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Long-Term Hormonal Secretion from the Autotransplanted Sheep Pancreas

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Summary. Seventy-five duct-ligated pancreatic segmental autotransplants were made into bipedicled skin loops on the necks of merino ewes by vascular suture to the carotid artery and jugular vein; the in situ pancreatic remnants apparently continued to function normally. Thirty-seven were found to be active hormone secretors (secretion-rate responses to Na butyrate greater than 1 mU/min for insulin or 5 ng/min for glucagon) when first tested approximately 1 month after transplantation; 12 remained active at 1 year, 5 at 2 years, and 4 at 3 years. At first testing, the responses were (mean ± standard errors): insulin, 12.3 ± 2.52 mU/min; glucagon, 52.6 ± 13.5 ng/min. It is concluded that this autotransplant can, on occasion, be relatively long-lived and that it is a useful model with which to study not only pancreatic physiology but also non-immunological factors involved in survival of endocrine function in pancreatic transplants.

Key words: Pancreas, endocrine pancreas, sheep, transplantation, autotransplantation, insulin, glucagon, alpha-cell, beta-cell.

In the merino ewe, autotransplantation of organs into the neck as an aid to their physiological study has been described for the adrenal [1, 2, 14], ovary [3] and pancreas [4, 5, 6]. Exteriorisation of the thyroid has also been described [7, 8, 9]. The organ is transplanted (or in the case of the thyroid, exteriorised) into a bipedicled skin loop on the left-hand side of the neck where its blood supply is anastomosed to the left carotid artery and left jugular vein, also contained in the loop (Fig. 1). By the use of appropriately inflated pneumatic cuffs, control of blood flow in and out of the loop is obtained so that infusions and sampling can be made at organ level, avoiding the dilutional effects of the whole circulation and the interference by other organs that occur in whole animal studies. Not only are such preparations conveniently accessible without anaesthesia, in contrast to the in situ organs, but they can continue to function apparently normally and without detriment to the health or activity of their hosts for up to several years, allowing many experiments on the one preparation.

Details have been published elsewhere of the surgical technique [10] and of physiological studies on

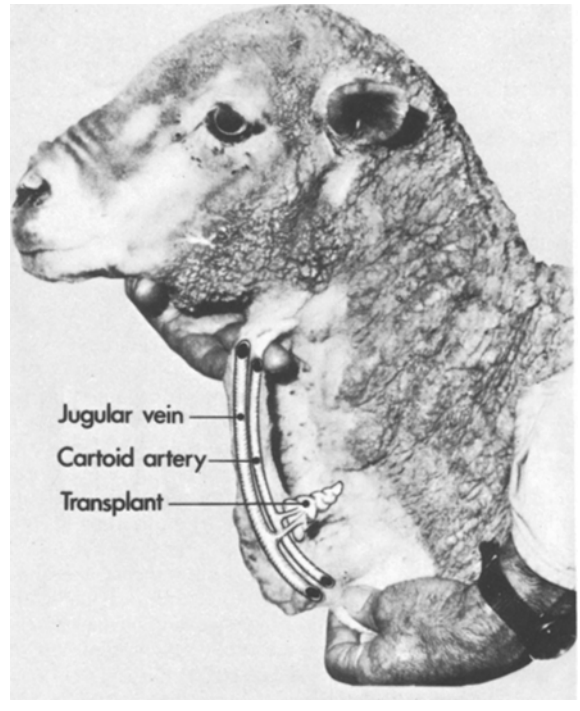

Fig. 1. General view of transplant

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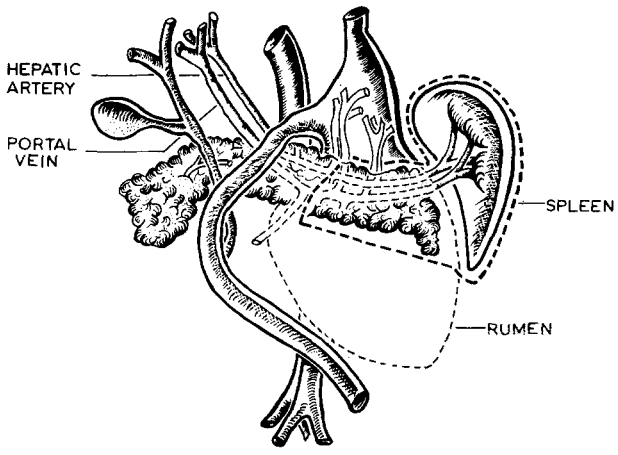

Fig. 2. Anatomical relationships of in situ ovine pancreas. The portions removed are outlined with the heavy dashed line

the secretion of insulin [11, 12, 6] and glucagon [4, 13]. The present communication summarises observations on transplant survival time.

