Aust. J. Biol. Sci., 1976, **29**, 223–36

Studies with the Autotransplanted Ovine Pancreas: Glucagon and Insulin Secretion

A. C. Arcus, M. Jane Ellis, R. D. Kirk, D. W. Beaven, R. A. Donald, D. S. Hart,[A] *G. W. Holland and C. Redekopp*

Department of Medicine, Christchurch Hospital and Christchurch Clinical School, Christchurch, New Zealand.
[A] Lincoln College, Lincoln, New Zealand.

Abstract

Basic studies on the secretion of glucagon and insulin by the ovine pancreatic autotransplant in the neck are described. Of the 17 transplants in the series none failed to secrete glucagon and only three failed to secrete insulin in detectable amounts. The longest surviving transplant actively secreted both hormones 3 years after transplantation and five other transplants were functional and the animals healthy after 16 months. Exocrine secretion disappears shortly after transplantation.

Sodium butyrate and alanine each promoted the secretion of both hormones by the transplant. Glucagon failed to promote insulin secretion by the transplant, although it apparently stimulated the ovine *in situ* pancreas. The immediate (presumably direct) effect of insulin was to inhibit transplant glucagon secretion. Hypoglycaemia induced by peripheral insulin administration failed to stimulate glucagon secretion by the transplant, although it did promote glucagon secretion by the ovine *in situ* pancreas. Heparin did not markedly suppress basal transplant secretion of either glucagon or insulin.

Phasic response patterns occurred with both hormones during long butyrate perfusions, although first-phase responsiveness was not a constant feature. In one trial, first-phase responses fell off with repeated short butyrate infusions. Glucagon and insulin secretory patterns in response to butyrate were remarkably alike, suggesting a common mechanism.

Loss of specific functions by the ovine pancreas after transplantation is discussed.

Introduction

Autotransplantation of a large portion of the pancreas of the Merino ewe into a specially prepared site in the neck for physiological studies has been described in full by Holland (1969) and Bell *et al.* (1970). Briefly, it involves the surgical fashioning on the left side of the neck of a bipedicled loop of skin within which the left carotid artery and left jugular vein are incorporated, and subsequently, at a later operation, the transfer of the relatively discrete tail portion of the pancreas, comprising one-third to one-half of the organ, to this loop, where the pancreatic blood vessels are anastomosed, end-to-side, to the carotid artery and the jugular vein respectively. The pancreatic remnant, which appears to remain fully functional, is left in place in the abdomen. Because of the small size of the blood vessels supplying the pancreatic tail, the attached, larger, splenic vessels are used for the anastomoses, which entails sacrificing the spleen. Such animals have survived in good health with actively secreting transplants for up to 3 years.

This preparation has proved especially convenient for the study of biochemical factors involved in insulin secretion (Bell *et al.* 1970) in that it is far more accessible than the *in situ* pancreas. Thus, experiments involving perfusions and blood sampling right at gland level can be carried out without anaesthesia or surgical trauma or any

other major stress to the animal. Moreover, a single animal can be experimented on repeatedly over periods of months and years. Also it is an easy matter to temporarily isolate the transplant by clamping off the blood vessels in the loop in such a way that short, closed-circuit perfusion experiments can be performed.

A preliminary report on glucagon secretion by this preparation in response to infusions of L-arginine and butyrate has been published by Ellis *et al.* (1973). The present paper describes further studies on both glucagon and insulin secretion.

Materials and Methods

Animals

The pedigree Merino ewes which were used were maintained out-of-doors on pasture except for short periods around the times of surgery and experimentation. The main pancreatic duct was not ligated at a preliminary operation, as was the practice earlier in this work (Bell *et al.* 1970). When ligation is not done, digestive secretion from the transplant forms a fistula in the loop through which it discharges for several weeks after the transplant operation; eventually this secretion ceases and the fistula heals. Sheep were brought into the laboratory the day before testing and food withdrawn until after the test.

Materials

The following materials were used: sodium butyrate (Pfaltz and Bauer; solutions for infusion were made up in isotonic saline); heparin injection BP, 25 000 i.u./ml (Weddel Pharmaceuticals Ltd); L-arginine (Sigma); glucagon antiserum (rabbit; K30 from Dr R. H. Unger, Dallas, Texas); insulin antiserum, guinea pig anti-porcine; insulin, crystalline ovine (Eli Lilly, batch No. PJ4499); glucagon, crystalline bovine-porcine (Eli Lilly, lot No. 258-234B-167-1); Trasylol, 10 000 Kallikrien Inactivator Units (K.I.U.)/ml (Bayer); activated charcoal, Norit-OL (Hopkin and Williams); and Dextran T-70 (Pharmacia).

Assays

Plasma insulin was measured by the charcoal-separation radioimmunoassay method of Albano and Ekins (1970), using ovine insulin as standard, human insulin [labelled with ^{125}I as described by Hunter and Greenwood (1962)] as tracer, and guinea pig anti-porcine insulin antiserum. Blood samples for insulin assay were collected into heparinized tubes in ice–water; the plasma was 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.* (1970), using his antiserum K30, reported to be highly specific for pancreatic glucagon (Unger *et al.*, personal communication), and crystalline bovine-porcine glucagon as standard and as tracer when labelled with ^{125}I as described by Hunter and Greenwood (1962). Blood samples for glucagon assay were collected into EDTA tubes containing Trasylol (500 K.I.U./ml of blood) in ice–water; the plasma was separated without delay at 0°C and stored at −20°C. It was shown that glucagon and insulin were both stable under these conditions.

Samples from any one experiment were always run in the same assay.

The coefficients of variation within the glucagon assays for replicate samples run in duplicate were 8·5 and 3·0% at the 96 and 334 pg/ml levels respectively. Concentrations of glucagon were adjusted before assay by sample dilution to fall below 400 pg/ml. The inter-assay coefficients of variation measured over 10 assays were 24·6 and 6·5% for the 96 and 334 pg/ml samples respectively.

