Progress report

Perfusion of the pancreas

The pancreas is both an exocrine and an endocrine organ. These two aspects of its function may be studied either independently, or simultaneously, in the isolated perfused gland¹. In this review, only the external secretion of the pancreas will be considered.

Why perfuse the pancreas? There is little advantage to be gained in performing experiments on isolated perfused organs which can equally well be carried out with less labour on the intact animal, nor should perfusion be solely an exercise for the experimental surgeon. Organs are perfused in isolation so that their function can be studied without possible interference from other organs. An example of such interference is found in the action of prostaglandin E₁, which is inhibitory to secretin-stimulated pancreatic secretion *in vivo* but stimulates pancreatic secretion in the isolated gland². A thorough investigation into pancreatic secretory mechanisms involves studying the effects of anoxia, of metabolic inhibitors and other drugs, and of altered extracellular milieu, experiments which are impossible in the intact animal due to effects on vital organs.

Technique

The pancreas receives its blood supply from a number of sources and it is not possible to perfuse the gland without considerable surgical preparation. The details have been described by Babkin and Starling³, Goldstein⁴, Nardi *et al*⁵, Hermon-Taylor⁶, Grenier *et al*¹, Rao and Emslie⁷, and Augier *et al*⁸ for the dog; by Case *et al*⁹ for the cat; and by Kanno¹⁰ for the rat. Good surgical technique is essential if a viable, sensitive preparation is to be obtained. The less the gland is handled the better the final result. For this reason it is better to select young lean animals so that the anatomy can be clearly seen.

After surgical isolation of the gland, the vascular system is perfused with whole blood⁶, with blood diluted with saline^{5,7,8}, or with physiological saline solutions⁹. Babkin and Starling³ were the first to describe an isolated perfused pancreas. They utilized two preparations. In one the gland was isolated but, because of their method of perfusion, only part of it was viable; in the other, the gland was perfused *in situ*, the whole gland being viable. In both preparations the perfusion pump and oxygenator was the heart-lung preparation. Therefore the pancreas cannot be regarded as truly isolated, for not only is the duodenum left in the perfusion circuit, but substances may be released from the heart which could possibly affect pancreatic function¹¹ and from the pancreas and duodenum which have an effect on the pancreatic vascular system^{12,13}.

The autotransplanted pancreas preparation^{14,15} can be placed in a similar category. Delezenne, Hallion, and Gayet¹⁴ and Houssay and Molinelli¹⁵ transplanted the pancreas into the neck by suturing the coeliac axis to the carotid artery and the portal vein to the jugular vein. Although this prep-

aration is denervated it is susceptible to humoral agents released from many tissues and is therefore of limited value.

Blood-perfused Preparations

Blood-perfused preparations lend themselves particularly to the study of the relationship between blood flow and secretion, and the vasomotor phenomena which are associated with secretion. In the isolated preparations of Hermon-Taylor⁶, Grenier *et al*¹, and, in part, of Augier *et al*⁸, the upper duodenum was also perfused. The amount of duodenum perfused in parallel with pancreas no doubt varied from preparation to preparation but could equal or even exceed, the weight of the pancreas⁶. Can results obtained with this pancreatic-duodenal preparation be attributed solely to the pancreatic vasculature? The duodenum may be the source of other vasoactive material in addition to secretin and cholecystokinin-pancreozymin (CCK-PZ). Mutt and Said¹⁶ have isolated a most potent vasoactive polypeptide from hog small intestine, and Hilton and Jones¹³ have demonstrated in the cat that addition of secretin to the perfusion fluid releases 5-hydroxytryptamine, a powerful vasoactive amine. In pancreatic-duodenal preparations, secretin causes an increase in blood flow due to a decreased vascular resistance (vasodilatation)^{1,5,6,7,8}. Removal of the duodenum has been shown by Rao and Emslie⁷ to abolish this vasodilator response to secretin, although this has not been confirmed by Augier *et al*⁸. Interpretation of results from isolated pancreatic-duodenal preparations must then be considered in the light of re-circulation of the perfusion fluid and appropriate control experiments⁸ carried out.

Though Babkin and Starling³ stated that secretory activity of the pancreas is extremely susceptible to the condition of the blood supply, there has been no universal agreement concerning the relationship of blood flow to secretion rate. The secretory response has usually been regarded as independent of blood flow above a certain critical flow rate¹⁷. However, experimentally induced variations in blood flow have been shown to cause parallel changes in secretion^{8,18,19}.

The isolated blood-perfused gland is a most suitable preparation in which to investigate the relationship between blood flow and secretion, as all the variables may be rigorously controlled. It allows also the vasomotor response of the pancreatic vessels to be studied free from secondary effects caused by alterations in the blood pressure^{18,19,20}. The isolated pancreas of the rat has been similarly used to investigate the vasomotor actions of the prostaglandins on the pancreatic circulation^{21,22}.

