A similar calculation may be made in Case 3, where there was a rise in plasma sodium from 123 to 131 mEq/l. over a six-hour infusion period with a fall in weight from 68 to 67.9 kg and a urinary sodium loss of 50 mEq/l. The discrepancy in this case is 2.8 litres. It is difficult therefore to escape the conclusion that sodium passed from the intracellular to the extracellular space during the treatment period. A rapid and direct action of the treatment on the myocardium is also suggested by the change of rhythm seen within an hour of starting the infusion in Cases 1 and 2 and the rapid recovery from cardiogenic shock in Case 1. It could be argued that some of the improvement seen in these patients was due to potassium gain. This is unlikely in view of the rapidity of response long before any substantial positive potassium balance could be achieved. In those patients who responded biochemically there was also a clinical improvement with an increase in appetite and feeling of well-being. Although such changes are difficult to express quantitatively they were a striking and not unimportant feature of the response to treatment.

We wish to thank Dr. G. Walters and Dr. R. D. Eastham for

the biochemical estimations performed in their laboratories at the Bristol Royal Infirmary and Frenchay Hospital. We also thank Dr. J. E. G. Pearson, Dr. D. W. Barritt, and Dr. S. C. Jordan for permission to study patients under their care. We are grateful to Dr. M. Hartog and Professor A. E. Read for help and encouragement during this study. The support of the Wellcome Trust for one of us (S.P.A.) is gratefully acknowledged.

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PRELIMINARY COMMUNICATIONS

Glucose and Insulin Metabolism after Pancreatic Transplantation

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British Medical Journal, 1972, 3, 678-681

Summary

After removal of the pancreas pigs develop hyperglycaemia and ketosis. Both orthotopic and heterotopic transplants prevent hyperglycaemia but the animals so treated display markedly different patterns of glucose and insulin metabolism. Orthotopic graft recipients have normal glucose tolerance curves with raised plasma insulin levels. The glucose tolerance curves of heterotopic graft recipients show significant hypoglycaemia and very marked hyperinsulinaemia. These results are explicable by the diversion of the two pancreatic hormones from the liver, and possibly by the effect of ischaemia and denervation on the pancreas.

Introduction

Insulin has remained the mainstay of treatment in human diabetes since its discovery 50 years ago. Although insulin prolongs life in insulin-dependent diabetics, the long-term features of the disease and its complications may not be pre-

vented. "Control" of glucose metabolism in insulin-dependent diabetics may be complicated by neutralizing antibodies to heterologous insulin injections produced in the recipient (Burson and Yalow, 1960). Furthermore, the injection of a single dose of a "depot" insulin results in a period when the hormone is released at a steady rate into the circulation, a situation which requires rigid dietary control to prevent hypoglycaemia. In short, exogenous insulin therapy can produce enough hormone for day-to-day needs but cannot replace the delicately and homoeostatically balanced secretion of insulin (present in the normal subject and absent in the insulin-dependent diabetic) which controls the level of circulating glucose in accordance with the body's needs.

Pancreatic grafts may temporarily restore to normal the glucose metabolism of animals rendered diabetic experimentally (De Jode and Howard, 1962; Reemstma *et al.*, 1964; Bergan *et al.*, 1965; Largrader, 1967; Mitchell and Davidson, 1967) and patients suffering from maturity onset or juvenile diabetes (Lillehei *et al.*, 1967; Lillehei *et al.*, 1970). Although the side effects of immunosuppression make the procedure unjustifiable in most cases it is likely that with improved methods of suppressing graft rejection pancreatic grafting may well become a useful therapeutic tool in the treatment of diabetes, particularly if improved control of carbohydrate metabolism can be shown to influence the chronic retinal, neurological, and vascular side effects of the disease. Meanwhile more information is required on the metabolic effects of the types of graft available.