Correspondingly, for the insulin assay the coefficients of variation were: within-assay, 10·5, 2·5 and 2·0% at the 4·8, 108 and 246 microunits/ml levels respectively; inter-assay, 50, 9·5 and 6·9% at the 4·8, 111 and 243 microunits/ml levels respectively, calculated over 11 assays. Concentrations of insulin were adjusted before assays by sample dilution to fall below 200 microunits/ml.

Plasma glucose was assayed by the glucose oxidase method (Beckman Glucose Analyser).

Perfusion Experiments

Transplant perfusion experiments were carried out as described in detail by Bell *et al.* (1970).

Briefly, infusions were made through a scalp-vein, or similar, needle inserted into the loop carotid artery on the thoracic side of the transplant, the artery being clamped off on the head side so that

all of the infusate passed into the transplant. Infusions were made with a Harvard syringe pump at a constant rate.

For transplant venous sampling, the jugular vein in the loop was clamped off on both sides of the venous anastomosis and blood in this blind segment withdrawn as fast as it accumulated through an indwelling catheter inserted at the start of the experiment. Blood flow rates through the transplant were measured intermittently by timing the venous collections. Transplant glucagon and insulin secretion rates were calculated from the arterio-venous increases in plasma levels across the transplant and the blood flow rates through it, allowance being made for red cell volume.

Blood samples for the measurement of transplant arterial levels of glucagon, insulin and glucose were taken either from the infusion needle, or, more conveniently, from the contralateral jugular vein, it having been shown that levels of these at the two sites were practically equal.

In earlier studies heparin (8000 units) was given intravenously to the animal at the start of the experiment to prevent blood clotting occurring in the needle and catheter, as well as in the blood samples. Later, however, because of the possibility of its affecting transplant secretion, heparin was confined to samples and catheters, a procedure which proved to be quite satisfactory.

Control measurements were made on samples taken at the end of a settling-down period (at least $1\frac{1}{2}$ h) and immediately before the start of an infusion.

Results

Blood flow through the transplants averaged about 20 ml/min, but it varied considerably not only between transplants but also during experiments—flow rates were increased up to twice control rates during glucagon and the stronger butyrate infusions, and decreased, sometimes almost to zero, during the stronger glucose infusions. These effects occurred with both secretors and non-secretors. Because of these variations responses were reported in terms of hormone secretion rates rather than in terms of venous levels, since the latter would be expected to be directly affected by blood flow rate.

Over all experiments, the control transplant secretion rates of glucagon averaged 1228 pg/min (range 0–12 500), and of insulin 91 microunits/min (range 0–760).

It was regularly found that peripheral plasma levels of insulin and glucose were markedly affected by the setting up of the sheep for an experiment, which involved confining it to the experimental stand and inserting the infusion needle and catheters for blood sampling. Plasma levels over all experiments in the series averaged as follows: basal (early sample taken from the contralateral jugular vein within 1 min of first disturbing the animal on the day): glucagon 122 pg/ml, insulin 9·7 microunits/ml, and glucose 57 mg/100 ml; first control sample (taken about 1 h later): glucagon 97 pg/ml, insulin 15·1 microunits/ml, and glucose 91 mg/100 ml. Subsequently the plasma glucose level returned to normal over several hours. The apparent fall in glucagon level may be insignificant.

Sodium butyrate was found to be a potent stimulant for the secretion of both hormones, which led to its being used generally for testing. All of the transplants cited in this communication responded actively to butyrate, as shown in Table 1. In addition to the tests included in the table, for the 11 other transplants in the series that were tested the average responses (greatest increases in hormone secretion rate) to a standard butyrate infusion test (0·048 mmol/min for 30 min) were as follows: glucagon, 18 140 pg/min (range 525–41 000); insulin, 3265 microunits/min [range 0 (3 transplants)–13 400]. The longest-surviving transplant (V5) actively secreted both hormones in response to butyrate 3 years after transplantation, and five other sheep in the present series of 17 had functional transplants after 16 months.

Representative examples of response patterns to prolonged infusions of butyrate are shown in Fig. 1. The phasic nature of the responses is evident. In the cases of transplants LG10 and H838, the transplant first responded to a steady infusion by bursts of secretion of both glucagon and insulin, beginning within 30 s of the stimulant

Table 1. Transplant responses to various stimuli

Infusate	Transplant No.	Age (months)	Dose or infusion rate	Glucagon secretion rates (pg/min)		Insulin secretion rates (microunits/min)	
				Control	Greatest change	Control	Greatest change
Sodium butyrate, direct to transplant	M20/64	10	180 μmol/min	—	—	0	+1 750
	V5	32	420 μmol/min	118	+13 600	0	+18 000
		36	210 μmol/min	0	+24 100	109	+12 500
	94/67	9	210 μmol/min	16	+9 000	0	+630
		19	105 μmol/min	12 500	+27 000	374	+1 506
	H831/66	3	210 μmol/min	0	+41 000	0	+2 400
		5	24 μmol/min	0	+11 700	0	+524
		14	2 μmol/min	0	+10 200	63	+443
		24	48 μmol/min	324	+38 000	24	+449
	H836/66	2	48 μmol/min	610	+2 670	160	+3 300
		20	48 μmol/min	2 220	+1 150	228	+317
	H840/66	16	48 μmol/min	1 400	+14 100	0	+1 640
Arginine, direct to transplant	LG27	16	280 μmol/min	0	+4 500	250	+1 400
	V5	32	280 μmol/min	196	+2 677	24	+4 280
Glucagon direct to transplant	M20/64	2	3·3 μg/min	—	—	0	0
		10	3·3 μg/min	—	—	0	0
	94/67	19	8·9 ng/min	—	—	163	+177
		20	70 ng/min	—	—	0	0
	H831/66	15	175 ng/min	—	—	0	0
Glucagon, stat to periphery	94/67	19	1 μg	—	—	404	−376
		20	20 μg	—	—	0	+142
	H836/66	13	20 μg	—	—	0	+50
Insulin, direct to transplant	94/67	9	17·5 microunits/min	296	−211	—	—
	H836/66	9	17·5 microunits/min	555	−336	—	—
		9	17·5 microunits/min	181	−35	—	—
Heparin, stat to periphery	H831/66	12	12 000 units	690	−51	24	−14

entering the transplant, reaching a peak within about 5 min, decreasing to low rates after about 20 min, and subsequently rising and falling in a succession of smaller waves. This was the response pattern most frequently seen during routine transplant testing with butyrate. In the cases of transplants Z12/65 and H831/66, initial responses lagged behind the start of the stimulant infusion by up to 15 min and rose more slowly. On stimulant withdrawal, secretion rates sometimes returned to basal values

immediately (e.g. glucagon secretion by transplant Z12/65), whilst at other times the return was delayed by up to half an hour (e.g. transplant H831/66).