Materials and Methods

Animals. Pedigree merino ewes (4–6 yr) were used and were maintained out-of-doors on pasture, except for short periods around the times of surgery and experimentation. Food was routinely withheld for 16 hours before experimentation and for 24 h before surgery.

Surgery. This has been described in detail previously [10]. A bipedicled loop of skin containing the carotid artery and jugular vein (the branches and tributaries having been ligated and divided) was created in the neck. Several weeks later (Fig. 2) the spleen was removed, the splenic vessels dissected out, and all branches other than those to the pancreas ligated and divided. The narrow neck of tissue connecting the right and left lobes of the pancreas was then doubly ligated and divided and the left lobe removed, together with the splenic vessels and their pancreatic branches. The splenic artery and vein were then anastomosed end-to-side to the carotid artery and jugular vein in the loop (Fig. 1). The average ischaemic time for the transplant was usually 14–18 min and no cooling or perfusion was carried out. Just before removal of the pancreas, 10,000 U of heparin was injected into the coeliac artery.

Measurement of Transplant Responsiveness. The greatest increase in hormone secretion-rate during a 30 min perfusion of the transplant with sodium butyrate at 0.048 mmol/min was taken as the standard response with respect to the hormone concerned (insulin or glucagon). Details of the technique for measuring secretion-rates have been published elsewhere [6]. After insertion of catheters, animals were allowed to stand quietly for 90 min before the beginning of the test. Secretion-rate measurements were made immediately before and at 1, 3, 6, 10, 15, 20, 25 and 30 min after the start of the butyrate infusion. Transplants giving standard responses of 1 mU/min or more for insulin secretion or 5 ng/min or more for glucagon were classed as "active", those giving smaller responses being discarded.

Hormone Assays. Plasma insulin was measured by the charcoal-separation radioimmunoassay method [43] with ovine insulin as standard, human insulin (labelled with 125-I as described by Hunter and Greenwood [44]) as tracer, and guinea-pig anti-porcine insulin antiserum. Blood samples for insulin assay were collected into heparinised tubes standing in ice-water, and the plasma separated without delay at 0° C and stored at −20° C. Plasma glucagon was measured by a modification of the method of Unger et al. [45] using his antiserum K30. Crystalline bovine-porcine glucagon was used as standard and, labelled as for insulin, as tracer. Blood samples for glucagon assay were collected into EDTA tubes standing in ice-water. Trasylol, 500 KIU/ml of blood, was included during the former part of the study but was dispensed with latterly as it was shown to be unnecessary for hormone stability. The plasma was separated and stored as for insulin. It was shown that both hormones were quite stable under these conditions. All samples from any one experiment were always run in the same assay; inter-assay variation was adjusted for by the use of replication standards of glucagon at 2 levels and insulin at 3 levels in the sheep plasma and hormone levels were adjusted by dilution before assay to ≤ 200 μU/ml for insulin ≤ 400 pg/ml for glucagon.

For the insulin assay, the within-assay coefficients of variation for duplicate samples were 10.5, 2.5 and 2.0% at the 4.8, 108 and 246 μU/ml levels respectively; interassay co-efficients of variation over 11 assays were 50, 9.5 and 6.9% at these 3 levels. Correspondingly, for glucagon, the coefficients were: within-assay, 8.5 and 3.0% at the 96 and 334 pg/ml levels; interassay, 24.6 and 6.5% for the same levels, measured over 10 assays. As the glucagon assay was not available during the early part of this study, glucagon secretion data are presented on only a proportion of the transplants.

Results

Post-operatively there was usually a small leak of pancreatic juice from a fistula that formed in the neck-loop. In every case this decreased to zero after several weeks, when the fistula healed over. In at least 4 cases, however, no fistula appeared in transplants that were subsequently shown to actively secrete both insulin and glucagon.

