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Ionic transport mechanisms underlying fluid secretion by the pancreas

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The pancreas is a 'leaky' epithelium and secretes a juice in which sodium and potassium have concentrations similar to those of plasma. The characteristic features of the secretion are its isosmolality and its high bicarbonate concentration. It is the latter that has attracted considerable attention. Secretion in the isolated cat pancreas is directly proportional to the bicarbonate concentration in the nutrient fluid. The ability of the gland to secrete weak acids has led to the view that because of the very different chemical nature of the anions, it is most likely that it is a component common to all buffers, the proton, that is subject to active transport. This is supported by the decrease in pH and the increase in p_{CO_2} of the venous effluent when secretion occurs and the sensitivity of secretion to the pH of the nutritional extracellular fluid. It is proposed that the cellular mechanisms are as follows: CO_2 diffuses into the cell and is hydrated to carbonic acid under the influence of carbonic anhydrase. The bicarbonate ion so formed diffuses into the ductular lumen and the proton is transported backwards through the epithelium with a proton pump (Mg^{2+} -ATPase) provisionally located in the luminal membrane and a hydrogen–sodium exchange carrier located in the basolateral membrane. Energy for the latter process is derived from the sodium gradient between extracellular fluid and cell. This gradient is maintained by a ($Na^+ + K^+$)-ATPase also located in the basolateral membrane. Chloride appears to be transported partly through a chloride–bicarbonate exchange mechanism, but largely passively together with a large sodium and potassium component through the paracellular pathway. Osmotic equilibrium is likely to occur in the small ductules.

The pancreas secretes a clear alkaline fluid that is isosmotic with blood. Its most striking feature is the high bicarbonate concentration, which is responsible for its alkalinity. The major cations are sodium and potassium and the major anions bicarbonate and chloride.

The source of pancreatic electrolyte secretion

Pancreatic juice arises from two cell types, the acinar cell and the ductular cells. It is well established that the acinar cell produces the exportable proteins (Davies *et al.* 1949). Secretagogues of enzyme secretion also stimulate a chloride-rich fluid from acinar cells (Case *et al.* 1969*a*; Dockray 1972; Sewell & Young 1975; Kanno & Yamamoto 1977; Petersen & Ueda 1977). Acinar electrolyte secretion requires the presence of extracellular calcium but is independent of CO_2/HCO_3^-. The ductular cells secrete a bicarbonate-rich fluid which is dependent upon extracellular bicarbonate but is less dependent than the acinar cell on calcium (Rothman & Brooks 1965; Case *et al.* 1970; Argent *et al.* 1973; Schulz *et al.* 1969; Schulz 1971). To confirm these loci of secretion, attempts have been made to destroy selectively acinar and ductular cells. The acute administration of ethionine damages acinar cells and depresses their responsiveness to enzyme stimulants without affecting the volume response to secretin.

However, in chronic experiments on dogs, both enzyme and volume responses are depressed Kalser & Grossman 1954). Alloxan causes vacuolization and damage to the ductular cells and also reduces the responsiveness of the pancreas to secretin, suggesting that they are the locus of bicarbonate secretion (Grossman & Ivy 1946). A more successful approach of this nature was that of Fölsch & Creutzfeldt (1975, 1977) who were able to destroy acinar cells without any apparent damage to the ductular elements. This was achieved by feeding rats on copper-free diets. After an appropriate interval, the animals failed to respond to enzyme secretagogues but responded to secretin with a bicarbonate-rich fluid. These experiments have recently been confirmed and extended by Case *et al.* (1980) and Smith *et al.* (1981).

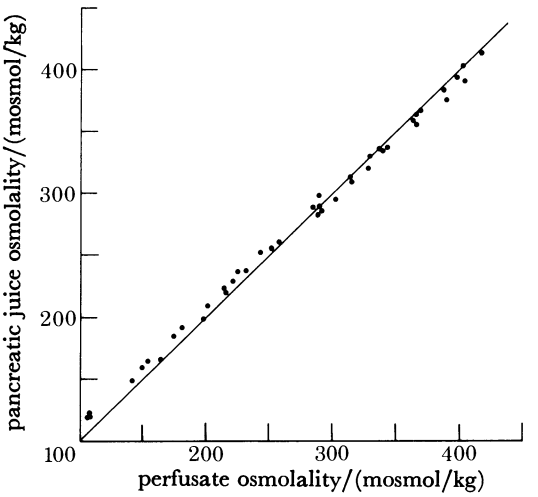

FIGURE 1. The relation of the osmolality of pancreatic juice to perfusate osmolality in the isolated cat pancreas. Perfusate osmolality was changed either by removal or addition of NaCl or by addition of sucrose. (From Case *et al.* (1968), with permission from the *Journal of Physiology*.)

THE OSMOLALITY OF PANCREATIC JUICE AND WATER TRANSPORT

The pancreatic juice is isosmolal with blood and with the nutrient extracellular fluid of the isolated perfused cat gland over a range of 450 mosmol/kg (figure 1) (Case *et al.* 1968). Most estimates of osmolality have been made by cryoscopic methods and whereas the above relation is generally true, careful examination of the data reveals that the juice is some 5–12 mosmol/kg hypertonic with respect to both the blood in intact animals and the perfusate of the isolated gland. However, in one of the few studies in which a vapour pressure method of Hill (1930) was used, Gilman & Cowgill (1933) demonstrated in 14 out of 15 observations that the juice was hypotonic with respect to blood by an equivalent of 2–11 mM NaCl.