Pancreatic grafts may be inserted heterotopically or orthotopically. In the heterotopic graft pancreatic venous blood drains into a systemic vein and therefore bypasses the liver; orthotopic grafts drain into the portal vein, whence the route of delivery of insulin and glucagon to the liver is normal. This paper presents a comparison of the glucose and insulin metabolism in apantectic pigs after these two procedures.

Animals

Large white Landrace or large white/Landrace (cross) pigs weighing 10-35 kg were used. These were obtained from the Agricultural Research Centre's pig colony at Babraham,

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Cambridgeshire, and from the University of Liverpool veterinary field research station at Leahurst, Cheshire.

Operative Techniques

Full details of the techniques are reported elsewhere (Calne and Sells, 1972). Pigs were anaesthetized by halothane and oxygen induction after 24 hours of starvation and were subjected to one of the procedures outlined below.

PANCREATECTOMY ALONE

A midline incision was made and the duodenum and upper jejunum were mobilized. The pancreas was dissected from the surrounding bowel, and by careful identification and ligation of all vessels coming from the splenic and pancreaticoduodenal arteries and by piecemeal dissection the gland was removed without interfering with the blood supplies of the spleen or duodenum. The abdomen was closed and the animals were put on a free diet without insulin substitution.

HETEROTOPIC PANCREATICODUODENAL ALLOGRAFTS

Donor Operation.—The donor gland was freed from adjacent bowel, taking care not to damage the delicate parenchyma. The splenic artery and vein were individually divided distal to the splenic lobe of the pancreas. The portal vein was freed for 1½ in (3.8 cm) above the entry point of the highest pancreatic vein. A 3-in (7.6-cm) segment of aorta was prepared bearing the coeliac artery and the superior mesenteric artery, the lumbar and hepatic branches being divided. The numerous divisions of the superior mesenteric vein and superior mesenteric artery were divided separately at the caudal edge of the body of the pancreas. The pancreas was then removed, together with a segment of duodenum, by dividing the aorta on each side of the coeliac axis and superior mesenteric artery and by cutting the portal vein above the pancreas. Care was taken to preserve the pancreatic duct papilla which is sited in the lower duodenum or upper jejunum.

FIG. 1—Heterotopic pancreaticoduodenal graft in situ.

Recipient Operation.—After removal of the animal's own pancreas the recipient abdominal vena cava and aorta were exposed for 2 in (5 cm) above their bifurcations. The cephalic end of the donor aortic segment was anastomosed end-to-side to the abdominal aorta of the recipient and the distal end of the aortic stump was oversewn. The donor portal vein was anastomosed end-to-side to the abdominal vena cava. The donor duodenal segment was either interposed distal to the recipient pylorus between the stomach and the duodenum or anastomosed end-to-side to the recipient jejunum, the upper end being oversewn. (A heterotopic pancreaticoduodenal graft in situ is illustrated in Fig. 1.)

ORTHOTOPIC PANCREATICODUODENAL ALLOGRAFTS

Donor Operation.—As above.

Recipient Operation.—After removal of the animal's own pancreas the segment of donor aorta was joined end-to-side to the recipient upper abdominal aorta, the distal end of the aortic segment being oversewn. The portal vein was anastomosed side-to-side to the recipient portal vein. Duodenal continuity with recipient bowel was restored in the same way as in the heterotopic grafts. (An example of an orthotopic pancreaticoduodenal graft in situ is given in Fig. 2.)

FIG. 2—Orthotopic pancreaticoduodenal graft in situ.

Postoperative Investigations

Intravenous glucose tolerance tests were performed under anaesthesia before operation (control) and at 2-20 days after pancreatic transplantation. Animals were starved for 24 hours, anaesthetized with halothane and oxygen, and an intravenous catheter was introduced through the jugular vein into the right atrium. Fifty per cent. dextrose was injected rapidly into the right atrium in a dose of 0.3 or 0.5 g/kg body weight. Blood samples were taken at 0 (fasting), 1, 5, 10, 20, and 30 minute after the glucose injection. Whole blood sugar estimations were performed on samples taken into fluoride tubes using the Technicon Autoanalyzer. Blood for insulin estimation was taken into lithium heparin bottles and the red cells were removed by centrifugation within two hours of removal. Immunoassay for plasma insulin was performed using the method of Hales and Randle (1963).