Averaged over all "active" tests in the study, basal insulin and glucagon secretion-rates (measured immediately before starting the butyrate infusion) were 3.0% and 5.0%, respectively of the corresponding standard responses.

Of the 75 transplants made, 37 proved to be active by the criteria given above when initially tested several weeks after transplantation. At these initial tests the mean responses (± standard errors) were, for insulin, 12.3 ± 2.52 mU/min; and for glucagon, 52.6 ± 13.5 ng/min, the highest responses being 56 mU/min and 260 ng/min respectively.

The table shows the lifetime distribution of the 37 initially active transplants, while Figure 3 shows the effect of transplant age on hormone responses for individual transplants. At last testing, 4 transplants were still active, aged 4, 5½, 11 and 12 months.

The responses of 2 transplants given the standard test immediately after transplantation, while their hosts were still under anaesthetic on the operating table, were respectively 38.4 and 266 mU/min for insulin secretion and 45.8 and 638 ng/min for glucagon; these results are shown on the zero ordinates in Figure 3.

Figure 4 shows insulin vs glucagon responses; the points referring to results on individual transplants at different ages are joined by straight lines.

Table 1. Lifetimes of initially active transplants. Criteria of activity given in Methods

Transplant Lifetime months	Number
0– 3	11
3–12	14
12–24	7
24–36	1
36–46	4

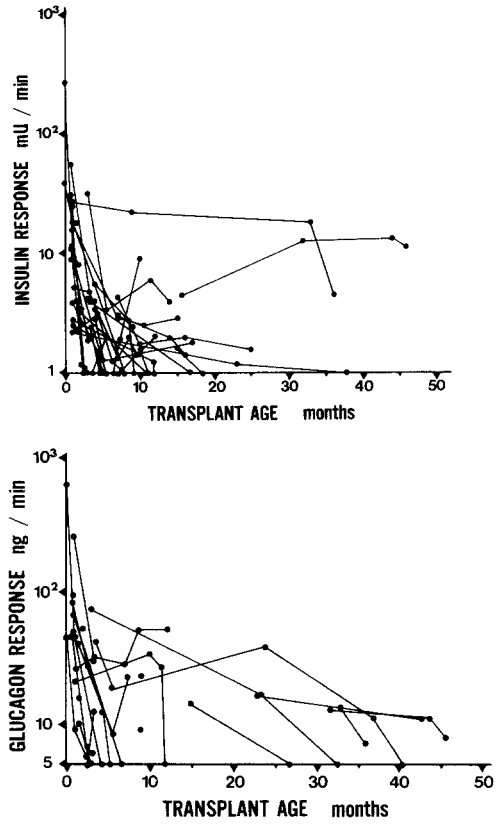

Fig. 3. Initially active transplants: Responsiveness as a function of transplant age. Criteria of activity and method of responsiveness measurement given in Methods. Zero-time data explained in Results

Discussion

Except possibly in the 4 cases in which no fistula formed, ligation of the cut end of the pancreatic lobe during transplantation, which was calculated to close off all branches of the pancreatic duct draining the area, did not result in the complete and immediate suppression of the exocrine function expected from reports on duct-ligation experiments in other species (e. g., 25, 28). Why this was so is not clear, but similar exocrine leakage from duct-ligated preparations has been reported in two studies on humans [36, 37].

Why this exocrine leak, once established, did not continue indefinitely, but invariably ceased after several weeks, is also not clear. Charters et al. [38] found that in rats, in which neither duct-ligation nor pancreatic sectioning was done, the normal exocrine function of the transplanted (presumably denervated) organ was maintained indefinitely. However, Rappaport et al. [22] described a canine preparation that behaved very like the ovine transplant, with initial discharge and healing of the fistula; the exocrine-secreting acinar tissue survived for a month or two longer but eventually it also disappeared. Rappaport's preparation had been sectioned, like ours, but not ligated. Thus it could have been that sectioning was the cause of the loss of exocrine function in our own and in Rappaport's transplants.

Sodium butyrate was used for testing hormone secretory activity because, in the ovine autotransplant, it is a potent secretagogue for both insulin and glucagon [4].