In the only experiment with three equal, short infusions of butyrate at a high level, successive responses by transplant H4/66 decreased markedly but the initial

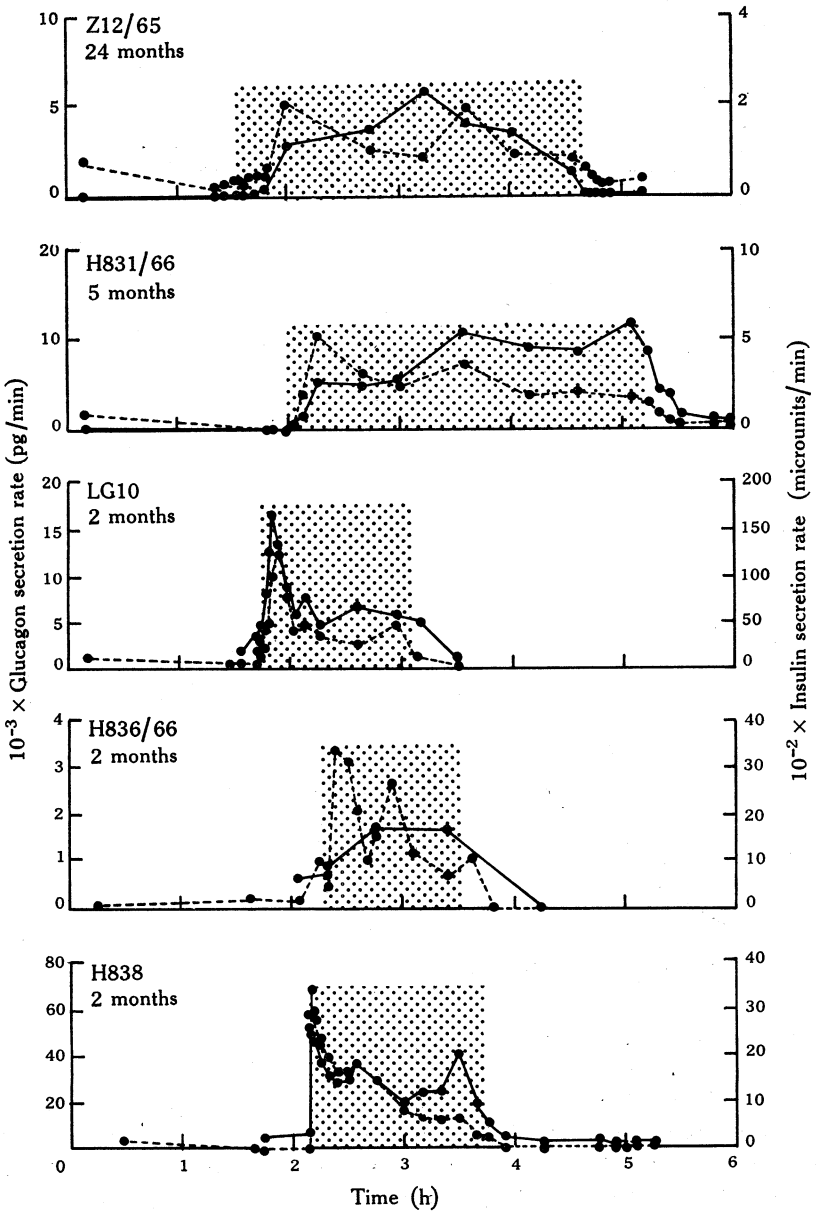

Fig. 1. Transplant responses to long infusions of butyrate. Infusion rates: 0·024 mmol/min for transplant H831/66, 0·048 mmol/min for all others. Transplant number and age is shown at the top of each graph. —— Glucagon. – – – Insulin.

high responses were largely restored, gradually, during a fourth, longer infusion at the same rate (Fig. 2).

In the only experiment made to examine the dose–response relationships, approximately logarithmic relationships were found for both glucagon and insulin secretion in response to butyrate (Fig. 3).

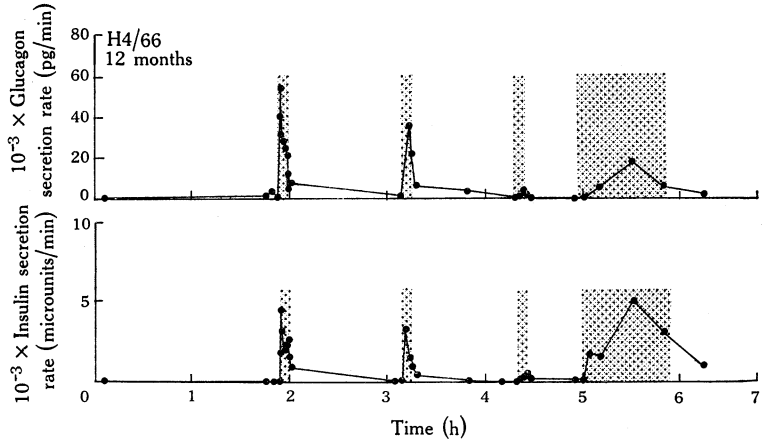

Fig. 2. Transplant responses to repeated infusions of butyrate at 0·21 mmol/min.