The Effect of Gastrointestinal Hormones on the Function of the Isolated Pancreas

SECRETIN

In all preparations, the addition of secretin to the perfusion fluid stimulates a profuse secretion of pancreatic juice^{1,3,4,5,6,7,8,9,14,15}. The juice has a similar composition to that secreted at the same rate by the same species *in vivo*.

Secretin has a more prolonged action *in vitro* than that *in vivo*. Whilst this is clear in the case of the cat pancreas⁹, it is not so certain in the case of

the dog as different doses and different preparations of secretin were used by the various workers. Nardi *et al*⁵ do not distinguish between the secretin manufactured by the Boots and Vitrum companies and seem to equate the units which makes comparison with other workers difficult. This prolonged action of secretin cannot be attributed solely to re-circulation of the hormone through the gland as it is observed in the saline-perfused cat pancreas in the absence of re-circulation⁹. Hermon-Taylor⁶ has stated that this prolonged action occurs only in preparations in which blood is diluted with saline which may act by preventing secretin destruction possibly due to the dilution of a 'secretinase'.

An increased oxygen uptake occurs on stimulation with secretin indicating an increased metabolic activity^{5,6,8}.

CHOLECYSTOKININ-PANCREOZYMIN, ACETYLCHOLINE, AND GASTRIN

In contrast to the long duration of secretin action, CCK-PZ has only a transient effect^{5,23}. As the kinetics of the action of CCK-PZ in the anaesthetized cat are similar to those in the isolated saline-perfused preparation^{23,24}, where no enzymic inactivation of CCK-PZ by plasma can occur, it would appear that the hormone is likely to be inactivated after combination with the receptor on the acinar cell.

Intraarterial injection of acetylcholine (1-100 μ g) was ineffective in stimulating enzyme secretion in the isolated dog pancreas⁵, even though a prompt vasodilatation was induced. However, in the saline-perfused cat pancreas preparation, acetylcholine is effective in stimulating enzyme secretion when infused in doses as low as 5 ng/min²⁵. Gastrin II, like CCK-PZ and acetylcholine, after a short latent period causes a sharp fall in perfusion pressure (vasodilatation) accompanied by enzyme secretion⁶.

Relationship of Oxygen Consumption to Ion Secretion

The blood-perfused pancreas would seem ideal for studying this important aspect of pancreatic physiology but few direct observations have been made. To relate oxygen consumption to ion secretion three major parameters must be measured simultaneously: the blood flow, the arteriovenous oxygen concentrations, and the rate of electrolyte secretion. The oxygen consumption has been measured in many studies^{5,6,8} but there are few, if any, direct measurements of the ratio of electrolyte transported to the increase in oxygen uptake following stimulation of secretion²⁶. From experiments of this type an upper limit to this ratio will determine the choice between various models which have been proposed to explain active transport (see Thaysen²⁶ for a discussion of this problem).

The Saline-perfused Pancreas

In the study of cellular mechanisms of pancreatic secretion the presence of red cells and protein in the perfusate presents certain difficulties in experimentation and in interpretation of results. For example, manipulation of electrolyte concentrations in the perfusate can only be achieved when red cells are absent. To overcome these problems a technique was devised using a perfusate consisting only of a balanced salt solution with added glucose⁹. The

necessary oxygen supply to the gland was obtained from that carried in physical solution during perfusion at high rates of flow. The low viscosity of the solution enables this to be achieved at modest hydrostatic pressures. The physiological status of the preparation has been discussed⁹.

The Secretion of Bicarbonate

One of the main characteristics of pancreatic juice is its high bicarbonate concentration. The saline-perfused gland has been largely instrumental in advancing knowledge of this central problem in pancreatic physiology, the secretion of bicarbonate. By experimental manipulation of the perfusate in the isolated saline-perfused preparation, by replacement of chloride with sulphate, bicarbonate is secreted virtually as the only anion in pancreatic juice²⁷. Concentrations as great as 160 mequiv HCO_3^- /l can be achieved. Pancreatic secretory rate is directly proportional to the concentration of bicarbonate ion in the perfusate; secretion completely, or almost completely, ceases when bicarbonate is omitted²⁸. Incorporation of ^{14}C bicarbonate into the perfusate results in its prompt appearance in the pancreatic juice concentrated four to five times. It has been concluded that about 95% of pancreatic juice bicarbonate is derived from plasma and little from the oxidative metabolism of the pancreatic cell²⁸.