The data of the Table show that of the 37 ovine transplants that were classified as active hormone secretors at the initial test according to the criteria stated above, 12 were still active at one year, 5 at 2 years and 4 at 3 years.

Whilst it is not possible to make precise comparisons between these results and those of other workers because of species differences (no other sheep studies have been reported) and details of the preparations (especially the probability than none of our animals depended on its transplant for survival), it is fair to say that a significant proportion of the ovine transplants have been relatively long-lived. In the case of allotransplants, short life-times have often been attributed to immunological rejection, but even with iso- and autotransplants, relatively few have survived for as long as a year. According to Mitchell et al., writing in 1966 [15] the longest documented survival of either homo-or autografts up to that time was 17 days, but since then a number of much longer lived preparations have been reported. Lampe [16]

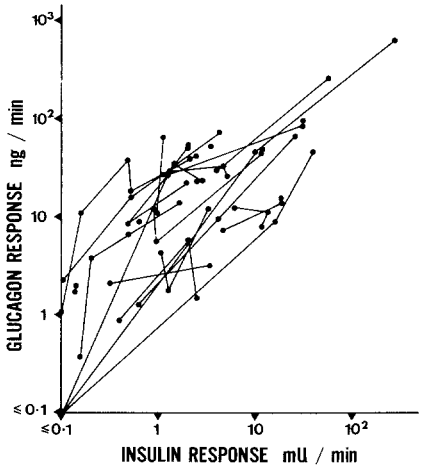

Fig. 4. Initially active transplants: Responsiveness with respect to insulin secretion *vs* that with respect to glucagon for transplants at different ages. Criteria of activity and method of responsiveness measurement given in Methods

reported, after autoimplantation of pancreatic fragments into the peritoneal cavity of the pig, one animal alive 6 months later with elevated blood sugar and low insulin levels, but with vascularised nodules of increasing size containing cells with aldehyde-fuchsin positive granules. Using diabetic rats, Orloff et al. [17] obtained survivals of up to 2 years, with normal blood insulin and sugar levels, by isografting pancreatico-duodenal and duct-ligated preparations; Weber et al. [18] obtained survivals of more than 10 months by intraperitoneal injection of isologous neonatal pancreas, while Leonard et al. [19] obtained survivals of 18 months with normoglycaemia by a similar procedure. Canine autotransplant survivals of up to 6 months were reported by Mitchell and Davidson [20] (pancreatic tail into leg), 7 months by Aquino et al. [21] (pancreatic tail into iliac fossa), 8 months by Rappaport et al. [22] (pancreatic tail into body-wall), 18 months with normoglycaemia by Dreiling and Ashikari [23] (part organ into neck), and 3 years with normal endocrine function by de Gruyl et al. [24]. Verschoor et al. [25] reported one autotransplanted dog still alive after 5 years and 2 iso-transplants still alive after more than 2 years. In spite of being under the threat of immunological rejection, some allotransplants have also been relatively long-lived: the longest human survival has been 4.2 years [26]. Idezuki et al. [27] described the survival of a single dog for 150 days after pancreatico-duodenal allotransplantation with immunosuppressive treatment, and Verschoor et al. [25] reported one allotransplanted dog on suppressive treatment still alive after more than 90 days.

Failure of hormone secretory activity in the ovine transplants typically took the form of a sharp decline in the first few weeks after transplantation (documented only in the two animals that were tested on the operating table but probably general) followed by a slower decline over periods of months. The reason for this failure, which is of interest because of its possible relevance to human pancreatic replacement in diabetes, is not at all clear. The transient exocrine secretion might have destroyed islet tissue – the exocrine discharge had a strongly corrosive action on the skin and wool of the loop in the vicinity of the fistula, and it might also have attacked the transplant itself. Furthermore, it would seem not unlikely that some exocrine secretion, resorbed internally, went on in the four animals in which no fistula developed, and also in others after fistula closure. Thus, in dogs, Rappaport et al. found acinar cells surviving some time after closure of the fistula [22]. Pancreatic duct-ligation in itself has usually been considered to lead to at least some reduction in endocrine function [24, 25, 29, 30, 31, 32], although Egdahl et al. [46] and Orloff et al. [17] found otherwise – in the latter paper the view is expressed that failures have been due rather to "technical difficulties related to transection of the pancreas and inadequate blood supply to the graft because of small vessel size and segmental vascular anatomy". Other factors, such as a mutual dependence of the exocrine and endocrine functions [33, 34, 35], or absence of nerve supply to the transplant, or autoimmune destruction, might also have played a part. Antibody studies to exclude this last mentioned possibility are in progress.