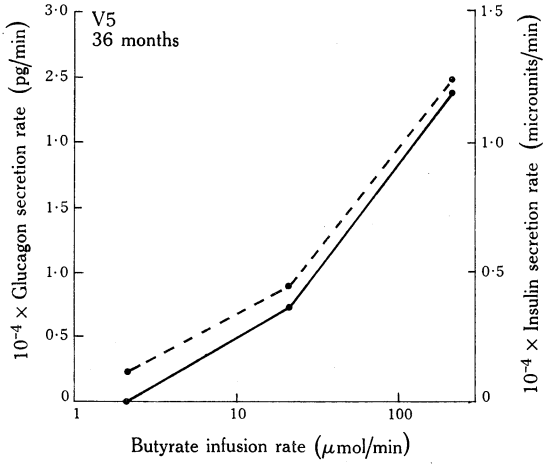

Fig. 3. Dose–response relationships from successive 6-min infusions of butyrate at intervals of 1 h and at increasing rates. —— Glucagon. – – – Insulin.

The cumulative effects of hormone secretion by both transplanted and remnant pancreas on peripheral hormone levels were small and caused no difficulties in the measurement of transplant secretion rates, even during the longest butyrate infusions to the transplants when most of the effluent, which contained butyrate as well as the hormones secreted by the transplant, was allowed to return to the general circulation. During the seven long butyrate infusions that were made, the mean increases in peripheral plasma hormone levels were from 51 to 88 pg/ml for glucagon, and from 13 to 14 microunits/ml for insulin. Stimulated transplant venous levels were of the order of ten times these values.

Arginine, like butyrate, promoted the secretion of both hormones (Table 1).

In Fig. 4, transplant glucagon responses (greatest changes in secretion rates) are plotted against insulin responses for all of the experiments in the series, the stimulants being sodium butyrate and arginine. These results show that in most cases the glucagon and insulin secretion rates were approximately proportional for a given transplant, but that the proportionality constant varied greatly from transplant to transplant.

Glucagon administration in various doses by either route had no marked effect on transplant insulin secretion (Table 1): in one instance (transplant 94/67) glucagon administered direct to the transplant appeared to slightly increase insulin secretion over *c.* 1 h, but in the same transplant peripheral glucagon administration was followed by a reduction of the high control secretion rate of insulin.

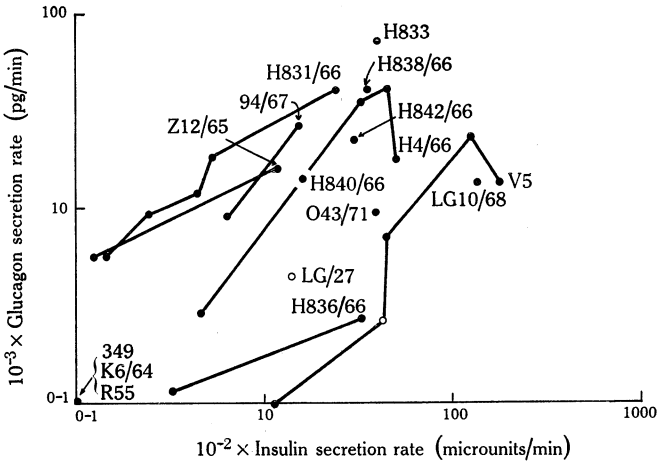

Fig. 4. Glucagon *v.* insulin responses (maximum secretion rate increases) to butyrate (●) and arginine (○).

The immediate (presumably direct) action of insulin on the transplant, whether given directly (Table 1) or peripherally (Fig. 5), was inhibition of control glucagon secretion. The hypoglycaemia that followed peripheral insulin administration was not accompanied by a marked increase in transplant glucagon secretion in any of the three cases studied—there were small increases towards control secretion rates, but these could equally well have been due to the decreasing insulin levels prevailing at these times as to the hypoglycaemia itself.

Table 1 shows that in one instance a bolus of 12 000 units of heparin administered to the periphery did not markedly affect control secretion rates of either hormone by a transplant that responded actively to butyrate, although a small decrease in insulin secretion may have occurred.

Table 2 and Fig. 5 show the changes in peripheral plasma glucagon and insulin levels, reflecting the activity of the pancreatic remnant, that followed peripheral administration of heparin, sodium butyrate, glucagon and insulin. In three experiments heparin administration caused no change in the glucagon level although slight decreases in insulin level may have occurred. Butyrate administration was followed by increases in the levels of glucagon and (except with the lowest butyrate dose)

insulin. Glucagon administration was followed in each instance by increases in the level of insulin. Insulin administration (Fig. 5) was followed by two distinct reactions in peripheral glucagon level following one another, i.e. an initial rapid decrease which was then followed by a slower rise to well above control levels approximately paralleling the insulin-induced hypoglycaemia.

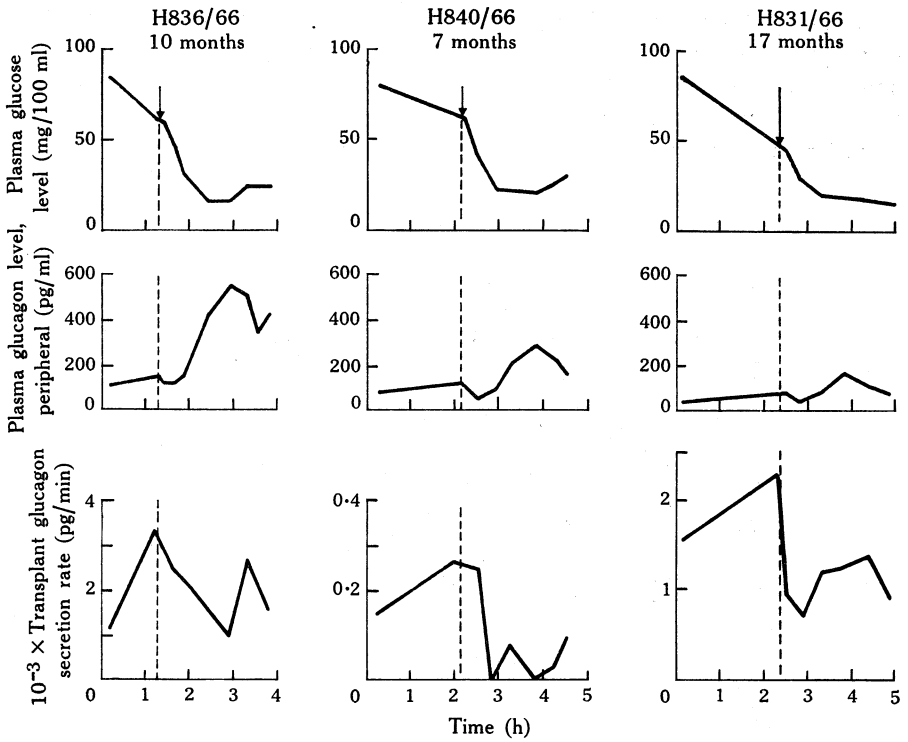

Fig. 5. Effects of peripheral administration of 10 units of insulin (shown by arrows) on three transplants.

