</td>
<td>181 ± 21 (14)</td>
<td>87.8 ± 5.7 (16)</td>
<td>92.4 ± 2.6 (18)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The efficiency of pancreatectomy is reflected by the IRI levels. Obviously some insulin was still circulating and, correspondingly, B cells were still found. But IRI levels in pancreatectomized foetuses were four to five times lower than those of the controls; moreover, part of these low levels might represent an artifact, for rat plasma proteins interfere in the double antibody procedure (Jacquot, Félix, Légrele, Sutter-Dub & Sutter, 1974). However, the sharp decrease in IRI levels after pancreatectomy provides direct experimental evidence of the endogenous origin of foetal insulin in the circulation.

**Blood glucose**

Injection of very large doses of insulin into the rat foetus at the end of gestation results in hypoglycaemia (Picon & Montane, 1968; and personal observations), and administration of anti-insulin antibody produces slight hyperglycaemia (Rabain & Picon, 1974; confirmed by personal observations). The present results show that when insulinaemia is drastically reduced by sub-total pancreatectomy, slight hyperglycaemia is present at 21 days p.c. It might be thought that reducing the insulinaemia of an adult rat to a quarter of its normal value would result in marked hyperglycaemia, except perhaps under severe dietary restriction. This discrepancy between the large decrease of IRI and the very moderate increase of blood glucose in the pancreatectomized foetus is puzzling. Perhaps these IRI levels, although reduced, are still able to maintain a normal glycemia, but then why are the normal IRI levels so high? Another explanation might be that insulin is not a potent regulator of glucose metabolism in the foetus: some unresponsiveness to insulin by foetal tissues (other than liver) has been suggested in vitro (Britton & Blade, 1970; Félix, Sutter, Sutter & Jacquot, 1971) and in vivo (Clark, Cahill & Soeldner, 1968; Manns & Brockman, 1969), but contradictory results have also been obtained in vitro (Fricke & Clark, 1973) and in vivo (Rabain & Picon, 1974). It could also be suggested that insulin increases the placental transfer of glucose to the foetus, an effect counteracting the hypoglycaemic action of insulin; here again very few experimental data are available (see, for instance, Rabain & Picon, 1974). Finally, the discrepancy between the large decrease of IRI and
the very moderate increase of glycaemia might be due to the fact that the placenta is virtually impermeable to insulin and freely permeable to glucose: any tendency to foetal hyperglycaemia would be counteracted by glucose back-diffusion to the maternal blood. But it is difficult, in this situation, to understand the hyperglycaemic effect of glucagon on the rat foetus (Girard, Caquet, Bal & Guillot, 1973; Girard, 1975). There is at present no explanation for the apparent lack of action of foetal pancreatectomy on the blood glucose level.

Liver glycogen metabolism

When pancreatectomy was performed at 18 days p.c., the liver contained less than 1% glycogen; 3 days later, despite the pancreatectomy, it contained roughly 7%. It seems therefore that pancreatic hormones, and especially insulin, are not an absolute requirement for glycogen storage, a result which has already been suggested (Jacquot, 1959). Indeed liver glycogen content is smaller in pancreatectomized than in sham-operated or control foetuses, but the major portion of their glycogen store is present. It seems therefore that insulin, in vivo, merely completes the action of glucocorticoids in building up the ability of the liver to store glycogen. Manns & Brockman (1969) and Rabain & Picon (1974) found that insulin injected into the foetus enhances glucose incorporation into liver glycogen. In-vitro results of Plas et al. (1973) and Eisen et al. (1973) also support this suggestion.

It is well known that after completion of glycogen storage the liver develops the faculty for glycogen mobilization during the perinatal period. Glucose-6-phosphatase is one of the key enzymes in such a process. In the rat, G-6-Pase activity increases rapidly on and after 20 days p.c. (see Jacquot & Kretchmer, 1964). Since the work of Greengard & Dewey (1967) it has often been suggested that pancreatic glucagon might be responsible for this rapid increase. Although it was not measured in the present study, one may assume that the blood glucagon level (from pancreatic or intestinal origin) was low in the pancreatectomized foetuses, as judged by their insulin level and by the sub-total absence of their gastro-intestinal tract; nevertheless their liver G-6-Pase activity at 21 days p.c. was only moderately reduced (see Table 1) and was in any case much greater than at 18 days p.c. (approximately 15 μmol P₁ / g at 30 min). It seems therefore that glucagon, even if playing a role in the regulation of G-6-Pase activity, is not an absolute requirement for its induction. Former work (Jacquot & Kretchmer, 1964; Jacquot, 1971) indicated that factors originating in the head are probably important, since decapitated foetuses have reduced G-6-Pase activity in their liver. This point will be discussed in a forthcoming paper.

Finally, our results provide some indication that the pancreas is involved in foetal growth. Mean body weights of pancreatectomized foetuses were 3:55 g, and those of sham-operated and control foetuses were 4:2 and 4:3 g respectively, the gastro-intestinal tracts of the foetuses having been excluded in each case. The differences between pancreatectomized and sham-operated or control foetuses are statistically significant (P < 0:01). It seems therefore that pancreatectomy adds some effect to the non-specific retardation of growth due to surgical trauma. Intervention of insulin in foetal growth has already been studied by Picon (1967), and Blazquez, Simon, Blazquez & Foa (1974) have also discussed this point. Our results are compatible with their conclusions.

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REFERENCES


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