Figure 3 shows a few instances of apparently increasing transplant responsiveness. While poor test reproducibility could perhaps explain these results, work by others suggests that functional hypertrophy of the transplant would not seem to be out of the question, especially if the in situ pancreatic remnant had partly failed following the surgery so that additional demands fell on the transplant. Thus a restoration of pancreatic endocrine function over a period of 2 years, after an initial fall following duct-ligation, was reported in dogs by de Gruyl et al. [24]; Lampe et al. [16] reported an increase in the size of nodules of pancreatic tissue and in the number of cells containing aldehyde-fuchsin positive granules over 7 months in a pig in which pancreatic fragments had been autotransplanted into the peritoneal cavity; Hegre et al. [39] showed that the endocrine cells of implants of neonatal pancreas placed under the kidney capsule in isologous rats continued to grow and that a similar preparation implanted intra-peritoneally probably did so also. Other similar observations have been reported [40, 41, 42].

Figure 4 shows that both the alpha- and the beta-cell activities of the ovine transplant fail together, in which respect this preparation does not resemble the diabetic pancreas with its specific beta-cell failure [47].

We feel that the ovine pancreatic autotransplant offers a generally useful alternative to other preparations for studying pancreatic transplantation as well as physiology. As a physiological model it permits study of the endocrine pancreas in relative isolation in an intact animal without the dilutional effects of the circulation on either infusates or secretions, or possible interference from other organs (including the exocrine pancreas) that occur in whole-animal studies. It also allows unequivocal distinction between transplant pancreatic glucagon and that from other sources. Furthermore short (5–20 min) closed-circuit perfusions can be carried out without the perfusate entering the general circulation. As a transplant, it provides a relatively long-lived model for ectopic, duct-ligated, pancreatic transplantation, one that is probably not complicated by immunological rejection and whose functions may be monitored easily and specifically at regular intervals in an unanaesthetised animal.