Table 2. Pancreatic remnant responses to various stimuli

Infusion (peripheral)	Transplant No.	Date of experiment	Glucagon level (pg/ml)		Insulin level (microunits/ml)	
			Control	Greatest change	Control	Greatest change
Heparin						
12000 units stat	349	22.ii.74	45	0	25	−9
	G10/67	1.iii.74	40	0	22	−6
	H831/66	8.iii.74	60	0	8	−5
Butyrate						
0·6 mmol/min	H836/66	29.iii.74	80	+75	32	0
2·4 mmol/min	H836/66	29.iii.74	80	+120	20	+44
1 g stat	H831/66	2.viii.74	70	+1820	30	+18
Glucagon						
1 μg stat	94/67	5.iv.74	57	—	29	+7
20 μg stat	94/67	24.v.74	30	—	23	+36
	H836/66	28.vi.74	246	—	7	+24

Discussion

Basal glucagon levels in our animals (average 122 pg/ml) were almost the same as those reported by Bassett (1972) for Merinos fasted overnight, viz. 120 pg/ml; Bassett presented evidence that his glucagon assay was specific for pancreatic glucagon and did not respond to glucagon-like factors secreted by the gut, which suggests that our glucagon assay was of comparable specificity. In sheep, basal glucagon levels reported are 400–1000 pg/ml (Kamalu 1970), 200–1000 pg/ml (Manns 1969), and 135–900 pg/ml (mean of 403 pg/ml) (Alexander *et al.* 1971), suggesting that these reported assays were less specific for pancreatic glucagon. Although doubts have been expressed (Weir *et al.* 1973) about the specificity of the K30 antiserum, which we used, ambiguity does not arise in the present studies because of the use of arterio–venous differences for calculating transplant secretion rates.

Interference, even in long transplant infusion experiments, due either to stimulation of the remnant pancreas by the infusate or to increases in peripheral hormone levels caused by the transplant secretions, was shown to be small and was easily allowed for by the use of arterio–venous differences in the calculation of secretion rates. Also, it is unlikely, as discussed by Bell *et al.* (1970), that the isotonic saline in which infusates were made up affected the pancreatic responses in any way. Orosz *et al.* (1974) and Gerich *et al.* (1974*a*) report inhibitions of both glucagon and insulin secretion by heparin, which was infused as an anticoagulant in the earlier experiments of this series, but present results show that at the levels used heparin did not markedly affect these secretions by either the ovine pancreatic remnant or the transplant.

The demonstration in the present study that sodium butyrate directly stimulated the ovine pancreatic transplant, as well as the *in situ* pancreatic remnant, to secrete both glucagon and insulin is consistent with observations by others on the secretion of these hormones in response to short-chain fatty acids in ruminants (Horino *et al.* 1968; Edwards *et al.* 1972). Thus in sheep and goats indirect evidence of pancreatic glucagon secretion in response to infusions of propionate, butyrate, isovalerate, hexanoate, octanoate, and possibly decanoate (but not acetate or lactate or dodecylsulphate) was found during earlier studies when it was noticed that hypoglycaemia was usually not produced by the infusion in spite of the resultant hyperinsulinaemia (Ash *et al.* 1964; Phillips *et al.* 1969; Ambo *et al.* 1973). Since then it has been shown by direct assay that in the sheep pancreatic glucagon is actively secreted in response to a peripheral infusion of propionate, butyrate or valerate, but not oleate (Manns 1969; Kamalu 1970; Bassett 1972). Our results show, apparently for the first time, that butyrate promotes glucagon secretion by direct action on the pancreas. Also, insulin secretion by the ruminant pancreas has been shown to be promoted by propionate, butyrate, valerate, isovalerate, hexanoate, octanoate, and slightly by acetate, probably by direct action in every case. The methods used in these studies included intravenous administration of the acid into intact animals (Bassett 1972; Ambo *et al.* 1973), pancreatic arterial infusions (Manns *et al.* 1967), and perfusion of the autotransplanted ovine pancreas (Bell *et al.* 1970). Bell *et al.* (1970) showed that in the present preparation butyrate is a more potent secretagogue for insulin than is glucose.

It has been suggested (see, for example, Manns *et al.* 1967) that insulin secretion in response to butyrate and other short-chain fatty acids in the ruminant could possibly occur in two stages, fatty acid promoting glucagon secretion followed by

glucagon promoting insulin secretion. But because he could detect no lag between the two secretions, Bassett (1972) concluded that they were probably promoted independently by the direct actions of the fatty acids on the pancreatic islet cells. Our own observations support Bassett's conclusion since we found that not only were the two hormones secreted simultaneously, but also, while the butyrate-promotes-insulin reaction occurred in the transplant, the glucagon-promotes-insulin reaction apparently did not, as discussed below.

Our findings that glucagon and insulin were both secreted by the transplants in response to infusions of arginine are consistent with observations in monogastric species by others (e.g. Edwards *et al.* 1972; Fajans *et al.* 1972; Unger and Lefebvre 1972; Eaton and Schade 1974). In ruminants also, increases in plasma insulin levels have been shown following the intravenous administration of amino acids, including arginine (Stern *et al.* 1971; Trenkle 1972), but the only report concerning their effects on ruminant glucagon secretion seems to be that of Kamalu (1970) who found no increase in plasma glucagon level following the infusion of a casein hydrolysate in a 26-kg lamb on two occasions. Kamalu's negative result was probably due to his glucagon assay being overwhelmingly sensitive to non-pancreatic glucagon-like materials. Again, as with butyrate, our results show that in the sheep glucagon does not mediate insulin secretion in response to arginine as suggested for other species (Kaneto and Kosaka 1971*a*, 1971*b*; Samols *et al.* 1972).

In spite of the direct reactivity of the ruminant endocrine pancreas to arginine and short-chain fatty acids, there is still considerable doubt as to what part, if any, these metabolites play in the physiological regulation of the pancreas (Bassett 1971, 1972, 1974; Trenkle 1972).

It is widely accepted that, in general, insulin inhibits pancreatic glucagon secretion by direct action on the islet cells (e.g. Samols *et al.* 1972) and, in the present study, whilst the effects were small and it was not possible to separate with certainty the direct effects of insulin administration from those of the subsequent hypoglycaemia, this generally seems to have been the case for both transplanted and remnant pancreas. However, the effect of plasma glucose level on glucagon secretion by the transplant was anomalous in that, contrary to findings in other species (e.g. Ohneda *et al.* 1968; Iversen 1971; Unger and Lefebvre 1972; Dobbs *et al.* 1974; Gerich *et al.* 1974*b*), and to our own in the ovine pancreatic remnant, insulin-induced hypoglycaemia did not promote transplant glucagon secretion. Bloom *et al.* (1975) report patterns of peripheral plasma glucagon changes after insulin administration to calves that are similar to our own in the sheep; their results show the same small initial decrease in glucagon level immediately after insulin administration, though this is not mentioned in text.

It is well established in monogastrics that insulin secretion can be promoted by the direct action of glucagon on the β-cell (e.g. Samols *et al.* 1972). We failed to demonstrate this reaction in the ovine transplant, showing that it cannot be responsible for mediating transplant insulin secretion in response to arginine or short-chain fatty acids. Evidence for its occurrence in the ruminant pancreas *in situ* is suggestive but inconclusive; both we (present study) and Bassett (1971) found in adult sheep, and Kamalu (1970) found in 38-kg lambs, that glucagon given intravenously caused plasma insulin levels to rise, but, as Bassett pointed out, this could have been secondary to the concomitant increase in plasma glucose level induced by the glucagon.

Thus the present study has shown up two respects in which the ovine pancreas may have been altered by transplantation, viz. loss of ability to secrete insulin in response to glucagon, and loss of response to hypoglycaemia by the glucagon-secreting cells. The reasons for these changes are not obvious but some changes in pancreatic function on transplantation are to be expected because of the known dependence of the endocrine pancreas not only on humoral factors, which should have equal access to both transplant and remnant, but also on both the pancreatic exocrine tissue (Pfeiffer *et al.* 1971; Youngs 1972; Grabner *et al.* 1974), which is apparently not functional in the transplant, and autonomic nervous connections (Hertelendy *et al.* 1969; Obara *et al.* 1971; Woods and Porte 1974), which will have been severed during surgery, possibly irreversibly. Some isolated pancreatic islet preparations of other species, which likewise have been dissociated from both exocrine and neural influences, have been shown to retain both the ability to secrete insulin in response to glucagon (Lacy *et al.* 1968; Coll-Garcia and Gill 1969; Hinz *et al.* 1971; Schatz *et al.* 1974) and also some sensitivity by the glucagon-secreting cells to glucose (e.g. Nonaka and Foà 1969), although Chesney and Schofield (1969) and Edwards and Taylor (1970) found reduced sensitivities to glucose in glucagon secretion by their isolated islet preparations. While reports have appeared suggesting involvement of the autonomic nervous system in the effects of glucose on pancreatic glucagon secretion, none apparently has claimed an absolute dependence on it (Vaughan *et al.* 1973; Bloom *et al.* 1974; Walter *et al.* 1974). It would also seem possible that exocrine secretion from the incised ducts during the first few weeks after the operation might affect the endocrine tissue. Heptner *et al.* (1974) found in dogs and rabbits that ligation of the pancreatic duct, which was calculated to cause atrophy and fibrosis of the exocrine pancreatic tissue, resulted in some impairment in both insulin secretion and glucose tolerance: insulin secretion by the canine pancreas in response to glucagon was almost completely suppressed (although they attributed this to a general decrease in secreting capability rather than to a qualitative change in the islet), whilst there was a fivefold reduction in the insulin content of the rabbit pancreas. Similar impairments have been reported in humans following pancreatitis (Lillehei *et al.* 1970; Grabner *et al.* 1974).

The ovine transplants showed the phasic responsiveness reported by other workers for both pancreatic glucagon (e.g. Assan *et al.* 1972; Iversen 1972) and insulin (e.g. Grodsky *et al.* 1971; Bittner *et al.* 1974) secretions in response to various stimuli in many species. In sheep, biphasic insulin secretion was apparent in the portal vein studies of Manns *et al.* (1967), which was confirmed by Bell *et al.* (1970) using the pancreatic transplant, and also by the present study. Phasic glucagon secretion was shown, apparently for the first time in ruminants, in the present study. Typically with an infusion of stimulant at constant rate, both responses began within 30 s of the stimulant entering the transplant, increased very rapidly to maxima about 5 min later and fell to relatively low values at about 15 min (Fig. 1, transplants LG10 and H838). Thereafter the secretion rates fluctuated considerably and apparently haphazardly, neither returning to the first-phase value nor levelling off. When the stimulant was withdrawn, return to baseline secretion rates did not always occur immediately but sometimes took up to 30 min (Fig. 1). Bergman (1971) noted a similar delayed return to baseline with insulin secretion after long perfusions in the isolated, perfused canine pancreas, whilst Weir *et al.* (1974) described alpha-cell 'glucose

memory' of *c.* 10 min in the perfused rat pancreas. First-phase responses were highest during the first stimulant infusion of the day and decreased with successive stimuli (Fig. 2; Bell *et al.* 1970), although they were sometimes absent even during the first stimulation (Fig. 1, upper two examples). Curry (1971), Ganda *et al.* (1975), and Donnelly *et al.* (1972) also reported progressively decreasing rates of insulin secretion with successive short stimuli of glucose or tolbutamide. It would seem that the stores of immediately available hormone are depleted by short infusions, whilst a longer infusion taps less accessible stores.