If the coupling between water and salt transport is osmotic, then at what site does the coupling occur? It is difficult to conceive of a double membrane theory, but a standing gradient of the Diamond & Bossert (1967) type is feasible, not in the intercellular space, but in the restricted ductular system. This problem has been investigated by Swanson (1977), who was unable to detect in the isolated rabbit pancreas a significant difference in the osmolality of spontaneously secreted fluid in the small (less than 70 μm diameter) and the large (more than 70 μm in diameter) extralobular ducts. During secretin stimulation, however, the fluid in the small extralobular ducts averaged 6 mosmol/kg H_2O greater than that in the larger extralobular ducts. Though highly significant, this small difference is very much less than would

have been expected from the calculations of Diamond & Bossert (1967). Several explanations are possible: the isolated rabbit pancreas is not very sensitive to secretin and only a small increase in flow above the spontaneous rate would be expected (osmotic equilibrium is likely to be dependent upon rate of flow); the gland is more 'leaky' than the gall bladder (Dewhurst *et al.* 1978; Jansen *et al.* 1980) and the difference in osmolality observed might have been greater if the sampling could have been made in more proximal ducts.

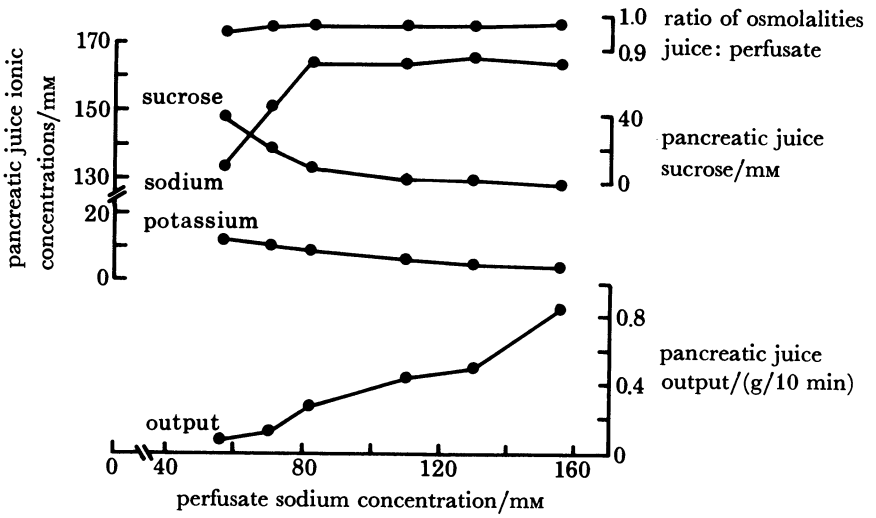

FIGURE 2. The effect of lowering the perfusate sodium chloride concentration on the rate and composition of pancreatic secretion from the isolated cat pancreas. The perfusate was maintained isosmolal by addition of appropriate amounts of sucrose. (From Case *et al.* (1968), with permission from the *Journal of Physiology*.)

THE COMPOSITION OF THE PANCREATIC JUICE

(*a*) *Cations*

The concentration of sodium is about 160 mM, that is about 10–12 mM greater than that of blood. This is true for the rabbit (Rothman & Brooks 1965), the cat (Case *et al.* 1969*a*), the pig (Hickson 1970) and man (M. G. Ashton, D. Hutson & T. Scratcherd, unpublished observations). This difference is not due to the protein content of either blood or pancreatic juice, as it occurs when the pancreas is perfused with protein-free fluids during stimulation with pure secretin (Case *et al.* 1968).

The concentration of potassium follows closely that of the blood or of the perfusate of the isolated gland (Rothman & Brooks 1965; Case *et al.* 1968; Case & Scratcherd 1974; Jansen *et al.* 1980). The concentrations of both sodium and potassium are constant, at constant extracellular fluid concentrations and independent of secretion rate.

Calcium

Calcium is secreted into the pancreatic juice through at least two pathways, though Moore (1976) in a theoretical analysis describes 15 possible modes. The first route is associated with protein secretion and originates from the acinar cell. The other is related to secretin-stimulated juice and is therefore of ductular origin. Unlike the monovalent cations, the concentration of Ca^{2+} in pancreatic juice is flow-dependent, with the concentration increasing as the flow rate decreases. At the lowest rates of flow, the concentration reaches 1.6 mM in the perfused gland

(perfusate concentration 2.5 mM) while at the highest rates of flow, it falls to 0.4 mM. At constant flow rate, the concentration of calcium in the juice is related to that of the perfusate.

(*b*) *Anions*

These are almost exclusively bicarbonate and chloride, though phosphate may make a significant contribution in sheep. Bicarbonate increases with increasing flow rate, while chloride decreases reciprocally (Hart & Thomas 1945; Bro-Rasmussen *et al.* 1956; Case *et al.* 1969*a*). This relation also holds in man (M. G. Ashton, D. Hutson & T. Scratcherd, unpublished observations) and the pig under both secretin and vagal stimulation (Hickson 1970). The sum $Cl^- + HCO_3^-$ is constant and independent of flow and is equal to the sum $Na^+ + K^+$ within a few millimoles per litre (Bernier & Lambling 1962).