Results

SURVIVAL

After pancreatectomy alone the animals appeared to suffer few ill effects during the first seven days. They then suffered weight loss, and the three longest surviving animals died with ketosis. The mean survival time in this group was 14.4 days. Orthotopic and heterotopic graft recipients (after pancreatectomy) did not exhibit such noticeable weight loss, and death due to rejection of the graft or intercurrent intestinal obstruction was often acute and unheralded by significant wasting. The mean survival time for all transplanted animals was 23 days (see Table).

Survival of Pigs after Pancreatectomy and Pancreatic Grafting (Days)

Pancreatectomy only (n = 5)	1, 10, 17, 18, 26 (mean 14.4)
Orthotopic pancreatic graft recipients (n = 6)	11, 15, 18, 23, 24, 50 (mean 23.5)
Heterotopic pancreatic graft recipients (n = 7)	5, 10, 11, 16, 21, 22, 49 (mean 19.1)

BLOOD SUGAR AND PLASMA INSULIN LEVELS AFTER OPERATION

Group 1.—Normal anaesthetized animals ($n=16$). After a glucose load the peripheral blood level fell rapidly and within 30 minutes had descended to a mean of 150 mg/100 ml. Plasma insulin levels were fairly constant at around 15–20 μ U/ml (Fig. 3).

Group 2.—Pancreatectomy alone ($n=5$). The mean blood sugar level remained higher than 280 mg/100 ml throughout the

FIG. 3—Group 1, normal pigs ($n=16$). Effect of glucose tolerance tests (0.3 g dextrose/kg body weight) on mean blood sugar concentrations and plasma insulin levels (± 1 S.D.).

FIG. 4—Group 2, apantretic pigs ($n=5$). Effect of glucose tolerance tests on mean blood sugar (●) and plasma insulin (○) levels.

glucose tolerance tests. No insulin could be detected in peripheral blood during the tests (Fig. 4).

Group 3.—Orthotopic pancreatic grafts ($n=6$). The mean blood sugar curve was indistinguishable from the normal mean glucose tolerance curve. However, the plasma insulin levels were raised throughout the test by comparison with normal (difference between means at 30 minutes $P < 0.05$) (Fig. 5).

Group 4.—Heterotopic pancreaticoduodenal allografts ($n=7$). In this group of pigs glucose tolerance tests were performed before the abdomen was opened and compared with those conducted two hours (and in four animals 2 and 10 days) after transplantation. The dextrose load was 0.5 g/kg body weight. At all times after transplantation the mean blood glucose

FIG. 5—Group 3, orthotopic pancreatic graft recipients ($n=6$). Effect of glucose tolerance tests on mean blood sugar concentrations and plasma insulin levels.

concentrations were lower than preoperative values during the tests. This difference was significant at 30 minutes ($P < 0.05$). The plasma insulin levels were greatly increased over normal ($P < 0.01$).

No significant departure from these patterns of glucose and insulin production was seen during rejection of the grafts, although hyperglycaemia was found in one group 3 and two group 4 animals immediately before death.

Discussion

The extensive literature on experimental transplantation of the pancreas contains much information on fasting blood glucose levels in normal, apantretic, and transplanted dogs; there have been occasional reports of animals having an unexpectedly low blood sugar level postoperatively after orthotopic (Largiadre

FIG. 6—Group 4, heterotopic pancreatic graft recipients ($n=7$). Effect of glucose tolerance tests (0.5 g dextrose/kg body weight) on mean blood glucose concentrations and plasma insulin levels before (●) and after (○) pancreatectomy and grafting.

et al., 1967) and heterotopic grafting. Bergan *et al.* (1965) commented on the consistently low blood sugar levels found in 10 out of 13 animals surviving heterotopic transplantation of the pancreas and suggested that hyperinsulinaemia consistently follows a one-hour period of total pancreatic ischaemia in dogs; experiments conducted in the same laboratory (Binder *et al.*, 1970) showed that the pancreas secretes larger amounts of insulin during the first week after heterotopic transplantation, although control data for comparison are lacking. The authors suggest that ischaemic damage to β -cell membranes results in increased permeability to insulin, causing insulin "leakage" from the islets analogous to potassium leakage from damaged mucosal cells. As insulin granule release is a process of emiocytosis (reverse pinocytosis) rather than diffusion, it is possible that increased insulin granule release is stimulated by agents other than passive diffusion across a "leaky" cell membrane.

This study shows that orthotopic pancreatic grafts restore normal glucose tolerance to apantretic pigs, although the plasma insulin level is persistently raised above normal for up to 10 days after transplantation. If this effect were due purely to the transient effects of ischaemic cell membrane damage, then a return to normal would be expected within a few days of transplantation, as in the series of dogs with ischaemic non-transplanted pancreases described by Cerra *et al.* (1970). In our series, however, the hyperinsulinaemia appeared to be permanent, the sole difference (apart from the species) being that the gland was denervated during the removal of the organ from the donor. Warner and her colleagues (Bunnag *et al.*, 1963) indicated from their experiments on murine islets of Langerhans that insulin release and hence the blood sugar concentration depends *inter alia* on the blood flow through the islet microvasculature. It is reasonable to postulate that the hyperinsulinaemia found after orthotopic pancreatic transplantation is caused by insular hyperaemia which follows denervation of the donor pancreas; this effect persists after the postoperative reactive hyperaemia caused by tissue ischaemia has passed off.

Heterotopic grafts cause a significant depression of the glucose tolerance curve, which is accompanied by a pronounced increase in the level of circulating insulin in the peripheral blood. These two phenomena result from the diversion of pancreatic vein blood away from the hepatic portal vein, with subsequent abnormalities in the distribution, site of action, and fate of its two major hormonal secretions, glucagon and insulin.

Portal vein glucagon normally stimulates glucose release from hepatic glycogen (but not muscle glycogen) by forming cyclic adenylic acid which activates glycogen-splitting phosphorylase (Guyton, 1971). The diversion of glucagon from the liver would thus inhibit the release of glucose from the liver and contribute to a hypoglycaemic state.

The level of active insulin is higher in human portal vein blood than in hepatic vein or peripheral blood (Samals and Ryder, 1961; Ryder, 1961; Anthoniades *et al.*, 1962). Samaan *et al.*

(1962) were able to show higher values for immunologically reactive insulin in portal vein blood than in hepatic vein blood after glucose administration. If it is assumed that conversion of immunologically reactive insulin to non-reactive insulin occurs in the liver, peripheral hyperinsulinaemia would be expected after diversion of pancreatic blood direct into a systemic vein.

Hypoglycaemia has not been reported in human heterotopic pancreatic graft recipients, but as all the patients received steroids postoperatively it seems likely that the blood glucose was maintained at an artificially high level. Lillehei *et al.* (1970) reported marked increase in blood sugar and daily insulin requirements in these patients when the dosage of prednisone was increased.

If pancreatic transplantation can ever be performed without necessitating the administration of steroids then the possible hazards of hypoglycaemia should be avoidable by orthotopic rather than heterotopic placement of the graft.

We are grateful to Miss Marion Bramley of the department of biochemistry University of Cambridge and Mr. S. H. Davison, of the John Bennett Clinical Laboratories, Addenbrooke's Hospital, for performing insulin assays. Expert technical help was provided by Miss D. Davies at Cambridge and Mrs. I. Tyrrell at Liverpool, and we express our most sincere thanks to Mrs. M. F. Allen, who drew the illustrations.

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