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ABSENCE OF A RELATIONSHIP BETWEEN ARTERIAL pH AND PANCREATIC BICARBONATE SECRETION IN THE ISOLATED PERFUSED CAT PANCREAS

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SUMMARY

1. The secretion rate of bicarbonate by the isolated saline-perfused cat pancreas was linearly related to the bicarbonate concentration of the arterial inflow at constant P_{CO_2} and at high volume rates of secretion.

2. Pancreatic bicarbonate secretion was independent of arterial inflow pH at constant bicarbonate concentrations when the pH was manipulated by alterations in the P_{CO_2} at high volume rates of secretion.

3. A small but statistically significant linear relationship existed between the pH of the arterial inflow and bicarbonate secretion at constant P_{CO_2} after inhibition of carbonic anhydrase by acetazolamide. Under the same conditions no relationship was found between bicarbonate secretion and arterial inflow pH when the perfusate bicarbonate concentration was kept constant and the P_{CO_2} varied.

4. When the volume rate of secretion was reduced by about 60–70 % of maximum no relationship was found to exist between arterial inflow pH and bicarbonate secretion at constant bicarbonate concentration in the perfusate. There was also no relationship between inflow pH and bicarbonate secretion at constant P_{CO_2} down to a pH of 7·3 until the bicarbonate concentration of the perfusate was reduced below 10 mM, when the secretion rate fell off rapidly.

5. A linear relationship was found to exist between the volume rate of secretion and the P_{CO_2} of the pancreatic juice and the output of lactate both in the isolated saline-perfused gland and the blood-perfused pancreas *in situ*.

6. At high rates of secretion the P_{CO_2} of the pancreatic juice was always higher than that of either the arterial inflow or the venous outflow. There is therefore no gradient for the passive movement of carbon dioxide between the arterial inflow and the pancreatic juice.

7. Inhibition of secretion with acetazolamide caused a fall in the P_{CO_2} of pancreatic juice and increased the output of lactate. The secretion of lactate was not due to hypoxia as it also occurred in the blood-perfused gland *in situ* which had normal haemoglobin concentrations and oxygen saturation.

8. It is concluded that the secretion of bicarbonate is independent of arterial pH but critically dependent upon the arterial concentration of the bicarbonate ion. These experiments do not support the concept that the secretion of protons over the basolateral membrane is the major primary event in pancreatic secretion of bicarbonate.

INTRODUCTION

Bicarbonate secretion from the cat pancreas is under the control of the hormone secretin. It is derived almost entirely from the extracellular fluid with only about 4 % arising directly from metabolism of the ductular cells (Case, Scratcherd & Wynne, 1970). Anions other than bicarbonate will support some secretion of pancreatic juice. In particular, sulphonamide, sulphamerazine (Schulz, 1971), formate, butyrate, proprionate and acetate (Swanson & Solomon, 1975; Hadi, Hotz, Scratcherd & Wynne, 1976) will substitute for bicarbonate to varying extents. However, their common feature is that they are all weak acids and lipid soluble and like bicarbonate are only one component of a buffer system. The proton is common to all and it is postulated that this is the actively transported ion (Scratcherd, Hutson & Case, 1981). Simultaneously with the secretion of bicarbonate into the ductular lumen, the pH of the fluid leaving in the venous drainage of the gland falls accompanied by an increase in the partial pressure of the carbon dioxide (Case, Scratcherd & Wynne, 1970). This has been interpreted as the secretion of protons backwards across the basolateral membrane. The energy for this process has been attributed to the sodium gradient through a Na^+–H^+ exchange carrier (secondary active transport) (Swanson & Solomon, 1975). If this is the case then lowering the pH of the extracellular fluid should inhibit bicarbonate secretion by creating a greater adverse proton gradient against which hydrogen ion movement must occur and conversely a more favourable gradient should augment secretion.

This view seems to have been largely accepted from experiments on intact animals in which the pH of plasma had been altered by creating either a state of acidosis, where secretion is inhibited, or a state of alkalosis where secretion is augmented (Rawls, Wistrand & Maren, 1963; Pak, Hong, Pak & Hong, 1966; Raeder, Mo & Aune, 1979; Raeder, Mo, Aune & Mathisen, 1980; Iijima, Yamagiashi, Iwatsuki & Chiba, 1986). However, in these experiments not only does the pH vary but also the partial pressure of the carbon dioxide P_{CO_2} and the bicarbonate concentration. This paper re-examines the problem by allowing the pH of the fluid perfusing the isolated cat pancreas to vary, either at constant P_{CO_2} or at constant bicarbonate concentrations.

METHODS

The isolated saline-perfused cat pancreas was used as described initially by Case, Harper & Scratcherd, 1968 and modified by Case, Hotz, Hutson, Scratcherd & Wynne, 1979. After isolation the gland was placed in a thermostatically controlled Perspex box and perfused through the coeliac axis. The effluent from the gland was drained by diverting the perfusate retrogradely through the superior mesenteric vein after tying the portal tract. The composition of the standard perfusion fluid was (mM): NaCl, 125; KCl, 4·7; $MgSO_4$, 1·0; $CaCl_2$, 2·5; $NaHCO_3^-$, 25; and glucose, 5; and was gassed with 95 % O_2, 5 % CO_2. The pH was changed either by keeping the bicarbonate concentration constant and altering the composition of the CO_2 gassing the fluid, or keeping the CO_2 constant and changing the bicarbonate concentration. Osmolality was kept constant at 290–300 mosmol/kg either by the addition or removal of NaCl where appropriate. The precise changes in the composition of the perfusion fluids are indicated in the text. The gland was stimulated to secrete at maximum rates except where stated in the text by the infusion of pure porcine secretin (from the Karolinska Institute, Stockholm) into the arterial cannula. Pancreatic juice was collected from a stainless-steel cannula inserted into the duct at the point at which it pierced the duodenal wall into tared tubes except when pH and P_{CO_2} were to be determined. In that case collection was made directly into

Corning blood-gas capillary tubing attached to the cannula which was filled over a predetermined time period dependent upon the rate of secretion. Samples of perfusate, both the inflow and the outflow from the gland were similarly collected at points close to entry and exit of the supplying vessels via a three-way tap into Corning blood-gas capillary tubing. The ends were immediately sealed with special rubber stoppers and the pH and P_{CO_2} were instantly measured on the fluid occupying the middle one-third of the tube anaerobically on a Corning 178 pH/Blood Gas Analyser. Bicarbonate concentration was measured by one of three methods depending on the amount of juice available for analysis either directly on a Natelson microgasometer, or indirectly by the Corning 178 pH/Blood Gas Analyser using the Henderson–Hasselbalch equation or from the expression $Na^+ + K^+ = Cl^- + HCO_3^-$. Chlorides were measured on the Buchler chloridimeter and sodium and potassium on the Corning 480 flame photometer. Lactate was measured by the method of Gutmann & Wahlefeld (1974).

In five animals the pancreas was perfused *in situ* and the operative procedures were identical to those described above except that the pancreas was isolated from all other abdominal organs other than the liver but left *in situ*. It received its blood supply from the coeliac axis and superior mesenteric arteries. The renal arteries and the common carotid on both sides were also tied. The portal vein and hepatic artery were not tied off and samples of venous blood were obtained from a catheter placed in the superior mesenteric vein. When samples of portal blood were collected the latter was temporarily occluded so that blood was diverted into the superior mesenteric catheter and collected in Corning blood-gas capillary tubing for analysis of pH, P_{CO_2}, P_{O_2}, oxygen saturation and haemoglobin concentration. Arterial blood samples were taken from a cannula placed in the aorta with its tip lying at the origin of the coeliac axis. Pancreatic secretion was stimulated by the continuous intravenous infusion of secretin. Because these preparations become acidotic, bicarbonate solutions were infused intravenously at a rate to keep the arterial blood pH within the normal range. Even so, both the arterial bicarbonate and P_{CO_2} levels were reduced below normal as the preparation had a compensated acidosis. The results were expressed as the mean ± 1 S.E. of mean and the regression lines calculated by the method of least squares.

RESULTS

The effect on bicarbonate secretion when the pH of the perfusion fluid was altered by adjusting the bicarbonate concentration at constant P_{CO_2}

In twenty-eight experiments on fourteen preparations the pH of the perfusate was varied over the range 6·9–7·6 either by the addition or removal of sodium bicarbonate, osmolality maintained between 290 and 300 mosmol/kg by the addition of sodium chloride when bicarbonate was removed, or its removal when bicarbonate was added above the standard 24 mM. The P_{CO_2} was kept constant at $5{\cdot}1 \pm 0{\cdot}06$ kPa. When adjusted in this way there was an apparent linear relationship between the pH of the perfusate inflow and the rate of bicarbonate secretion in the pancreatic juice (Fig. 1*A*).

The effect on bicarbonate secretion on altering the pH of the perfusion fluid by keeping the bicarbonate concentration constant at 24 mM, and varying the P_{CO_2} *between 2·80 and 13·21 kPa*

In twenty-nine experiments on ten preparations the pH was also varied over the pH range 6·9–7·6 but in this case the results were quite different, the rate of bicarbonate secretion by the gland was independent of the pH of the perfusion fluid (Fig. 1*B*).

The influence of acetazolamide

In the intact animal it has been claimed that the relationship between pancreatic bicarbonate secretion and arterial pH seen during acidosis and alkalosis is abolished

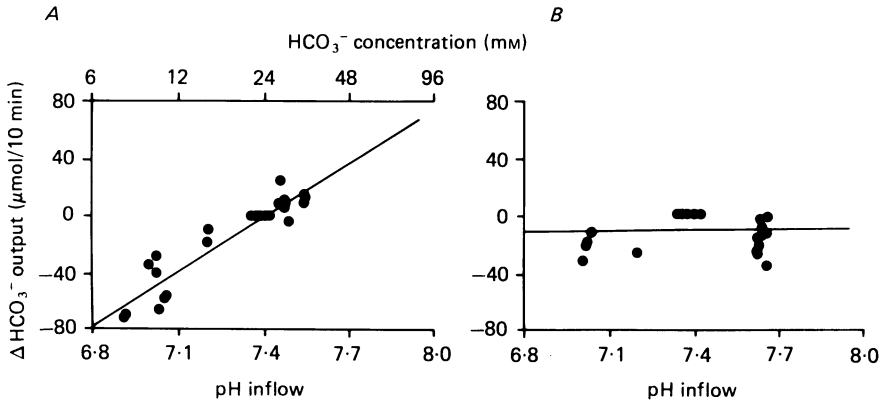

Fig. 1. The effect on the increment of bicarbonate secretion (positive for stimulation, negative for inhibition): on the left (*A*) varying the pH of the perfusate by altering the concentration of bicarbonate whilst keeping the P_{CO_2} constant. Equation for the regression line: $Y = -936 + 126X$ $r = 0{\cdot}94$ (d.f. = 26) $t = 14{\cdot}0$. On the right (*B*) by varying the pH of the perfusate at constant bicarbonate concentration. In this Figure and those which follow ΔHCO_3^- output was measured by recording the increment or decrement in output when compared with the previous control period.

during carbonic anhydrase inhibition (Raeder & Mathisen, 1982). The above experiments were then repeated when the perfusion fluid had added to it the carbonic anhydrase inhibitor acetazolamide in a concentration of 10^{-3} or 10^{-4} M.

This procedure caused an immediate inhibition by $72{\cdot}7 \pm 3{\cdot}1$ % in eight experiments on eight preparations. The inhibition was sustained so long as the gland was exposed to the inhibitor. When a steady rate of secretion had been established, usually within 30 min, the pH of the perfusion fluid was varied as before by altering the bicarbonate concentration between 10 and 35 mM but keeping the P_{CO_2} at $5{\cdot}12 \pm 0{\cdot}08$ kPa (twenty-five experiments in eight preparations).

In this case also there was an apparent linear relationship (with a positive slope) between perfusate pH and pancreatic bicarbonate secretion (Fig. 2*A*). Although the slope of the line relating these two parameters was only just discernible it nevertheless was highly significant ($P < 0{\cdot}001$). When the experiment was repeated but in this case the pH varied by keeping the bicarbonate concentration constant at 24 mM but varying the P_{CO_2} between 4·5 and 18·2 kPa then secretion of bicarbonate appeared independent of pH in fourteen experiments in eight preparations (Fig. 2*B*).

Twenty-two experiments in nine preparations were then carried out in the absence of acetazolamide but at a similar rate of secretion as that which occurred after carbonic anhydrase inhibition, brought about by infusing secretin at lower rates, then the secretion rate of bicarbonate was independent of pH at constant bicarbonate concentration. When the experiments were repeated (twenty-one experiments in nine preparations) at constant P_{CO_2} there was no fall off in secretion rate until the pH of the perfusate fell below a pH of 7·3 at that level the bicarbonate concentration was 10 mM and the secretion rate was so low that bicarbonate secretion could not be measured (Fig. 3*A* and *B*). These experiments suggest that pH may not play as dominant a role as previously thought, and that the secretion of bicarbonate in the

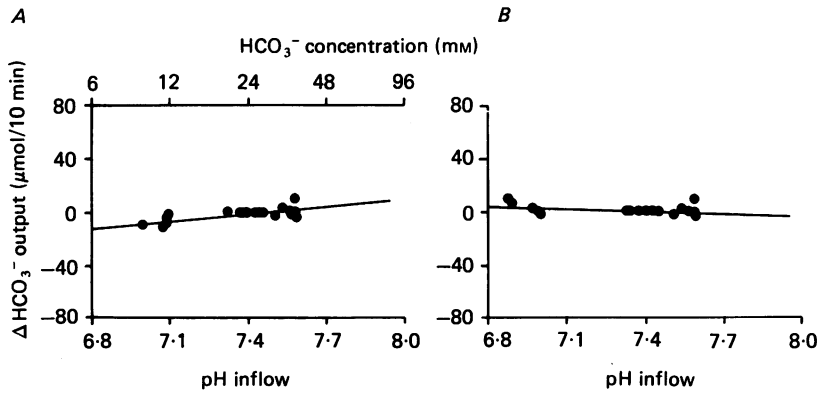

Fig. 2. The effect of acetazolamide on the secretion of bicarbonate: on the left (*A*) when the pH of the perfusate was varied by altering the concentration of perfusate bicarbonate at constant P_{CO_2}: equation of regression line is $Y = -124 + 16{\cdot}6X$ $r = 0{\cdot}75$ (d.f. = 23) $t = 5{\cdot}44$. On the right (*B*) when the pH of the perfusate was varied by altering the P_{CO_2} but keeping the bicarbonate concentration constant.

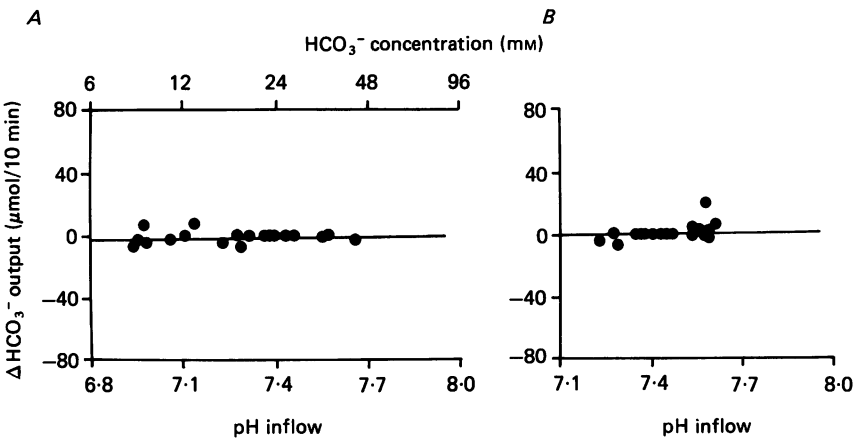

Fig. 3. The relationship between inflow pH, bicarbonate concentration and P_{CO_2} on the secretion of bicarbonate at low volume rates of secretion (about 30 % of maximum rate). On the left (*A*) the inflow pH was varied by altering the bicarbonate concentration but keeping the P_{CO_2} constant. On the right (*B*) the inflow pH was varied by altering the P_{CO_2}, but keeping the bicarbonate concentration constant. Compare with Fig. 1 *A* and *B*.

pancreatic juice is influenced more by the bicarbonate concentration in the perfusate (extracellular fluid) than the pH or the P_{CO_2} of the extracellular fluid.

The transpancreatic carbon dioxide concentration gradient

It has been proposed that the hydrogen ion which is secreted across the basolateral membrane reacts with bicarbonate of the plasma or extracellular fluid to produce carbon dioxide, which in its turn diffuses into the pancreatic cell. Here it becomes hydrated under the influence of carbonic anhydrase to form carbonic acid which on dissociation forms bicarbonate and hydrogen ions. The former enters the juice and

the latter passes back out of the cell in exchange for sodium. For the carbon dioxide to enter the cell there must be a favourable pressure gradient. In all experiments it was found that the P_{CO_2} of pancreatic juice was elevated above that both of the arterial inflow and the venous outflow. There was also found to be a linear relationship between the secretion rate of pancreatic juice and its P_{CO_2}, such that with increasing rates of flow the P_{CO_2} increased. In seventeen experiments on five preparations the P_{CO_2} of the perfusate inflow and outflow together with that of the pancreatic juice secreted simultaneously were measured. The P_{CO_2} of pancreatic juice was flow dependent, increasing with increasing rates of secretion. The perfusate inflow P_{CO_2} had a mean value of $5{\cdot}56 \pm 0{\cdot}1$ kPa and the P_{CO_2} of juice was always in excess of this with the difference being highly significant ($P < 0{\cdot}001$). There was even a difference between the P_{CO_2} of the effluent leaving the gland and the juice and although small ($0{\cdot}74 \pm 0{\cdot}09$ kPa) was highly significant. The P_{CO_2} of the pancreatic juice at low flow rates ($0{\cdot}18 \pm 0{\cdot}04$ g/10 min) was $6{\cdot}93 \pm 0{\cdot}15$ kPa with a lactate output of $3{\cdot}9 \pm 1{\cdot}6$ μg/10 min; at flow rates of $0{\cdot}6 \pm 0{\cdot}03$ g/10 min, the corresponding figures were $7{\cdot}28 \pm 0{\cdot}11$ kPa for carbon dioxide and $8{\cdot}09 \pm 2{\cdot}6$ μg/10 min for lactate. At high secretion rates ($1{\cdot}06 \pm 0{\cdot}06$ g/10 min) the P_{CO_2} of the juice was $7{\cdot}84 \pm 0{\cdot}19$ kPa and the lactate output $14{\cdot}5 \pm 1{\cdot}2$ μg/10 min.

There was therefore no gradient which would favour the passive transport of carbon dioxide into the pancreatic juice. The cause of the increase in P_{CO_2} of the juice is uncertain but as it is known that lactate appears in pancreatic juice it might simply be due to an overspill of lactic acid from metabolism of the cell. Lactate was found to appear in the juice at a rate proportional to the rate of pancreatic secretion. Although lactate may contribute to the rise in P_{CO_2} it is unlikely to be the sole explanation. In the experiments in which acetazolamide was added to the perfusate it was observed that not only did inhibition of secretion occur but also a fall in the P_{CO_2} of the juice secreted from $7{\cdot}07 \pm 0{\cdot}25$ to $6{\cdot}19 \pm 0{\cdot}27$ kPa ($n = 5$; $P > 0{\cdot}01$) and at the same time an increase in both the concentration and output of lactate occurred from $11{\cdot}1 \pm 3{\cdot}5$ to $17{\cdot}0 \pm 4{\cdot}7$ μg/10 min ($n = 5$; $P > 0{\cdot}02$). Quite clearly as P_{CO_2} had fallen in the face of a rise in lactate output, the lactate could not be the sole contributor to the P_{CO_2} effect. It might be argued that in the isolated saline-perfused preparation the lactate may have been the result of hypoxia as the perfusion fluid did not contain an oxygen carrier. These experiments were repeated on the pancreas perfused with blood *in situ* in the anaesthetized cat with qualitatively similar results.

Again there was an increase in the P_{CO_2} and an increase in output of lactate with increasing flow rates of pancreatic juice. At low rates of flow ($0{\cdot}17 \pm 0{\cdot}03$ g/10 min) the P_{CO_2} of the juice was $3{\cdot}72 \pm 0{\cdot}37$ kPa with a lactate output of $13{\cdot}4 \pm 4{\cdot}0$ μg/10 min, the corresponding figures for flow rate at $0{\cdot}38 \pm 0{\cdot}02$ g/10 min were $4{\cdot}91 \pm 0{\cdot}51$ kPa for carbon dioxide and $43{\cdot}2 \pm 14{\cdot}1$ μg/10 min for lactate. When the mean flow rate was further increased to $0{\cdot}69 \pm 0{\cdot}09$ g/10 min, the P_{CO_2} of the juice reached a mean of $5{\cdot}04 \pm 0{\cdot}46$ kPa and the lactate output was $124{\cdot}1 \pm 37{\cdot}4$ μg/10 min.

These animals did not suffer from hypoxia as the mean saturation of the haemoglobin with oxygen was $97{\cdot}1 \pm 0{\cdot}31$ % ($n = 5$), the mean P_{O_2} was $12{\cdot}82 \pm 0{\cdot}45$ kPa and the mean haemoglobin concentration $11{\cdot}52 \pm 0{\cdot}89$ g/dl. It is unlikely therefore that the lactate in the juice is the result of hypoxia.

The perfusate juice–bicarbonate difference

Bicarbonate must enter the secretory cell either as the anion bicarbonate or as molecular carbon dioxide, but whatever the mechanism the amounts which appear in the pancreatic juice must disappear from the perfusate. These experiments were carried out immediately the preparation was isolated. In ten experiments on two preparations the rate of disappearance of bicarbonate from the perfusate and its appearance in the juice were simultaneously measured. It was found that whereas $11{\cdot}03 \pm 0{\cdot}94$ μmol/min were lost only some $7{\cdot}98 \pm 0{\cdot}32$ μmol/min appeared in the pancreatic juice, a difference which was statistically significant ($P > 0{\cdot}02$). From these experiments it can be concluded that bicarbonate must be dissipated as carbon dioxide and is further confirmation that hydrogen ions are secreted over the basolateral membrane.

DISCUSSION

The concept that arterial plasma pH is the controlling factor in bicarbonate secretion by the pancreas arose from experiments in which acid–base balance was acutely disturbed by creating an acidosis or an alkalosis either by infusions of acids or bases into the circulation or by hyperventilating the animal on a respiratory pump or by the inhalation of carbon dioxide. In such experiments even when the pH is kept reasonably constant usually both the P_{CO_2} and the bicarbonate concentrations in the plasma vary (Rawls *et al.* 1963; Raeder *et al.* 1980; Iijima *et al.* 1986). These circumstances contrast with those for isolated preparations where strict control can be kept for long periods. When the effects of constant P_{CO_2} and constant bicarbonate concentrations in the arterial inflow of the perfused pancreas are compared as in the experiments reported in this paper it is clear that when pH is changed by altering bicarbonate concentration there is an effect on bicarbonate secretion. This is also true at constant pH where the rate of secretion and bicarbonate output in the juice are proportional to the bicarbonate concentration in the perfusate (Case *et al.* 1968; Schulz, 1971). When bicarbonate concentrations in the perfusate were kept constant and pH altered by manipulating the P_{CO_2}, the secretion of bicarbonate in the pancreatic juice was independent of pH. Raeder & Mathisen (1982) claimed that the secretion of bicarbonate was related to arterial pH during acidosis and alkalosis and that this effect was abolished by the inhibition of carbonic anhydrase. From the results presented in this paper the effects are almost entirely explained not as in Raeder and Mathisen's view due to the action of acetazolamide but as a consequence of a low rate of secretion. It is also clear that secretion can be independent of arterial pH, but is critically dependent upon the bicarbonate concentration of the plasma or extracellular fluid. The importance of the role of bicarbonate was also pointed out by Pak *et al.* (1966) in their experiments on intact dogs.

If the mechanism of secretion involves the passage of carbon dioxide across the basolateral membrane (produced from extracellular bicarbonate as the result of hydrogen ion secretion by the pancreatic cell) to a locus where it is hydrated, there must exist a gradient across the secretory cell down which carbon dioxide can pass. Under normal conditions, particularly at high secretion rates the P_{CO_2} of pancreatic

juice is always higher than either that of the arterial inflow or the venous outflow. Therefore no gradient exists for the passive movement of carbon dioxide between perfusate and juice, unless some barrier exists within the cell. Such evidence casts doubts on the theory stated above and suggests that it may be that bicarbonate ion transport across the basolateral membrane is more important than carbon dioxide in the secretory mechanism. There are some anomalies, for example Schulz (1971) noted that when the concentration of the carbon dioxide gassing the perfusate of the isolated cat pancreas (under the influence of acetazolamide) was increased, the secretion of bicarbonate increased but this effect was small and not consistent. Similar effects were recorded in the experiments reported in this paper but as Schulz reported only at low rates of secretion. It was noted that at constant bicarbonate concentrations of the arterial inflow, increasing the P_{CO_2} to lower the pH did have a small stimulating effect on the secretion rate of bicarbonate. In four out of ten experiments when the pH was lowered in this way to a mean of $7{\cdot}13 \pm 0{\cdot}06$ by increasing the P_{CO_2} to $9{\cdot}78 \pm 1{\cdot}33$ kPa bicarbonate secretion increased by $3{\cdot}43 \pm 1{\cdot}94$ μmol/10 min. During this period the pancreatic juice P_{CO_2} was $10{\cdot}5 \pm 1{\cdot}01$ kPa which was not significantly different from that of the arterial inflow ($P < 0{\cdot}5$). In this case changing from the control perfusion fluid when the P_{CO_2} of the secreted juice was $6{\cdot}93 \pm 1{\cdot}5$ kPa to one in which the pH had been lowered by increasing the P_{CO_2} of perfusate to a mean of $9{\cdot}78 \pm 1{\cdot}33$ kPa must, at least from some time, have created a gradient favourable for the passive movement of carbon dioxide into the cell. The failure to observe the same effect at high rate of flows could be accounted for by the increase in P_{CO_2} of the juice with increasing rates of flow. The same phenomenon was also seen when the secretion rate was reduced with acetazolamide, in this circumstance however, in three out of five experiments the pancreatic juice P_{CO_2} ($13{\cdot}9 \pm 0{\cdot}82$ kPa) was always lower than that of the arterial inflow ($16{\cdot}92 \pm 1{\cdot}17$ kPa: $P < 0{\cdot}05$). These effects however, were always very small and when taken together with all the results for increasing P_{CO_2} at constant bicarbonate concentration did not show a statistically significant change. Nevertheless when there was no counter gradient an increase in P_{CO_2} of the arterial inflow was capable of eliciting a small increase in bicarbonate secretion. Using the *in vitro* rabbit pancreas Swanson & Solomon (1975) observed that decreasing the concentration of carbon dioxide bubbling the fluid in the organ bath at constant bicarbonate concentration did cause a small increase in the secretion rate, but in this instance neither the P_{CO_2} of the bath fluid nor the pancreatic juice was measured. The only other measurements of the P_{CO_2} of arterial blood or extracellular fluid and the pancreatic juice are by Caflisch, Solomon & Galey (1979) and Raeder & Mathisen (1982) who noted lower values in the pancreatic juice of the anaesthetized rabbit and dog, respectively. On the other hand, Hubel (1967) using the *in vitro* rabbit pancreas, noted higher values. However, in none of these reports were the P_{CO_2} relationships systematically studied. The cause of the higher P_{CO_2} of the pancreatic juice is not immediately obvious. It would not appear to be due to lactate spill over into the pancreatic juice as the lactate output rises but the P_{CO_2} falls on carbonic anhydrase inhibition, though it might be due to the action of carbonic anhydrase, for when the latter is inhibited the difference between arterial inflow and juice is reduced. Also in the pancreas perfused *in situ* with blood, the lactate concentrations in the pancreatic juice were considerably higher and

the P_{CO_2} of the juice slightly less than that in the isolated gland. These differences were due to the presence of the liver and absence of the kidneys as the preparation was a pancreas–liver–heart–lung preparation. The blood lactate would be contributed by the liver and as lactate is secreted by the pancreas its concentration in the juice is increased over and above that secretion due to endogenous production by the gland. The state of compensated acidosis would account for the relatively low secretion rates and lower P_{CO_2} of the pancreatic juice.

There is more bicarbonate lost from the perfusate than can be accounted for appearing in the pancreatic juice and therefore it must be dissipated as carbon dioxide and escape from the preparation by diffusion. This is further evidence that protons are secreted backwards over the basolateral membrane as first suggested by Case *et al.* (1968). Swanson & Solomon (1975) proposed that protons were exchanged for sodium. In other tissues a Na^+–H^+ exchange carrier has been found to be inhibited by amiloride, but in the pancreas this has not been the case (Kuijpers, Van Nooy, De Pont & Bonting, 1984). As the drug competes with sodium for the carrier, the experiments have to be carried out at low sodium concentrations which in themselves will cause inhibition of pancreatic secretion (Case *et al.* 1968). Consequently such experiments have been indecisive. If proton movement is coupled to sodium influx by a Na^+–H^+ carrier, or indeed is associated with secretion, is as yet unproven. The independence of bicarbonate secretion on pH of the arterial inflow at moderate to high secretory rates of pancreatic juice would support the latter view.

In summary there are two possible mechanisms whereby bicarbonate enters the secretory cell across the basolateral membrane, either as molecular carbon dioxide or as ionic bicarbonate. It would appear from the present study that both could occur but with the latter by far the major process. At very low rates of secretion the P_{CO_2} of the perfusate may determine secretion of bicarbonate into the juice, but above about 10% of the maximal secretory rate no favourable gradient for the transfer of carbon dioxide exists and therefore bicarbonate ion must be the transported species. This work therefore supports the hypothesis of Kuijpers *et al.* 1984, who propose that a bicarbonate carrier mechanism exists on the basolateral membrane.

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