The ionic requirements and the mechanism of secretion

Almost all the information on this aspect has been obtained so far using two preparations, the isolated rabbit pancreas (Rothman & Brooks 1965) and the isolated perfused pancreas of the cat (Case *et al.* 1968). Whereas many of the properties of the two are identical, they differ in one important respect: the rabbit pancreas secretes spontaneously and is insensitive to the hormone secretin, whereas the cat preparation does not secrete spontaneously and is exquisitely sensitive to secretin (Scratcherd *et al.* 1975).

(*a*) *Cations*

(i) *Sodium*

There is an absolute requirement for Na^+ in the perfusate. The secretion of Na^+ is directly proportional to the sodium concentration in the perfusate (figures 2 and 3).

Replacement of the sodium in the perfusate by isosmotic amounts of sucrose in either the isolated cat pancreas (Case *et al.* 1968) or the rabbit pancreas (Bonting *et al.* 1980) causes a reduction in the secretion rate. At a sodium concentration of about 80 mM in the perfusate, a concentration gradient between perfusate and juice of about 90 mM was established. When the perfusate sodium concentration was lowered further (in the cat), the sodium concentration in the juice fell rapidly and osmotically equivalent amounts of sucrose appeared in the juice (figure 2). The establishment of a large Na^+ concentration gradient between perfusate and juice might at first be interpreted as evidence for an active Na^+ transport but, as the transductal electrical potential was not measured, such a conclusion cannot be made. If the distribution of sodium was passive, then a transtubular potential of about 19 mV must be established with the lumen negative. Bonting *et al.* (1980) maintain that all sodium moves passively through the paracellular pathway, as the ratio of sodium and potassium in the secreted fluid is always about equal to that in the bathing medium. Removal of sodium without isosmotic replacement results in an increase in the rate of secretion until the sodim concentration reaches between 75 and 80 mM, after which the secretion rate rapidly falls. Replacement of sodium with lithium did not support fluid secretion (Rothman & Brooks 1965; Case & Scratcherd 1974).

(ii) *Potassium*

Omission of potassium from the bathing medium or perfusion fluid reduced the rate of secretion to about 35% of control: the inhibition was immediate and sustained and was completely and rapidly reversible (figure 3). Maximum secretion rates were observed when

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the potassium concentration in the perfusate was between 10 and 15 mM. When the potassium in the perfusate was varied between 0 and 120 mM (sodium being adjusted to maintain isosmolality), the concentrations of potassium in the juice and perfusate were almost identical. This suggests that the ion was passively distributed and that movement into the juice was via the paracellular pathway. Rubidium was a complete substitute for potassium (figure 3*b*) (Case & Scratcherd 1974).

The requirement for sodium and potassium, the stimulating effect on secretion of potassium, the ability to replace potassium with rubidium and the failure of Li^+ to substitute for Na^+, support the view that sodium might be actively transported by a sodium pump. This is consistent with the earlier observations of Ridderstap & Bonting (1969) and Case & Scratcherd (1974) that ouabain, the specific inhibitor of $(Na^+ + K^+)$-ATPase (Glynn 1964; Skou 1965), inhibited pancreatic secretion. The dose–response curves relating the inhibition of $(Na^+ + K^+)$-ATPase and pancreatic secretion were closely parallel. However, using [^{3}H]ouabain to locate

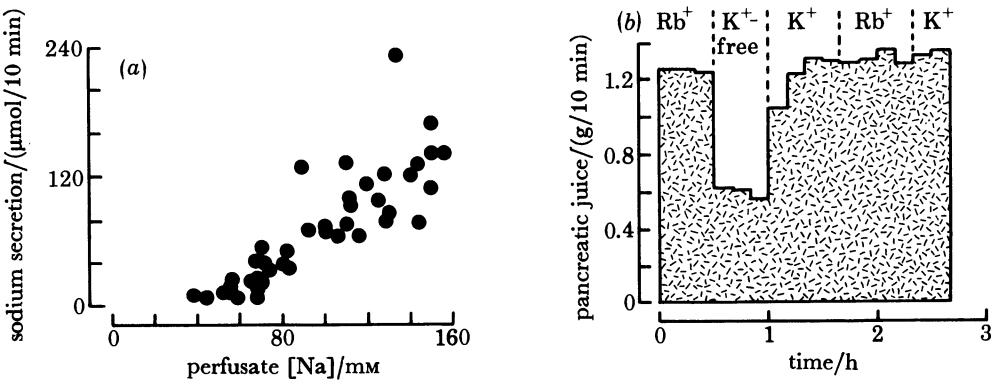

FIGURE 3. The requirements of pancreatic secretion for (*a*) sodium and (*b*) potassium, in the perfusate of the isolated pancreas.

$(Na^+ + K^+)$-ATPase, Bundgaard *et al.* (1981) have observed that binding was confined largely to the basolateral membrane of intercalated and interlobular ducts. There were few binding sites located on the basolateral membranes of acinar cells and none on the luminal membrane of either group of cells. The direction of sodium transport must therefore be towards the extracellular fluid and not towards the duct lumen (figure 7).