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References

1. Beaven, D. W., Espiner, E. A., Hart, D. S.: The suppression of cortisol secretion by steroids, and the response to corticotrophin, in sheep with adrenal transplants. J. Physiol. (Lond.) **171**, 216–230 (1964)
2. Wright, R. D., Blair-West, J. R., Coghlan, J. P., Denton, D. A., Funder, J. W., Godin, J. R., Nelson, J. F., Scoggins, B. A.: The structure and function of adrenal autotransplants. Aust. J. Exp. Biol. Med. Sci. **50**, 873–892 (1972)
3. Goding, J. R., McCracken, J. A., Baird, D. T.: The study of ovarian function in the ewe by means of a vascular autotransplantation technique. J. Endocrinol. **39**, 37–52 (1967)
4. Arcus, A. C., Ellis, M. J., Kirk, R. D., Beaven, D. W., Donald, R. A., Hart, D. S., Holland, G. W., Redekopp, C.: Studies with the autotransplanted ovine pancreas: glucagon and insulin secretion. Aust. J. Biol. Sci. **29**, 223–236 (1976)
5. Beaven, D. W., Salamonsen, L. A., Hart, D. S., Holland, G. W., Espiner, E. A.: Insulin secretion in sheep with autotransplants of the pancreas. Nature **233**, 955 (1969)
6 Bell, J. P., Salamonsen, L. A., Holland, G. W., Espiner, E. A., Beaven, D. W., Hart, D. S.: Autotransplantation of the pancreas in sheep: insulin secretion from the transplant. J. Endocrinol. **48**, 511–525 (1970)
7. Falconer, I. R.: The exteriorization of the thyroid gland and measurement of its function. J. Endocrinol. **26**, 241–247 (1963)
8. Falconer, I. R., Hetzel, B. S.: Effect of emotional stress and TSH on thyroid vein hormone level in sheep with exteriorized thyroids. Endocrinology **75**, 42–48, (1964)
9. Turner, J. G.: An evaluation of lithium in the treatment of thyrotoxicosis. MD Thesis, University of Otago, 1977
10. Holland, G. W.: Pancreatic autotransplantation in sheep. Aust. N. Z. J. Surg. **39**, 75–77 (1969)
11. Beaven, D. W., Bell, J. P., Harty, P., Hart, D. S.: The effect of certain gastro-intestinal hormones on insulin secretion from pancreatic auto-transplants. N. Z. Med. J. (Proceedings) **74**, 338 (1971)
12. Bell, J. P., Beaven, D. W.: Biphasic insulin release, experimental and clinical aspects. Aust. N. Z. J. Med. **1**, 107 (1971)
13. Ellis, M. J., Arcus, A. C., Beaven, D. W., Donald, R. A., Hart, D. S., Holland, G. W.: Glucagon secretion by the autotransplanted pancreas in the sheep. Proc. Univ. Otago Med. Sch. **51**, 22–24 (1973)
14. McDonald, I. R., Goding, J. R., Wright, R. D.: Transplantation of the adrenal gland of the sheep to provide access to its blood supply. Aust. J. Exp. Biol. Med. Sci. **36**, 83–95 (1958)
15. Mitchell, R. I., Rappaport, A. M., Davidson, J. K.: Autotransplantation of the pancreas. Can. J. Surg. **9**, 192–198 (1966)
16. Lampe, E. W., II, Sutherland, D. E. R., Najarian, J. S.: Autotransplantation of porcine islets of Langerhans. Surgery **79**, 138–143 (1976)
17. Orloff, M. J., Lee, S., Charters, A. C., Grambort, D. E., Storck, L. G., Knox, D.: Long term studies of pancreas transplantation in experimental diabetes mellitus. Ann. Surg. **182**, 198–206 (1975)
18. Weber, C., Zatriqi, A., Weil, R., McIntosh, R., Hardy, M. A., Reemtsma, K.: Pancreatic islet isografts, allografts and xenografts: comparison of morphology and function. Surgery **79**, 144–151 (1976)
19. Leonard, R. J., Lazarow, A., McEvoy, R. C., Hegre, O. D.: Islet cell transplantation. Kidney Int. [Suppl. 1] **6**, 169–178 (1974)
20. Mitchell, R. I., Davidson, J. K.: Heterotopic autotransplantation of the canine pancreas. Surgery **62**, 454–461 (1967)
21. Aquino, C., Ruiz, J. O., Schultz, L. S., Lillehei, R. C.; Pancreatic transplantation without duodenum in the dog. Am. J. Surg. **125**, 240–244 (1973)
22. Rappaport, A. M., Vranic, M., Wrenshall, G. A.: A pedunculated subcutaneous autotransplant of an isolated pancreas remnant, for the temporary deprivation of internal pancreatic secretion in the dog. Surgery **59**, 792–798 (1966)
23. Dreiling, D. A., Ashikari, H.: Physiologic studies of the heterotopic autotransplanted pancreas. Surg. Forum **17**, 203–205 (1966)
24. de Gruyl, J., Verschoor, L., Vriesendorp, H. M., Hörchner, P., Westbroek, D. L., Hulsmans, H. A. M.: DL-A and isolated pancreas allograft. Br. J. Surg. **59**, 315 (1972)
25. Verschoor, L., Hulsmans, H. A. M., de Gruyl, J., Westbroek, D. L., MacDicken, I.: Endocrine function of the canine pancreas: The effect of duct ligation and transplantation of the total duct ligated pancreas. Acta Endocrinol. (Kbh.) **80**, 302–318 (1975)
26. ACS/NIH Organ Transplant Registry: Final Newsletter, June 30, 1977
27. Idezuki, Y., Feemster, J. A., Dietzman, R. H., Lillehei, R. C.: Experimental pancreaticoduodenal preservation and transplantation. Surg. Gynecol. Obstet. **126**, 1002–1014 (1968)
28. Zeligs, J. D., Aaron, J., Dumont, A. E.: The course and nature of acinar cell death following pancreatic ligation in the guinea pig. Am. J. Pathol. **80**, 203–226 (1975)
29. Grambort, D. E., Lee, S., Storck, L. G., Chandler, J. G., Charters, A. C., Elliott, M. L., Orloff, M. J.: Pancreatic duct ligation and long-term endocrine function of pancreas transplants. Surg. Forum **24**, 299–302 (1973)