The question has been posed, How does bicarbonate from plasma reach the pancreatic juice and in what form is it transported? The isolated saline-perfused gland has shed some light on this problem. When secretion is initiated by secretin there is a fall in pH and a rise in pCO_2 of the effluent perfusate^{27,28}. This observation supports the concept that the H^+ ion is secreted across the contraluminal cell membrane into the perfusion fluid. This secretion of H^+ ion causes the liberation of CO_2 from the plasma bicarbonate which diffuses into the pancreatic cell. The primary event in bicarbonate secretion would therefore appear to be the back secretion of H^+ after separation of H^+ and OH^- from water. The CO_2 so liberated by H^+ ion combines with OH^- in the luminal membrane under the influence of carbonic anhydrase to form bicarbonate which, on passing through the luminal membrane, becomes trapped on ionization and cannot diffuse back.

The concept that bicarbonate is actively transported from the pancreatic cell to the duct system must now be questioned. Schulz *et al*²⁹, using the isolated saline-perfused preparation, have shown that a number of weak organic acids can replace bicarbonate in the perfusion fluid and support the secretion of electrolytes. The chemical composition of these weak acids is so diverse that it is unlikely that they would be actively transported^{29,30}. The appearance of these organic acids in pancreatic juice is not determined by their chemical composition, but by their pK value (a value near to 7 being optimal) and their lipid solubility. This work supports the concept of the active transport of H^+ back across the pancreatic cell. However, there is considerable evidence that an active inward transport of sodium may also be involved^{9,27}, possibly coupled to hydrogen ion transport into extracellular fluid³⁰.

If the current view is accepted that the bicarbonate of pancreatic juice is derived from bicarbonate of plasma and enters the cell as carbon dioxide, then the presence of red blood cells would make the analysis of the secretory mechanism more difficult. It would be possible with the red cells present for

the carbon dioxide to be derived from bicarbonate by a reversed chloride-bicarbonate shift, and so obscure the back secretion of hydrogen ion.

Cation Dependence of Secretion

Electrolyte secretion is critically dependent on the presence in the perfusate not only of bicarbonate, but also of Na^+ and K^+ , whose removal causes an abrupt inhibition of secretion. In the long term, removal of Ca^{2+} also inhibits both electrolyte and enzyme secretion²⁵. Even when using the isolated organ care must be taken in interpreting results. When the potassium concentration in the perfusion fluid is raised above 30 mequiv/l, the pancreas is stimulated to secrete amylase²⁴. It might be argued that, as in the case of the posterior pituitary³¹, potassium acts directly on the acinar cell. However, this is not so. In the pancreas, potassium acts on nerve terminals to release acetylcholine which then stimulates protein secretion²⁴. Similar arguments apply when potassium-free solutions are used. Thus the possibility of acetylcholine release must always be considered when protein secretion is observed after an experimental procedure involving the use of a drug or a change in perfusate composition.

The Osmolality of Pancreatic Juice

Pancreatic juice is always (within experimental error) equiosmolal with the fluid perfusing the vascular system²⁵. This is achieved by altering the concentration of ions appearing in the juice. Under physiological conditions the cations of the pancreatic juice ($\text{Na}^+ + \text{K}^+$) are secreted at concentrations similar to those found in plasma. When the osmolality of the perfusate is increased by the addition of sodium chloride, the concentration of sodium plus anion in the juice increases to maintain osmolalities of juice and perfusate identical⁹. If the osmolality of the perfusate is increased by the addition of an impermeant molecule which does not appear in the juice, eg, sucrose, the total electrolyte concentration of the juice increases so as to exceed that of the perfusate by an amount equivalent to the increased osmolality of the perfusate. Removal of sodium chloride from the perfusate results in the concentration of sodium plus anion in the juice being correspondingly decreased⁹. In the isolated perfused gland, the range over which the experimental conditions may be varied is considerably greater than that possible *in vivo*, for not only would other vital organs be stressed and secondarily have effects on pancreatic function, electrolyte concentrations in the plasma from red cell damage would make precise control of experimental conditions impossible.

The Isolated Perfused Pancreas as an Assay Organ

The isolated saline-perfused pancreas is extremely sensitive to secretin and can be used to assay secretin in biological fluids and tissue extracts³². The gland can be made even more sensitive, by a factor of up to 10, by the addition of theophylline to the perfusion fluid. This agent inhibits phosphodiesterase, the enzyme responsible for the hydrolysis of adenosine 3',5'-monophosphate (cyclic AMP). Secretin probably acts by stimulating adenylate cyclase, an enzyme bound to the plasma membrane, to produce cyclic AMP from

ATP, and theophylline therefore allows a greater accumulation of cyclic AMP for a given hormonal stimulus^{33,34}. Cyclic AMP then triggers off the electrolyte-secreting mechanism. A sensitive preparation will detect as little as 250 pg of pure secretin. Although only the saline-perfused gland has been used for this purpose it would seem possible that the blood perfused preparation would also be suitable.

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