(*b*) *Anions*

(i) *Bicarbonate*

A requirement for bicarbonate in the bathing medium was first demonstrated by Rothman & Brooks (1965). Pancreatic fluid and bicarbonate secretion are directly proportional to the concentration of bicarbonate ion in the perfusate (figure 4) (Case *et al.* 1970; Schulz 1971). Using the data of Davies *et al.* (1949) for the Q_{O_2} of cat pancreas (and assuming that secretin only stimulated bicarbonate secretion and that the cells responsible for the secretion were alone responsible for the increased oxygen consumption), Case *et al.* (1970) calculated that not more than 7% of the bicarbonate of pancreatic juice could have its origin in metabolic CO_2. This prediction was tested by taking advantage of the fact that reasonable secretion rates can be maintained when external bicarbonate ions are replaced by acetate. Under these conditions, the bicarbonate concentration in the juice fell from 122.2 ± 5.7 mM ($n = 10$) with an output of

16.23 ± 1.77 µmol/min, to a concentration of 6.5 ± 0.38 mM ($n = 21$) and a corresponding output of 0.25 ± 0.032 µmol/min (s.e.). These figures are very close to the predicted values and demonstrate that the major part of bicarbonate in the juice comes from the extracellular fluid.

In considering the mechanism of bicarbonate secretion, much insight has been gained from observations of the way in which the pancreas secretes buffers other than HCO_3^-. Weak organic acids such as sulphamerazine, glycodiazine (Schulz *et al.* 1969; Schulz 1971) or the organic anions formate, propionate, butyrate and acetate (Swanson & Solomon 1975; Case *et al.* 1979) (figure 5) can substitute for bicarbonate and promote secretion of fluid and buffer. The secretion rate is proportional to the anion concentration and, at a constant anion concentration, is sensitive to pH, i.e. secretion rate increases as the pH rises (figure 6). Buffer is actively transported and must take a transcellular route. Because of the different chemical nature of the buffers, it seems unlikely to be the anionic form of the buffer that is actively transported but rather the proton.

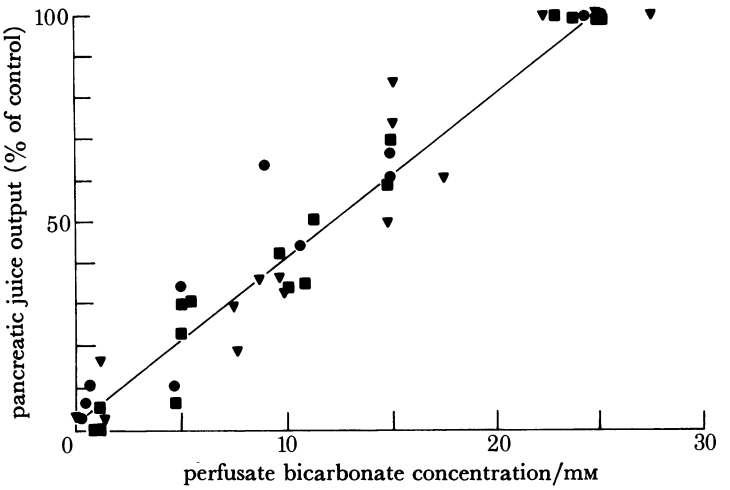

FIGURE 4. The effect of perfusate bicarbonate concentration on the rate of secretion from the isolated pancreas. In two experiments (●) the perfusate was of normal composition, in four experiments (■) it contained 6% dextran, and in four others (▼) it contained either 6% dextran and 2% haemoglobin (three experiments) or 6% haemoglobin (one experiment). The rate of secretion is expressed as a percentage of the rate at normal perfusate bicarbonate concentration (about 25 mM). The line is the calculated regression line. (From Case *et al.* (1970), with permission from the *Journal of Physiology*.)

Active transport must occur across at least one face of the cell. To localize the site(s) of active transport, it is necessary to determine the electrical potential difference across the membrane faces, the activities of the transported ions and the presence in the membranes of appropriate pump sites. The membrane potentials are amenable to direct measurement but the ionic activities, e.g. hydrogen ion concentration (pH), have been determined only by indirect means and are therefore subject to some uncertainty. The potential difference across the duct system is only a few millivolts (-2 to -9 mV) with the duct negative to the interstitium. Secretin stimulation increases this duct negativity by a few more millivolts but so far this measurement has only been made across the main duct (i.e. distal to the site of salt secretion) (Schulz *et al.* 1969; Way & Diamond 1970; Swanson & Solomon 1973). From the Nernst equation, it has been calculated that only with HCO_3^- does transport take place against an electrochemical gradient (Way & Diamond 1970; Schulz & Ullrich 1978). Of the ionic pumps, two have been located. The presence of the $(Na^+ + K^+)$-ATPase on the basolateral membrane and its absence from

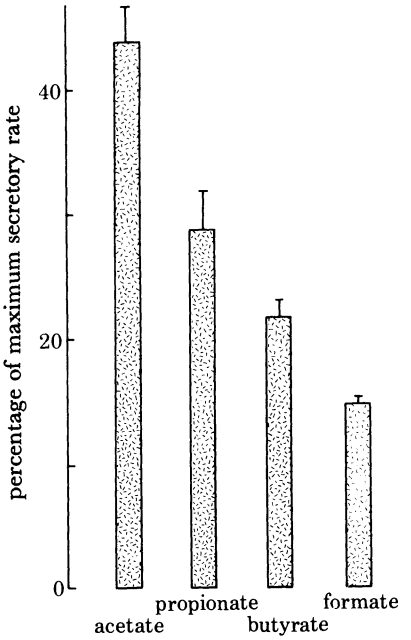

FIGURE 5. The effectiveness of weak organic acids as substitutes for bicarbonate (25 mM) in the perfusate in sustaining secretion in the isolated cat pancreas.