30. de Gruyl, J., Westbroek, D. L., Dijkhuis, C. M., Vriesendorp, H. M., MacDicken, I., Elion-Gerritsen, W., Verschoor, L., Hulsmans, H. A. M., Hörchner, P.: Influence of DL-A matching, ALS, and 24-hour preservation on isolated pancreas allograft survival. Transplant. Proc. **5**, 755–759 (1973)
31. Heptner, W., Neubauer, H. P., Schleyerback, R.: Glucose tolerance and insulin secretion in rabbits and dogs after ligation of the pancreatic ducts. Diabetologia **10**, 193–196 (1974)
32. Idezuki, Y., Goetz, F. C., Lillehei, R. C.: Late effect of pancreatic duct ligation on beta cell function. Am. J. Surg. **117**, 33–39 (1969)
33. Grabner, W., Phillip, J., Strigl, P.: The diagnostic value of oral glucose tolerance test and combined B-cell stimulation in chronic pancreatitis. Am. J. Dig. Dis. **18**, 1055–1060 (1973)
34. Pfeiffer, E. F., Fussganger, R., Hinz, M., Raptis, S., Schleyer M., Straub, K.: Extrapancreatic hormones and insulin secretion. In: Diabetes: Proc. 7th Congr. Int. Diabetes Fed., Buenos Aires. Rodriguez, R. R., Vallance-Owen, J. (Eds.). Excerpta Medica Int. Congr. Series **231**, 460–471 (1971)
35. Youngs, G.: Progress report: hormonal control of pancreatic endocrine and exocrine secretion. Gut **13**, 154–161 (1972)
36. Gliedman, M. L., Tellis, V., Soberman, R., Rifkin, H., Freed, S. Z., Veith, F. J.: Pancreatic transplantation in New York. Kidney Int. [Suppl. 1] **6**, 164–168 (1974)
37. Brynger, H.: Studies on the duct-ligated pancreas as allograft for islet cell function. Acta Chir. Scand. [Suppl.] **453**, 28 (1975)
38. Charters, A. C., Lee, S., Storck, G., Chandler, J. G., Orloff, M. J.: Long-term exocrine function of the pancreas transplant. Am. J. Surg. **129**, 16–22 (1975)
39. Hegre, O. D., Leonard, R. J., Schmitt, R. V., Lazarow, A.: Isotransplantation of organ-cultured neonatal pancreas: reversal of alloxan diabetes in the rat. Diabetes **25**, 180–189 (1976)
40. Boquist, L., Edström, C.: Ultrastructure of pancreatic acinar and islet parenchyma in rats at various intervals after duct-ligation. Virchows Arch. [Pathol. Anat.] **349**, 69–79 (1970)
41. Edström, C., Falkmer, S.: Pancreatic morphology and blood glucose level in rats at various intervals after duct-ligation. Virchows Arch. [Pathol. Anat.] **345**, 139–153 (1968)
42. Zweens, J., Bouman, P. R.: Neoformation of insulin-producing islets following ligation of the pancreatic ducts in normal and alloxan-diabetic rats. Acta Physiol. Pharmacol. Neerl. **14**, 529–531 (1967)
43. Albano, J. D. M., Ekins, R. P., Maritz, G., Turner, R. C.: A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta Endocrinol. (Kbh.) **70**, 487–509 (1972)
44. Hunter, W. M., Greenwood, F. C.: Preparation of iodine – 131 labelled human growth hormone of high specific activity. Nature **194**, 495–496 (1962)
45. Unger, R. M., Aguilar-Parada, E., Müller, W. A., Eisentraut, A. M.: Studies of pancreatic alpha cell function in normal and diabetic subjects. J. Clin. Invest. **49**, 837–848 (1970)
46. Egdahl, R. H., Hiebert, J. M., Mack, E.: Insulin secretion in the duct-ligated pancreas. J. Clin. Invest. **47**, 28 (1968)
47. Barnes, A. J., Bloom, S. R.: Pancreatectomized man: A model for diabetes without glucagon. Lancet **1976 I**, 219–221

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