The response patterns (Fig. 1) show a degree of parallelism between the time courses of the secretion rates of the two hormones by the ovine transplant that might suggest a connection between them. In ruminants some connection is also suggested strongly by the parallel way in which both secretions depend on chain length of the fatty acid stimulant (Bassett 1972; Ambo *et al.* 1973). Iversen (1971, 1972) also found simultaneous and approximately parallel secretions of glucagon and insulin by the isolated perfused canine pancreas in response to various stimuli, but he did not check directly on whether glucagon promoted insulin secretion by infusing it. Although our results suggest, as discussed above, that in the ovine transplant neither hormone is secreted in response to the other, this does not necessarily preclude a common mediator, or possibly even production of one hormone in response to *intracellular* production of the other.

The rough proportionality of insulin and glucagon responses to stimulation by butyrate seen with particular transplants (Fig. 2) is what one would expect from tissue containing a given proportion of glucagon-secreting to insulin-secreting cells, the proportion varying considerably from transplant to transplant.

In the present study, the results do not allow any conclusions to be made about feedback effects of either hormone, when infused, on its own secretion (cf. Hahn and Michael 1970; Iversen and Miles 1971) since the high plasma levels of the hormone obscured its production by the transplant.

We have so far been unable to measure how well the islet function survives transplantation, but the great variability in responsiveness from transplant to transplant, as well as the observation that prolonged butyrate stimulation of the transplant has little effect on peripheral hormone levels, suggests that there is a considerable loss.

Up till now poor reproducibility of transplant responses has meant that only rough estimates could be made of, for example, dose–response relationships, relative potencies of stimulants, and transplant longevity. One factor that would clearly affect reproducibility is the decline in responsiveness that was found with successive stimulations in the one day (Fig. 2; Bell *et al.* 1970). Another factor affecting reproducibility would seem to be the variability, both spontaneous and infusate-induced, in transplant blood flow rate. This would certainly affect the infusate level within the transplant, and it might also affect transplant responsiveness in the manner reported for other preparations (Rappaport *et al.* 1971; Semb and Aune 1971). (The effects on flow rate of glucose, butyrate and glucagon noted in the present work would seem to be non-specific for pancreatic tissue as the effects also occurred with old transplants that had largely lost the ability to secrete glucagon and insulin.) Finally, the changes in peripheral levels of glucose, insulin, and possibly glucagon that were regularly seen during the setting up of animals for infusion tests, presumably originating in some psychological stress, emphasize that as yet uncontrolled factors

affect the pancreatic remnant and it is likely that at least some such factors would affect the responsiveness of the transplant also.

In spite of difficulties with reproducibility of response and some possibly anomalous features, the ovine pancreatic autotransplant clearly affords a useful alternative to other preparations for the study of pancreatic physiology in the sheep. On the one hand it permits the study of the gland in relative isolation without the diluting effects of the circulation on samples and infusates and the interference from other organs of whole-animal studies, whilst at the same time retaining the natural circulation. In the studies reported here this has been specially advantageous in defining unequivocally the secretion of pancreatic glucagon as distinct from glucagon and glucagon-like materials from other organs. On the other hand, in contrast to *in vivo* and *in vitro* studies made on the isolated pancreas, experimentation on a transplant does not involve anaesthesia or surgery, is not limited in duration by failure of the preparation, and may be repeated continually for periods up to months and years.

Acknowledgments

We acknowledge the valuable technical assistance of Miss J. R. Smith, Miss A. Thomson, Mrs M. Jensen and Mr L. D. Barrier. This work was supported by grants from the Medical Research Council of New Zealand.

References

Albano, J., and Ekins, R. P. (1970). In 'In Vitro Procedures with Radioisotopes in Medicine'. (IAEA: Vienna.)

Alexander, D. P., Assan, R., Britton, H. G., and Nixon, D. A. (1971). *J. Endocrinol.* **51**, 597.

Ambo, K., Takahashi, H., and Tsuda, T. (1973). *Tohoku J. Agric. Res.* **24**, 54.

Ash, R. W., Pennington, R. J., and Reid, R. S. (1964). *Biochem. J.* **90**, 353.

Assan, R., Boillot, J., Attali, J. R., Soufflet, E., and Ballerio, G. (1972). *Nature (London) New Biol.* **239**, 125.

Bassett, J. M. (1971). *Aust. J. Biol. Sci.* **24**, 311.

Bassett, J. M. (1972). *Aust. J. Biol. Sci.* **25**, 1277.

Bassett, J. M. (1974). *Aust. J. Biol. Sci.* **27**, 167.

Bell, J. P., Salamonsen, L. A., Holland, G. W., Espiner, E. A., Beaven, D. W., and Hart, D. S. (1970). *J. Endocrinol.* **48**, 511.

Bergman, R. N. (1971). *Diss. Abstr. Int. B* **32**, 1803.

Bittner, R., Beger, H. G., and Kraas, E. (1974). *Horm. Metab. Res.* **6**, 423.

Bloom, S. R., Edwards, A. V., Hardy, R. N., Malinowska, K. W., and Silver, M. (1975). *J. Physiol. (London)* **244**, 783.

Bloom, S. R., Vaughan, N. J. A., and Russell, R. C. G. (1974). *Lancet* **2**, 546.

Chesney, T. McC., and Schofield, J. (1969). *Diabetes* **18**, 627.

Coll-Garcia, E., and Gill, J. R. (1969). *Diabetologia* **5**, 61.

Curry, D. L. (1971). *Am. J. Physiol.* **220**, 319.

Dobbs, R., Fujita, Y., Gotto, A., Faloona, G., and Unger, R. (1974). *Clin. Res.* **22**, 466A.

Donnelly, P. E., Hayles, J., and Turtle, J. R. (1972). *Proc. Endocrine Soc. Aust.* **15**, 21.

Eaton, R. P., and Schade, D. S. (1974). *Metab. Clin. Exp.* **23**, 445.

Edwards, J. C., and Taylor, K. W. (1970). *Biochim. Biophys. Acta* **215**, 310.

Edwards, J. C., Asplund, K., and Lundqvist, G. (1972). *J. Endocrinol.* **54**, 493.

Ellis, M. Jane, Arcus, A. C., Beaven, D. W., Donald, R. A., Hart, D. S., and Holland, G. W. (1973). *Proc. Univ. Otago Med. Sch.* **51**, 22.

Fajans, S. S., Floyd, J. C. Jr., Knopf, R. F., Pek, S., Weissman, P., and Conn, J. W. (1972). *Isr. J. Med. Sci.* **8**, 233.

Ganda, O. P., Kahn, C. B., Soeldner, J. S., and Gleason, R. E. (1975). *Diabetes* **24**, 354.

Gerich, J. E., Langlois, M., Schneider, V., Karam, J. H., and Naocco, C. (1974*a*). *J. Clin. Invest.* **53**, 1284.
Gerich, J. E., Schneider, V., Dippe, S. E., Langlois, M., Noacco, C., Karem, J. H., and Forsham, P. H. (1974*b*). *J. Clin. Endocrinol.* **38**, 77.
Grabner, W., Phillip, J., and Strigl, P. (1974). *Diabetes* **23**, 635.
Grodsky, G. M., Lee, J., Licko, V., and Landahl, H. (1971). In 'Diabetes'. Proc. 7th Congr. Int. Diabetes Fed., Buenos Aires, 1970. (Eds R. R. Rodriguez and J. Vallance-Owen.) p. 427. (Excerpta Medica Int. Congr. Ser. No. 231.)
Hahn, H. J., and Michael, R. (1970). *Horm. Metab. Res.* **2**, 119.
Heptner, W., Neubauer H. P., and Schleyerbach, R. (1974). *Diabetologia* **10**, 193.
Hertelendy, F., Machlin, L., and Kipnis, D. M. (1969). *Endocrinology* **84**, 192.
Hinz, M., Katsilambros, N., Schweitzer, B., Raptis, S., and Pfeiffer, E. F. (1971). *Diabetologia* **7**, 1.
Holland, G. W. (1969). *Aust. N.Z. J. Surg.* **39**, 75.
Horino, M., Machlin, L. J., Hertelendy, F., and Kipnis, D. M. (1968). *Endocrinology* **83**, 118.
Hunter, W. M., and Greenwood, F. C. (1962). *Nature* (*London*) **194**, 495.
Iversen, J. (1971). *J. Clin. Invest.* **50**, 2123.
Iversen, J. (1972). *Isr. J. Med. Sci.* **8**, 760.
Iversen, J., and Miles, D. W. (1971). *Diabetes* **20**, 1.
Kamalu, T. M. (1970). Ph.D. Thesis, Univ. Microfilms Inc., Ann Arbor, Michigan, No. 71-7288.
Kaneto, A., and Kosaka, K. (1971*a*). *Endocrinology* **88**, 1239.
Kaneto, A., and Kosaka, K. (1971*b*). *Endocrinology* **91**, 691.
Lacy, P. E., Young, D. A., and Fink, C. J. (1968). *Endocrinology* **83**, 1155.
Lillehei, R. C. *et al.* (1970). *Can. Med. Assoc. J.* **172**, 405.
Manns, J. G. (1969). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **28**, 491.
Manns, J. G., Boda, J. M., and Willes, R. F. (1967). *Am. J. Physiol.* **212**, 756.
Nonaka, K., and Foà, P. P. (1969). *Proc. Soc. Exp. Biol. Med.* **130**, 330.
Obara, Y., Sasaki, Y., Watanabe, S., and Tsuda, T. (1971). *Tohoku J. Agric. Res.* **22**, 156.
Ohneda, A., Parada, E., Eisentraut, A. M., and Unger, R. H. (1968). *J. Clin. Invest.* **47**, 2305.
Orosz, L., Fischer, U., Hommel, H., and Fiedler, H. (1974). *Acta Endocrinol.* (*Copenhagen*) **75**, 531.
Pfeiffer, E. F., Fussganger, R., Hinz, M., Raptis, S., Schleyer, M., and Straub, K. (1971). In 'Diabetes'. Proc. 7th Congr. Int. Diabetes Fed., Buenos Aires, 1970. (Eds R. R. Rodriguez and J. Vallance-Owen.) p. 460. (Excerpta Medica Int. Congr. Ser. No. 231).
Phillips, R. W., House, W. A., Miller, R. A., Mott, J. L., and Sooby, D. L. (1969). *Am. J. Physiol.* **217**, 1265.
Rappaport, A. M. *et al.* (1971). *Am. J. Physiol.* **221**, 343.
Samols, E., Tyler, J. M., and Marks, V. (1972). In 'Glucagon'. (Eds P. J. Lefebvre and R. H. Unger.) p. 151. (Pergamon Press: New York.)
Schatz, H., Otto, J., Hinz, M., Maier, V., Nierl, C., and Pfeiffer, E. F. (1974). *Endocrinology* **94**, 248.
Semb, L. S., and Aune, S. (1971). *Scand. J. Clin. Lab. Invest.* **27**, 105.
Stern, J. S., Baile, C. A., and Mayer, J. (1971). *J. Dairy Sci.* **54**, 1052.
Trenkle, A. (1972). *J. Dairy Sci.* **55**, 1200.
Unger, R. H., Aguilar-Parada, E., Müller, W. A., and Eisentraut, A. M. (1970). *J. Clin. Invest.* **49**, 837.
Unger, R. H., and Lefebvre, P. J. (1972). In 'Glucagon'. (Eds P. J. Lefebvre and R. H. Unger.) p. 213. (Pergamon Press: New York.)
Vaughan, N. J. A., Bloom, S. R., Ogawa, O., Bircham, P. M. M., and Edwards, A. V. (1973). *Experientia* **29**, 805.
Walter, R. M., Dudl, R. J., Palmer, J. P., and Ensinck, J. W. (1974). *J. Clin. Invest.* **54**, 1214.
Weir, G. C., Knowlton, S. D., and Martin, D. B. (1974). *Clin. Res.* **22**, 482A.
Weir, G. C., Turner, R. C., and Martin, D. B. (1973). *Horm. Metab. Res.* **5**, 241.
Woods, S. C., and Porte, D., Jr. (1974). *Physiol. Rev.* **54**, 596.
Youngs, G. (1972). *Gut* **13**, 154.

Manuscript received 10 October 1975