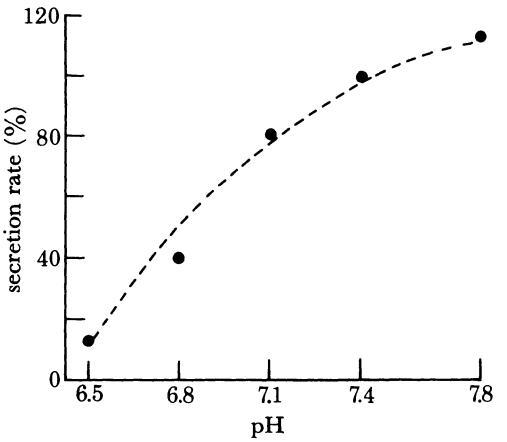

FIGURE 6. The effect of pH on pancreatic secretion when bicarbonate in the perfusate is replaced by acetate. The secretion rate is expressed as a percentage of that at pH 7.4.

the luminal membrane seems now to be established (Bundgaard *et al.* 1981). A Mg^{2+}-dependent ATPase has been described in duct cells and this has been provisionally located in the luminal membrane as an ATP-driven H^+ pump (Schulz 1980). Swanson & Solomon (1972, 1975) proposed that a Na–H exchange carrier existed across the contraluminal membrane in which the energy for H^+ extrusion was obtained from the sodium gradient, maintained by the $(Na^+ + K^+)$-ATPase of the basolateral membrane referred to earlier (figure 7). Of crucial importance to this theory of Swanson & Solomon (1975) and supported by Schulz (1980) is the transport back into the extracellular fluid of H^+ through the Na–H carrier. Direct evidence for H^+ transport has been reported by Case *et al.* (1970). They described a fall in pH and an increase in the p_{CO_2} of the perfusion fluid issuing from the gland when secretion was established by stimulation with secretin (figure 8). Rawls *et al.* (1963) had previously found that the p_{CO_2}

of pancreatic juice was 10–18 mmHg (*ca.* 1.3–2.4 kPa) less than that of plasma. These two sets of results indicate that H^+ ions are transported into the plasma, and that a gradient favourable to the diffusion of CO_2 into the cell exists. However, the question must be posed: are these values for 'back-secretion' of hydrogen ion adequate to account for bicarbonate secretion? What is the stoichiometry of the reaction proposed? These questions, together with an explanation for the role of chloride, must be answered before the above model can be even provisionally accepted.

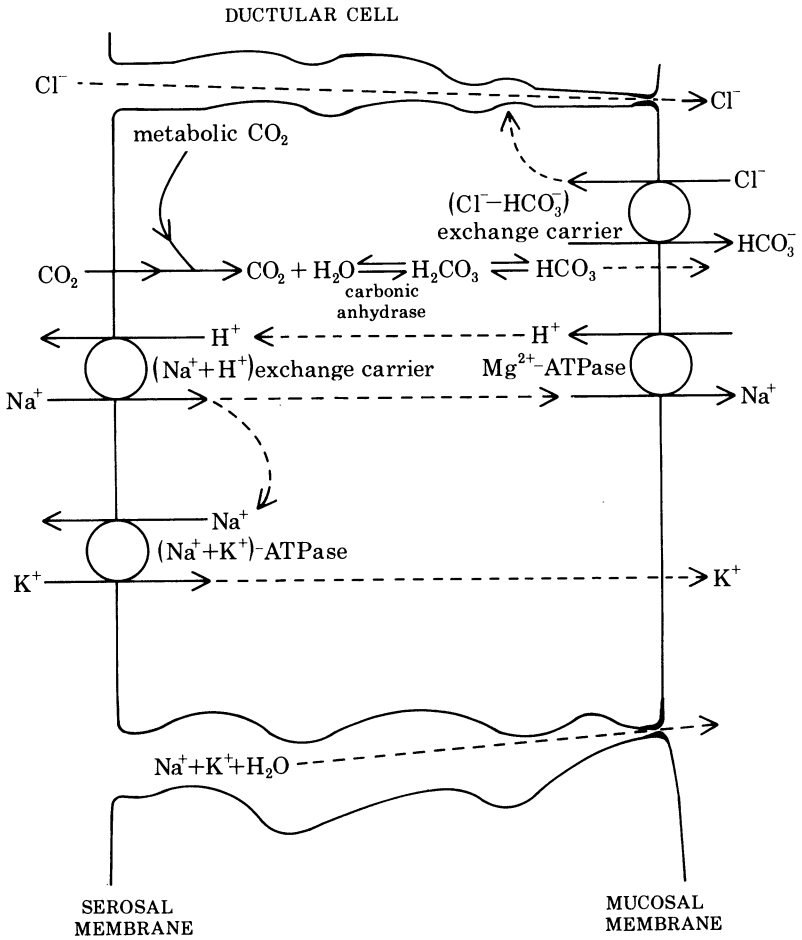

FIGURE 7. A schematic representation of the proposed cellular mechanisms for pancreatic secretion by the ductular cells.

(ii) *Chloride and its role in pancreatic secretion*

In published hypotheses of pancreatic secretion, chloride is either assigned a passive role or is totally ignored. It is clear that chloride plays a permissive role at least, and an important one. Rothman & Brooks (1965) demonstrated that when chloride of the bathing medium was replaced with sulphate, secretion from the rabbit pancreas *in vitro* was reduced by about 70%. This effect was confirmed and extended to other inorganic and organic anions (Case *et al.* 1969*b*, 1979). The degree of effectiveness of the replacement anions followed the following sequence: chloride = bromide ⩾ nitrate > iodide > sulphate > methyl sulphate > isethionate. When the chloride of the perfusate of the isolated cat pancreas is replaced in steps by methyl sulphate,

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isethionate or cyclamate, the secretion rate is linearly reduced in proportion to the amount of chloride remaining in the perfusate (figure 9). The rate of secretion at a particular chloride concentration was different for the three substituting anions, with methyl sulphate being the most effective and cyclamate the least. Extrapolation of the regression lines to zero chloride concentration cuts the secretion axis at 26% of the maximal secretory rate for methyl sulphate, 40% for isethionate and 55% for cyclamate. When chloride was totally substituted experimentally, the corresponding figure for methyl sulphate was 58% and for cyclamate 70% (Case *et al.* 1979). The latter figures are considerably higher than the former, suggesting that the presence of chloride may facilitate the secretion of bicarbonate.

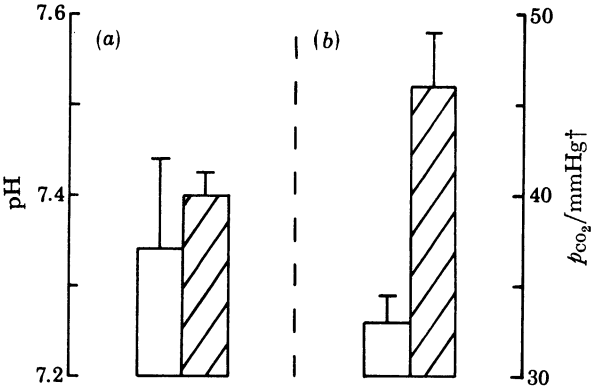

FIGURE 8. The pH and p_{CO_2} of the perfusate from the isolated pancreas, (*a*) before and (*b*) after stimulation of secretion by secretin. Hatched columns, p_{CO_2}. † 1 mmHg ≈ 133 Pa.

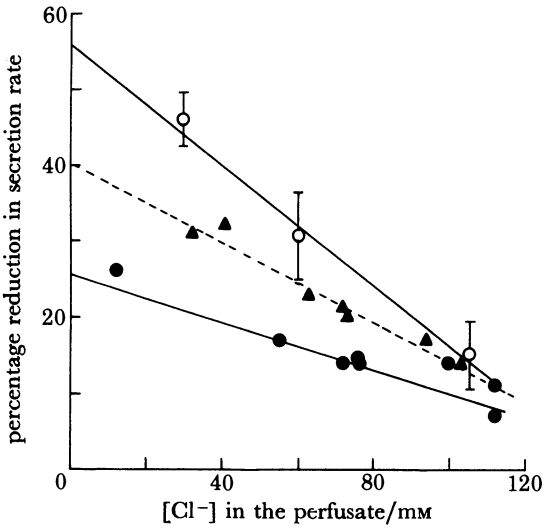

FIGURE 9. The effect on pancreatic secretion of partial replacement of chloride of the perfusate of the isolated pancreas with cyclamate (○), isethionate (▲) and methyl sulphate (●).

Using indirect approaches to examine the role of chloride, Scratcherd & Hutson (1981) have concluded that, although the pancreas does not have a specific requirement for chloride, it must be taken into account in discussing any theory of pancreatic secretion. Only bromide and possibly nitrate are complete substitutes. The effectiveness of other anions depends upon the degree to which they enter the pancreatic juice and therefore depends less on their chemical nature and more upon their size. It seems likely that a chloride–bicarbonate exchange carrier

may be involved for the distilbene SITS (regarded as a highly specific blocker of carrier-mediated anion exchange) (Cabantchik & Rothstein 1972) inhibits pancreatic secretion by about 30% (Scratcherd & Hutson 1981).

On this and other evidence (Case *et al.* 1979), Scratcherd & Hutson (1981) propose that a chloride–bicarbonate exchange mechanism could account for a small part (perhaps one-third) of the chloride movement. At the moment, there is no evidence that it is actively transported or that an electrically neutral co-transport (secondary active transport) is involved. It seems that passive diffusion down its electrochemical gradient is the most probable explanation for the major part of chloride transport.

The paracellular pathway and pancreatic secretion

It has been proposed by Bonting *et al.* (1980) that the paracellular pathway is an important route for water and passive ion transport. The pancreas is a 'leaky' epithelium as judged by most of the criteria of Frömter & Diamond (1972). It is normally partly permeable to large organic molecules such as sucrose and mannitol (Dewhurst *et al.* 1979; Jansen *et al.* 1979). Isosmotic replacement of sodium with sucrose causes a reduction in the secretion rate and a gradual increase in the sodium concentration gradient between juice and perfusion fluid or bathing medium, as described earlier in this paper. The K^+ concentration in the pancreatic juice also increases even though it remains constant in the bathing medium. There is a linear correlation between the sodium–potassium ratio, in the secreted fluid and the bathing medium. These data may indicate that Na^+ and K^+ largely enter pancreatic secretion via the paracellular route.

References (Scratcherd *et al.*)

Argent, B. E., Case, R. M. & Scratcherd, T. 1973 Amylase secretion by the perfused cat pancreas in relation to the secretion of calcium and other electrolytes and as influenced by the external ionic environment. *J. Physiol., Lond.* **230**, 575–593.

Bernier, J. J. & Lambling, A. 1962 Relations between the volume and output of bicarbonate in pancreatic secretion. In *The exocrine pancreas* (ed. A. V. S. De Reuck and M. P. Cameron), pp. 138–144. London: J. & A. Churchill Ltd.

Bonting, S. L., De Pont, J. J. H. H. M. & Jansen, J. W. C. M. 1980 The role of sodium in pancreatic fluid secretion in the rabbit. *J. Physiol., Lond.* **309**, 533–546.

Bro-Rasmussen, F., Killman, S.-A., Thaysen, J. S. 1956 The composition of pancreatic juice as compared to sweat, parotid saliva and tears. *Acta physiol. scand.* **37**, 97–113.

Bundgaard, M., Møller, M. & Poulsen, J. H. 1981 Localization of sodium pump sites in cat pancreas. *J. Physiol., Lond.* **313**, 405–414.

Cabantchik, Z. I. & Rothstein, A. 1972 The nature of the membrane sites controlling anion permeability of human red blood cells as determined by studies with disulphonic stilbene derivatives. *J. Membr. Biol.* **10**, 311–330.

Case, R. M., Charlton, M., Smith, P. A. & Scratcherd, T. 1980 Electrolyte secretory processes in exocrine pancreas and their intracellular control. In *Biology of normal and cancerous exocrine pancreatic cells* (ed. A. Ribet, L. Pradayrol & C. Susini), pp. 41–54. Amsterdam: Elsevier/North Holland Biomedical Press.

Case, R. M., Harper, A. A. & Scratcherd, T. 1968 Water and electrolyte secretion by the perfused pancreas of the cat. *J. Physiol., Lond.* **196**, 133–149.

Case, R. M., Harper, A. A. & Scratcherd, T. 1969*a* The secretion of electrolytes and enzymes by the pancreas of the anaesthetized cat. *J. Physiol., Lond.* **201**, 335–348.

Case, R. M., Harper, A. A. & Scratcherd, T. 1969*b* Water and electrolyte secretion by the pancreas. In *The exocrine glands* (ed. S. Y. Botelho, F. P. Brooks & W. B. Shelley), pp. 39–56. Philadelphia: University of Pennsylvania Press.

Case, R. M., Holtz, J., Hutson, D., Scratcherd, T. & Wynne, R. D. A. 1979 Electrolyte secretion by the isolated cat pancreas during replacement of extracellular bicarbonate by organic anions and chloride by inorganic anions. *J. Physiol., Lond.* **286**, 563–576.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Case, R. M. & Scratcherd, T. 1974 The secretion of alkali metal ions by the perfused cat pancreas as influenced by the composition and osmolality of the external environment and by inhibitors of metabolism and $Na^+ + K^+$-ATPase activity. *J. Physiol., Lond.* **242**, 415–428.

Case, R. M., Scratcherd, T. & Wynne, R. D. A. 1970 The origin and secretion of pancreatic juice bicarbonate. *J. Physiol., Lond.* **210**, 1–15.

Davies, R. E., Harper, A. A. & Mackay, I. F. S. 1949 A comparison of the respiratory activity and histological changes in isolated pancreatic tissue. *Am. J. Physiol.* **157**, 278–282.

Dewhurst, D. G., Hadi, N. A., Hutson, D. & Scratcherd, T. 1978 The permeability of the secretin stimulated exocrine pancreas to non-electrolytes. *J. Physiol., Lond.* **277**, 103–114.

Diamond, J. M. & Bossert, W. H. 1967 Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. *J. gen. Physiol.* **50**, 2061–2083.

Dockray, G. J. 1972 The action of secretin, cholecystokinin–pancreozymin and caerulein on pancreatic secretion in the rat. *J. Physiol., Lond.* **225**, 679–692.

Fölsch, U. R. & Creutzfeldt, W. 1975 Electrolyte secretion by a pancreatic model in the rat in vivo and accumulation of cyclic adenosine 3′,5′-monophosphate *in vitro* in response to gastrointestinal hormones. In *Stimulus–secretion coupling in the gastrointestinal tract* (ed. R. M. Case & H. Goebell), pp. 381–384. Lancaster: MTP Press.

Fölsch, U. R. & Creutzfeldt, W. 1977 Pancreatic duct cells in rats: secretory studies in response to secretin, cholecystokinin-pancreozymin, and gastrin. *Gastroenterology* **73**, 1053–1059.

Frömter, E. & Diamond, J. M. 1972 Route of passive ion permeation in epithelia. *Nature, new Biol.* **235**, 9–13.

Gilman, A. & Cowgill, G. R. 1933 Osmotic relations between blood and body fluids. IV. Pancreatic juice, bile and lymph. *Am. J. Physiol.* **104**, 476–479.

Glynn, I. M. 1964 The action of cardiac glycosides on ion movements. *Pharmac. Rev.* **16**, 381–407.

Grossman, M. I. & Ivy, A. C. 1946 Effect of alloxan upon the external secretion of the pancreas. *Proc. Soc. exp. Biol. Med. N.Y.* **63**, 62–63.

Hart, W. M. & Thomas, J. E. 1945 Bicarbonate and chloride of pancreatic juice secreted in response to various stimuli. *Gastroenterology* **4**, 409–420.

Hickson, J. C. D. 1970 The secretion of pancreatic juice in response to stimulation of the vagus nerves in the pig. *J. Physiol., Lond.* **206**, 275–297.

Hill, A. V. 1930 A thermal method of measuring the vapour pressure of an aqueous solution. *Proc. R. Soc. Lond.* A **127**, 9–19.

Jansen, J. W. C. M., De Pont, J. J. H. H. M. & Bonting, S. L. 1979 Transepithelial permeability in the rabbit pancreas. *Biochim. biophys. Acta* **551**, 95–108.

Kalser, M H. & Grossman, M. I. 1954 Pancreatic secretion in dogs with ethionine-induced pancreatitis. *Gastroenterology* **26**, 189–197.

Kanno, T. & Yamamoto, M. 1977 Differentiation between the calcium-dependent effects of cholecystokinin-pancreozymin and the bicarbonate-dependent effects of secretin in exocrine secretion of the rat pancreas. *J. Physiol., Lond.* **264**, 787–799.

Moore, E. W. 1976 Comprehensive formal models for pancreatic calcium secretion. *Am. J. digest. Dis.* **21**, 766–775.

Petersen, O. H. & Ueda, N. 1977 Secretion of fluid and amylase in the perfused rat pancreas. *J. Physiol., Lond.* **264**, 819–835.

Rawls, J. A., Wistrand, P. J. & Maren, T. H. 1963 Effects of acid-base changes and carbonic anhydrase inhibition on pancreatic secretion. *Am. J. Physiol.* **205**, 651–657.

Ridderstap, A. S. & Bonting, S. L. 1969 Na^+–K^+-activated ATPase and exocrine pancreatic secretion *in vitro*. *Am. J. Physiol.* **217**, 1721–1727.

Rothman, S. S. & Brooks, F. P. 1965 Pancreatic secretion in vitro in 'Cl-free', 'CO_2-free' and low Na^+ environment. *Am. J. Physiol.* **209**, 790–796.

Schulz, I. 1971 Influence of bicarbonate-CO_2 and glycodiazine buffer on the secretion of the isolated cat's pancreas. *Pflügers Arch. Eur. J. Physiol.* **329**, 283–306.

Schulz, I. 1980 Bicarbonate transport in the exocrine pancreas. *Ann. N.Y. Acad. Sci.* **341**, 191–209.

Schulz, I. & Ullrich, K. J. 1978 Transport processes in the exocrine pancreas. In *Membrane transport in biology*, vol. 4A (ed. G. Giebisch, D. C. Tosteson & H. H. Ussing), pp. 811–852. Berlin: Springer-Verlag.

Schulz, I., Yamagata, A. & Weske, M. 1969 Micropuncture studies on the pancreas of the rabbit. *Pflügers Arch. ges. Physiol.* **308**, 277–290.

Scratcherd, T., Case, R. M. & Smith, P. A. 1975 A sensitive method for the biological assay secretin and substances with 'secretin-like' activity in tissues and biological fluids. *Scand. J. Gastroent.* **10**, 821–828.

Scratcherd, T. & Hutson, D. 1981 The role of chloride in pancreatic secretion. In *Electrolyte and water transport across gastrointestinal epithelia* (ed. R. M. Case, A. Garner, L. Turnberg & J. A. Young). Raven Press. (In the press).

Sewell, W. A. & Young, J. A. 1975 Secretion of electrolytes by the pancreas of the anaesthetized rat. *J. Physiol., Lond.* **252**, 379–376.

Skou, J. C. 1965 Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.* **45**, 596–617.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Smith, P. A., Sunter, J. P. & Case, R. M. 1981 Progressive atrophy of pancreatic acinar tissue in rats fed a copper-deficient diet supplemented with D-penicillamine or triethylenetetramine: morphological and physiological studies. *Digestion* (In the press).

Swanson, C. H. 1977 Isotonic water transport in secretory epithelia. *Yale J. Biol. Med.* **50**, 153–163.

Swanson, C. H. & Solomon, A. K. 1972 Evidence for Na–H exchange in the rabbit pancreas. *Nature, Lond.* **236**, 183–184.

Swanson, C. H. & Solomon, A. K. 1973 A micropuncture investigation of the whole tissue mechanism of electrolyte secretion by the in vitro rabbit pancreas. *J. gen. Physiol.* **62,** 407–429.

Swanson, C. H. & Solomon, A. K. 1975 Micropuncture analysis of the cellular mechanisms of electrotype secretion by the in vitro rabbit pancreas. *J. gen. Physiol.* **65**, 22–45.

Way, L. W. & Diamond, J. M. 1970 The effect of secretin on electrical potential difference in the pancreatic duct. *Biochim. biophys. Acta* **203**, 298–307.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES