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THE ORIGIN AND SECRETION OF PANCREATIC JUICE BICARBONATE

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SUMMARY

1. The rate of secretion from a saline-perfused preparation of the cat's pancreas is directly proportional to the perfusate bicarbonate concentration. When all bicarbonate is omitted, secretion completely or almost completely ceases.

2. Incorporation of [^{14}C]bicarbonate into the perfusion fluid results in its prompt appearance in the juice. The radioactive label is concentrated four to five times in the juice just as the total juice bicarbonate is four to five times greater than perfusate bicarbonate.

3. These two observations suggest that about 95 % of pancreatic juice bicarbonate is derived from perfusate (plasma) bicarbonate.

4. The inhibition of pancreatic secretion from the perfused gland by acetazolamide is similar to that observed in the intact animal.

5. There is a fall in pH and rise in P_{CO_2} in the perfusion fluid leaving the gland which is greater during secretion than at rest.

6. It is therefore suggested that during secretion, hydrogen ions pass from the gland into the perfusate (plasma), thus increasing the production of carbon dioxide from circulating bicarbonate. This carbon dioxide diffuses into the cell, is rehydrated (partly under the influence of carbonic anhydrase) and finally is secreted, thus establishing the necessary gradient for the continued diffusion of carbon dioxide into the cell.

INTRODUCTION

Pancreatic juice bicarbonate can have two possible origins: plasma bicarbonate, and carbon dioxide generated intracellularly by oxidative metabolism. Early studies, in which the carbon dioxide content of blood was measured before and after it had passed through the pancreas, led

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Still, Bennett & Scott (1933) to conclude that the principal source was metabolic carbon dioxide synthesized by the gland. The detection of carbonic anhydrase in the gland by Van Goor (1940) lent support to this view. However, the evidence of Ball, Tucker, Solomon & Vennesland (1941) seemed to refute it. Intravenously injected bicarbonate, labelled with radioactive carbon (^{11}C), appeared promptly in the juice. The ratios of total carbon dioxide in juice and serum, and of radioactive carbon in juice and serum, were approximately equal. These authors thus suggested that plasma bicarbonate was the chief source of juice bicarbonate. Conclusive though these experiments may have seemed, they did not explain the presence of carbonic anhydrase in the gland; this was subsequently shown by Birnbaum & Hollander (1953) to be an important factor in the secretion of pancreatic juice. Using the carbonic anhydrase inhibitor acetazolamide, Birnbaum & Hollander were able to reduce both the volume and bicarbonate concentration of canine pancreatic juice. The reduction in volume was about 60%. In man (Dreiling, Janowitz & Halpern, 1955) and in the cat (Case, Harper & Scratcherd, 1969*a*) results of acetazolamide injection are similar. In a recent review on carbonic anhydrase, Maren (1967) has concluded that all of the pancreatic juice bicarbonate is derived from the pool of carbon dioxide in pancreatic tissue, which is in diffusional equilibrium with blood carbon dioxide; there is no residual process of active bicarbonate transport from blood.

Using a saline-perfused preparation of the cat's pancreas (Case, Harper & Scratcherd, 1968*a*) attempts have been made to clarify this somewhat confusing situation in a number of ways. First, the effect of variations in perfusate bicarbonate concentration has been tested, together with the effect of acetazolamide, on pancreatic secretory rate. Secondly, the work of Ball *et al.* (1941) has been repeated using, in place of ^{11}C, the more stable isotope ^{14}C (^{14}C has a half-life of 5760 years whereas that of ^{11}C is 20 min). Lastly, measurements have been made of the pH and P_{CO_2} levels in perfusion fluid before and after passage through the quiescent and secreting gland.

Preliminary accounts of some of the work reported here have already been published (Case, Harper & Scratcherd, 1966, 1968*b* and 1969*b*).

METHODS

General preparation. The saline-perfused preparation of the cat's pancreas used in all the experiments to be reported, has previously been described in detail (Case *et al.* 1968*a*). After the pancreas had been surgically isolated, perfusion fluid was led from a reservoir, through a heat-exchange coil and, by means of a roller pump, was infused into the gland's arterial supply via a cannula in the aorta. The effluent was collected in a retrograde fashion from the superior mesenteric vein, after occluding the portal tract. The standard perfusion fluid, isosmolal with cat plasma, has the

following composition in mM: NaCl 125, KCl 4·3, $NaHCO_3$ 25, $MgCl_2$ 1·0, NaH_2PO_4 1·0, $CaCl_2$ 2·5, and glucose 5. Alterations in the composition of this fluid are described at the appropriate point in the Results section. Oxygen (95 %) and carbon dioxide (5 %) were bubbled through the reservoir of perfusion fluid unless stated otherwise. A bank of four reservoirs allowed rapid changes in the composition of the perfusion fluid.

Collection and analysis. Continuous, maximal stimulation of the pancreas was achieved by infusing secretin (prepared by the method of Crick, Harper & Raper, 1949) into the arterial cannula using a motor-driven syringe. Samples of juice were collected in tared Pyrex tubes and their masses determined gravimetrically. Samples of perfusion fluid were also taken and their osmolality checked on the osmometer (Model G-62, Fiske Associates, Inc.). The bicarbonate concentration of 0·01 ml. samples of pancreatic juice and perfusion fluid was measured using a Natelson microgasometer (Model 600, Scientific Industries, Inc.), which adapts the classical Van Slyke manometric technique (Van Slyke & Neill, 1924) to microanalysis. Since only 1 % of pancreatic juice carbon dioxide exists as the gas (Ball, 1930) the measured carbon dioxide is all assumed to have been released from bicarbonate, and the results expressed as m-equiv HCO_3^-/l. Chloride was determined potentiometrically using 0·05 M silver nitrate (Sanderson, 1952), and sodium and potassium concentrations were measured by flame photometry (Mark II, Evans Electroselenium, Ltd.).

Analyses were performed on the day following the experiment, samples being sealed and stored overnight in the refrigerator, except in the series of experiments involving radioactive bicarbonate, when total bicarbonate estimations were made at the conclusion of the experiment, and radioactivity was counted overnight. When required, effluent from the gland was collected under paraffin oil and the pH and P_{CO_2} determined immediately on a blood gas analyser (Model 48c, Electronic Instruments, Ltd.).

Preparation and counting of radioactive fluids. Sodium [^{14}C]bicarbonate was supplied in aqueous solution, pH 8·8, at a concentration of 1·0 mc/ml. (The Radiochemical Centre). The amount of this solution added to 500 ml. of otherwise normal perfusion fluid varied in different experiments but was never greater than 0·1 ml., thus avoiding over-all changes in pH, osmolality and total bicarbonate concentration. The radioactivity of samples of pancreatic juice and perfusion fluid was measured using a Tri-carb Liquid Scintillation Counter (Model 3003, Packard). It is known that precipitates or turbidity can affect the counting efficiency of liquid scintillation systems. Since, in the experiments to be carried out, the ratio of radioactivity in pancreatic juice, a protein-containing fluid, was to be compared with that in protein-free perfusion fluid, it was important to estimate any difference in the counting efficiency in the two fluids. The scintillation system used was a mixture of 10 % toluene and 90 % β-phenylethylamine, containing 2,5-diphenyloxazole (PPO; 0·4 %) and 1,4-di-[2-(5-phenyloxazolyl)]-benzene (POPOP; 0·004 %). Francis & Hawkins (1967) have claimed that this system gives a homogeneous mixture with protein concentrations of up to 3·2 mg/ml. of scintillator, without loss of counting efficiency. To test the accuracy of counting in pancreatic juice and perfusion fluid, identical amounts of radioactive bicarbonate were added to equal volumes of both fluids, and 0·1 ml. samples of each were mixed with 6·0 ml. of scintillator. (These volumes were found to be optimal and were used throughout, unless otherwise stated.) The mean ($\pm$ S.D.) of ten estimations on perfusion fluid was 49·82 $\pm$ 0·44 c.p.m., while that on pancreatic juice was 49·37 $\pm$ 0·52 c.p.m. The difference between means of 0·45 c.p.m. was just significant ($P < 0·05$). This slightly lower efficiency in pancreatic juice was probably due to the quenching effect of the protein present. To overcome it, a correction factor of 49·82 $\div$ 49·37 (i.e. 1·009) was applied when calculating the ratio between radioactivity in juice and perfusate.

RESULTS

Effect of varying perfusate bicarbonate concentration. In ten experiments the effect of step-wise reductions in the concentration of bicarbonate in the perfusion fluid was tested. Isosmolality was maintained by addition of appropriate amounts of sodium chloride. Predictably, these changes in bicarbonate concentration produced quite large changes in the pH of the fluid. In early experiments such changes were controlled in some measure

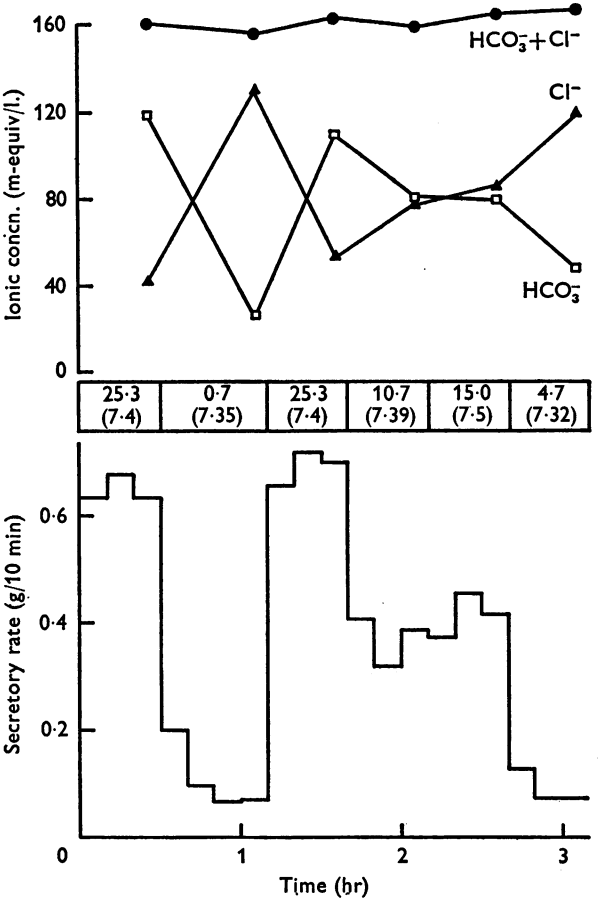

Fig. 1. One experiment illustrating the effect of varying perfusate bicarbonate concentration on the volume and composition of the secretion from the isolated pancreas. The values for perfusate bicarbonate concentration (m-equiv/l.) and pH (in brackets) are shown in the centre of the diagram. The relatively large secretion during perfusion with fluid containing 0·7 m-equiv HCO_3^-/l. is somewhat atypical (see Fig. 2). Sodium and potassium concentrations are not shown; they were constant (mean 158 and 5·2 m-equiv/l. respectively) throughout the experiment.

by adding phosphate buffer to the perfusion fluid. Gassing the solution with 95% oxygen and 5% carbon dioxide was continued, though pure oxygen was used when all bicarbonate was omitted. In later experiments a finer control of pH was achieved by altering the carbon dioxide concentration of the gassing mixture. The required carbon dioxide concentrations were calculated by substituting values of the bicarbonate concentrations in the Henderson–Hasselbach equation. One of these later experiments is illustrated in Fig. 1. Reducing the perfusate bicarbonate concentration

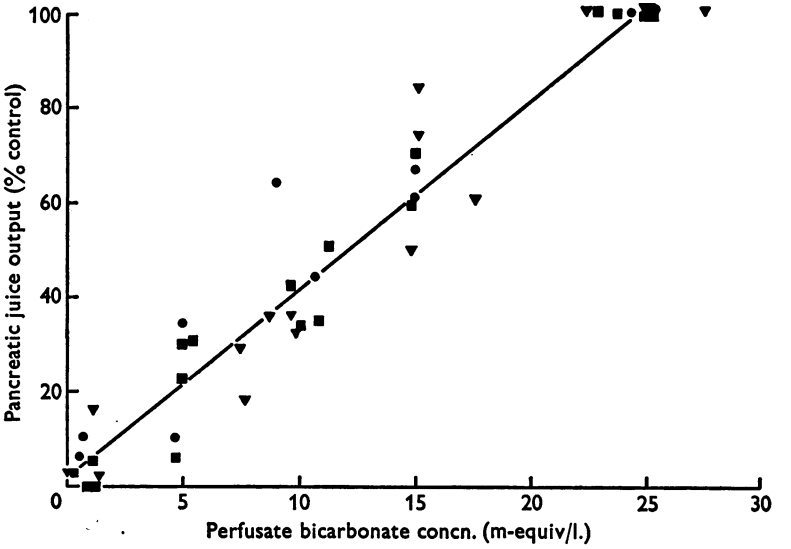

Fig. 2. The effect of perfusate bicarbonate concentration on the rate of secretion from the isolated pancreas. In two experiments (●) the perfusate was of normal composition; in four experiments (■) it contained 6% Dextran and in four others (▼) it contained either 6% Dextran and 2% haemoglobin (three 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 m-equiv/l.). The line is the calculated regression line.

caused a decline in secretion rate, so that the output of pancreatic juice was proportional to the amount of bicarbonate in the perfusion fluid. In agreement with observations we have already published (Case *et al.* 1968*a*), the fall in secretory rate was accompanied by a fall in juice bicarbonate concentration. There was a corresponding rise in the chloride concentration so that the sum of the two anions remained constant. Sodium and potassium concentrations remained constant throughout, and their sum was slightly above that of the anions. This experiment is atypical in one respect: a small secretion was still maintained after virtually all bicarbonate had been omitted from the perfusion fluid. Such omission usually

resulted in complete, or almost complete, cessation of secretion. This can be judged from Fig. 2, the combined observations from all experiments, in which, for comparison, the output of pancreatic juice is expressed as a percentage of control output (i.e. when the perfusate contained 25 m-equiv HCO_3^-/l.). The rate of secretion is linearly related to perfusate bicarbonate concentration, with the calculated regression line passing through a point close to zero on both ordinate and abscissa.

It could be argued that during saline perfusion the metabolism of the gland was lowered as a result of insufficient oxygenation. The gland would then be unable to manufacture bicarbonate from metabolic carbon dioxide. Although, at the high perfusion rates employed, this seems unlikely to us, to overcome this objection the perfusion fluid was modified in most experiments by the addition of either Dextran, to delay the onset of oedema in the gland, or haemoglobin (prepared by the method of D'Silva & Neil, 1954), to increase the oxygen-carrying capacity of the perfusate, or a mixture of both these substances. The identity of these experiments in Fig. 2 is revealed in the legend to that illustration. The behaviour of the gland during perfusion with dextran- or haemoglobin-enriched solutions was apparently no different from that in the remaining two experiments when the perfusion fluid was of normal composition.

Addition of extra bicarbonate (to 35 m-equiv/l.) in three of these experiments had either little or no potentiating effect on secretory rate.

Radioactive bicarbonate secretion. The pattern of radioactive bicarbonate secretion in each of the five experiments comprising this series was very similar. One experiment is illustrated in Fig. 3. After a steady state of secretion had been established, 3 min samples of pancreatic juice were collected before, during and after perfusion with labelled bicarbonate solution. The volume and total bicarbonate concentration were constant throughout, remaining within the range 0·250–0·261 g/3 min and 121·1–122·1 m-equiv/l. respectively. Within 3 min of perfusion with the test solution, the level of radioactivity in the juice was already half maximal. The maximal level was attained after about 9 min perfusion. In a single experiment, a drop-by-drop analysis was performed (0·01 ml. samples of juice and perfusate being counted for 100 min). An increase in juice radioactivity was detected about $1\frac{1}{2}$ min after changeover to radioactive perfusion fluid. As can be seen in Fig. 1, the decline in juice radioactivity on reverting to normal perfusion fluid was equally sharp, equilibrium again being attained in about 9 min.

From observations on the phenomenon of enzyme 'wash-out' (see Case *et al.* 1969*a*) an indirect estimate of the volume of the cat pancreatic duct system can be made. An average value obtained in this way is about 0·5 ml. If this is so, at a secretory rate of 0·25 ml./3 min, approximately 6 min is

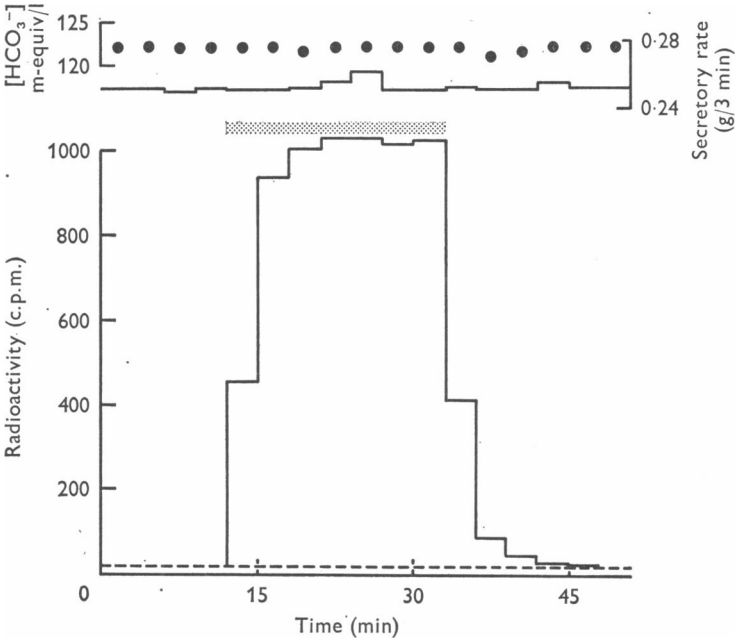

Fig. 3. The composition of pancreatic juice before, during and after perfusion with fluid containing [^{14}C]bicarbonate. The duration of perfusion with radioactive fluid is indicated by the bar. Juice radioactivity is expressed as total channel counting rate, the interrupted line indicating the background channel counting rate. The secretion rate (upper histogram) and total bicarbonate concentration (●) remained constant throughout the experiment.

TABLE 1. Comparison of the ratios of total bicarbonate in juice and perfusate, and of radioactive carbon in juice and perfusate, in five experiments

^{14}C (c.p.m.)			Total HCO_3^- (m-equiv/l.)			Cellular HCO_3^-
Perfusate	Juice	J/P	Perfusate	Juice	J/P	(%)
90	440	4·93	26·9	138·5	5·15	4·3
235	1116	4·79	25·5	127·5	5·00	4·2
215	1007	4·73	24·8	122·0	4·92	3·9
140	580	4·18	26·4	114·5	4·33	3·5
200	938	4·73	25·5	125·5	4·92	3·9
					Mean	3·96
					S.E.	0·14

In each experiment, the level of radioactivity in juice and perfusate is the net channel counting rate, obtained by subtracting the background channel counting rate from the total channel counting rate. The juice: perfusate (J/P) ratio for ^{14}C has been corrected for differences in counting efficiency in pancreatic juice and perfusate (see Methods). Column 7 is a calculation of the amount of bicarbonate not derived from perfusate, which presumably arises from cellular metabolism.

required to clear completely the duct space. It can be seen that, despite any oedema which may have developed in the gland, 6 min after return to normal perfusion fluid the amount of radioactivity in the juice had returned to near control levels.

The observations made in all five experiments are summarized in Table 1. The ratios between radioactivity in juice and perfusate and between total bicarbonate in juice and perfusate have been calculated. It is immediately obvious that there is a four- to fivefold concentration of both radioactive and total bicarbonate in the juice. The ratio of total bicarbonate is always slightly greater than that of radioactive bicarbonate, indicating that a small proportion of pancreatic juice bicarbonate is derived from a source other than circulating bicarbonate. A comparison of the two ratios allows the magnitude of this proportion to be expressed as a percentage of the whole (Table 1, column 7). The mean value so obtained was 4%.

Effect of acetazolamide. Since acetazolamide is known to reduce the output and bicarbonate concentration of pancreatic juice in acute and chronic animals and in man, its effect on the isolated gland has been determined in six preparations. Single injections of 2 mg of the drug resulted in a short-lasting partial inhibition of secretion. In three experiments, the drug was continuously infused at the rate of 0·25 mg/min (i.e. 2×10^{-4}M), a concentration calculated to be approximately equal to the blood concentration produced by injecting doses of 20 mg/kg into a peripheral vein of anaesthetized animals. The inhibition so produced was 59, 75 and 66% respectively, values close to those seen in the anaesthetized cat (where inhibition in six animals was 58 ± S.E. 2·6%; Case *et al.* 1969*a*). Inhibition was maintained only as long as acetazolamide was infused. Following return to normal perfusion fluid, full recovery occurred after about 30 min (Fig. 4). Changes in juice bicarbonate concentration again paralleled those of volume, though, since the bicarbonate concentration tends to decrease with time in this preparation, as we have previously reported (Case *et al.* 1968*a*), return to pre-inhibition bicarbonate concentrations was not complete.

Changes in pH and P_{CO_2} in the effluent from the perfused gland. In five experiments, the pH and P_{CO_2} were measured in perfusion fluid before it entered the gland. Similar measurements were made on effluent from the resting gland, and then later on effluent from the gland stimulated maximally with secretin (Table 2). The fall in pH, and corresponding rise in P_{CO_2}, of the fluid leaving the quiescent gland became more marked when the gland was secreting.

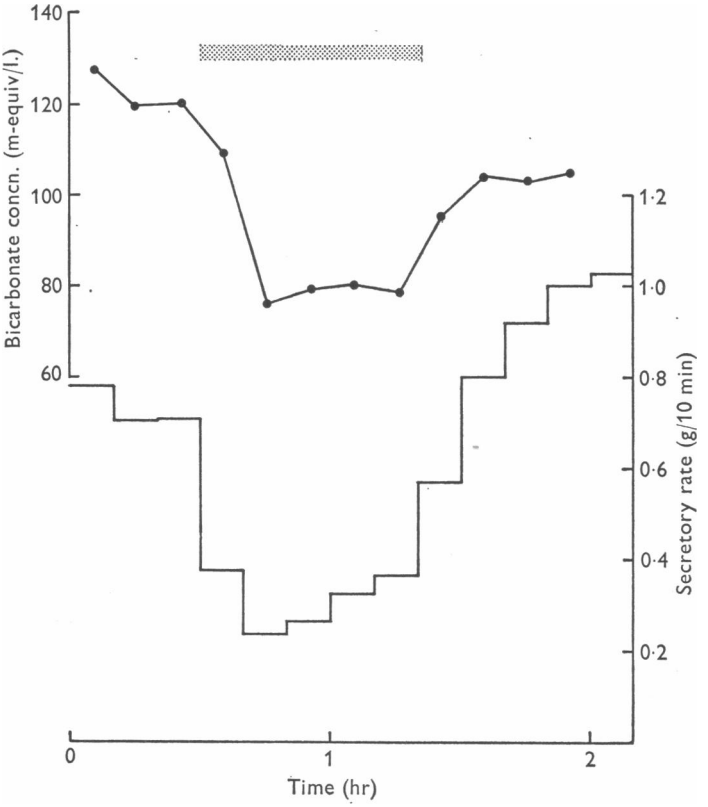

Fig. 4. An example of the effect of acetazolamide on the rate and bicarbonate concentration of secretion from the perfused pancreas. For the duration of the bar, the perfusion fluid contained acetazolamide at a concentration of 2×10^{-4} M.

TABLE 2. Changes in the pH and P_{CO_2} in the effluent from the perfused pancreas produced at rest and during secretion

	pH			P_{CO_2}		
	Control	Resting	Secreting	Control	Resting	Secreting
	7·40	7·30	7·22	33·5	39·0	46·0
	7·45	7·35	7·30	33·0	42·0	47·0
	7·42	7·43	7·30	33·0	33·5	43·0
	7·51	7·25	7·20	32·0	44·5	46·0
	7·44	7·30	7·28	32·0	39·0	44·0
Mean	7·44	7·34	7·26	32·7	40·0	45·2
S.E.	0·019	0·095	0·021	0·20	1·52	0·73

DISCUSSION

The use of perfusion techniques allows a more rigorous investigation of pancreatic secretory mechanisms than is possible in the intact animal. The physiological significance of observations made on the saline-perfused cat pancreas has already been assessed (Case *et al.* 1968*a*).

Reduction in the bicarbonate concentration of the perfusion fluid caused an inhibition of pancreatic secretory rate. The inhibition produced was directly proportional to the perfusate bicarbonate concentration, so that when all bicarbonate was omitted secretion completely, or almost completely, ceased. It seems, therefore, that in the absence of extracellular bicarbonate, metabolic carbon dioxide produced in the secretory cell is sufficient to support only a minimal secretion of pancreatic juice. The amount of secreted bicarbonate derived from this metabolic carbon dioxide can be estimated from Fig. 2 by measuring the intercept of the regression line with the ordinate at zero perfusate bicarbonate concentration. A value of 2·1 % is obtained by this method. These experiments suggest that bicarbonate in the perfusion fluid is the chief source of juice bicarbonate. The observation of Rawls, Wistrand & Maren (1963) and of Pak, Hong, Pak & Hong (1966) imply that plasma bicarbonate concentration can also influence the rate of pancreatic secretion *in vivo*. Both groups of workers showed that, during metabolic acidosis, canine pancreatic secretion was reduced by half when the plasma bicarbonate concentration was lowered to 16 mM, a reduction similar to that observed in this paper. The effect of lowering extracellular bicarbonate concentration on pancreatic secretion *in vitro* has been reported only once previously, by Rothman & Brooks (1965*b*), who used an isolated preparation of the rabbit's pancreas (Rothman & Brooks, 1965*a*). In three experiments, these authors observed an incomplete inhibition of secretory rate when the preparation was bathed in a 'CO_2-free environment' and concluded that about 10 % of pancreatic juice bicarbonate might be derived from metabolic carbon dioxide. The discrepancy between the work reported here and that of Rothman & Brooks may be due to a number of differences in experimental design. First, Rothman & Brooks observed the effects of changing the ionic environment on the spontaneous pancreatic secretion which occurs in the rabbit. There is no such basal secretion in the cat, and in the experiments reported here the pancreas was stimulated maximally throughout using secretin. Secondly, the mild agitation of the bathing solution in which the rabbit pancreas was suspended (Rothman & Brooks, 1965*a*) could result in the formation of unstirred layers around the cells, permitting the accumulation of sufficient intracellular carbon dioxide to provide for a small secretion of bicarbonate. Finally, it is possible that not

all the carbon dioxide had been removed from the solution bathing the rabbit pancreas, since the carbon dioxide concentration of this fluid was not measured (Rothman, 1969).

Support for extracellular bicarbonate as the chief source of pancreatic juice bicarbonate was obtained by labelling the perfusate with [^{14}C]-bicarbonate. When such a labelled solution was perfused through the gland, radioactivity appeared rapidly in the juice. On return to normal perfusate, radioactivity in the juice returned to control levels in the time required to clear the duct space of the gland. The incorporation of the radioactive isotope into organic molecules (which could then be secreted in the juice) seems unlikely to occur in the short time the gland is exposed to the label, and would be contrary to the findings of Ball *et al.* (1941). The ratio between radioactivity in juice and perfusate is very similar to that between total bicarbonate in juice and perfusate, there being four to five times more radioactivity in juice than in perfusate. An accurate comparison of the two ratios in individual experiments reveals that the ratio of radioactivity is no average, 4 % less than that of total bicarbonate. It is suggested that this might represent the contribution of metabolic carbon dioxide to the total secreted bicarbonate.

From experiments in which [^{11}C]bicarbonate was injected intravenously in dogs, Ball *et al.* (1941) concluded that not more than 20 % of pancreatic juice bicarbonate was derived from metabolic carbon dioxide. However, Ball and his colleagues were handicapped in two ways: firstly, because the level of radioactivity in the blood was not constant, as carbon dioxide was being lost, mainly through the lungs, and, secondly, because the half-life of ^{11}C is only 20 min. These inherent difficulties led to a lowering of the accuracy of these experiments, which was assessed by the authors to be in the region of 10 %.

Such difficulties do not arise during perfusion of the cat's pancreas with solutions containing [^{14}C]bicarbonate. One objection is encountered however: from the instant that radioactive bicarbonate is added to the perfusion fluid, the uncatalysed reaction:

$$[^{14}C]HCO_3^- + H^+ \rightleftharpoons [^{14}C]CO_2 + H_2O$$

will occur with a velocity constant of about 0·1 sec^{-1} (Edsall & Wyman, 1958). Radioactive equilibration will be approached exponentially since the backward reaction will also occur with the same velocity constant and a velocity depending on the concentration of radioactive carbon dioxide. It can be calculated that 99 % equilibration will occur within 1 min, so that in fact the pancreas is being perfused by a solution containing both radioactive bicarbonate and radioactive carbon dioxide. It would, therefore, be possible for the latter to exchange with metabolic carbon dioxide

across the contra-luminal cell membrane and thus enter the bicarbonate secretory mechanism. This explanation for the appearance of radioactive bicarbonate in pancreatic juice allows the conclusion that metabolic carbon dioxide could be a major source of secreted bicarbonate. However, from the figures in Table 1 it can be calculated that about 5 μ-mole dissolved carbon dioxide was perfused through the pancreas each minute whilst bicarbonate was secreted at the rate of about 10 μ-mole/min, so that, at the most, only half of the juice radioactivity could be accounted for in this way. This hypothesis is rendered completely untenable for two other reasons: it ignores the fact that secretion ceases when all bicarbonate is omitted from the perfusion fluid and, as will be explained in the discussion that follows, the total quantity of carbon dioxide produced metabolically by the pancreas is much less than that secreted in the juice.

A direct estimate of the proportion of juice bicarbonate derived from metabolism is theoretically possible using clearance techniques, in which the quantity of bicarbonate secreted is compared with the quantity removed from the perfusion fluid. This direct measurement is difficult because, at the high perfusion rates employed, only a very small difference exists between the concentration of bicarbonate in the perfusate and that in the effluent. An alternative method of estimating the probable carbon dioxide production by the gland would be to measure its oxygen consumption. In sixty-nine samples of isolated cat pancreatic tissue, Davies, Harper & Mackay (1949) found a mean Q_{O_2} of $-4{\cdot}27$, a value which increased by 24% on incubating with a 0·2% solution of Crick–Harper–Raper secretin. Similar figures have been reported for mouse pancreas by Dickman & Morrill (1957). Using these figures, and assuming for the small cats used in this study a dry, fat-free pancreatic weight of 1·0 g (mean of eleven cats), the maximum rate of bicarbonate production from metabolic carbon dioxide would be 43 μ-mole/10 min (a respiratory quotient of unity was used in calculating this figure, since it is known that glucose is an essential substrate in the perfused cat pancreas; Case *et al.* 1969*b*). This rate of carbon dioxide production is less than a third of the rate at which it is secreted in the pancreatic juice. A similar conclusion has been reached by Hokin (1967). However, this calculation assumes that all the carbon dioxide produced by the pancreas enters into the secretory mechanism. This seems very unlikely, for both the acinar cells, which constitute the major part of the pancreatic tissue, and the islet tissue would be utilizing oxygen and the carbon dioxide produced by these cells would probably not be available for bicarbonate production by the centro-acinar and duct cells—the major bicarbonate-secreting cells in the pancreas (Schultz, Yamagata & Weske, 1969). If these latter cells are confirmed as the only source of pancreatic juice bicarbonate, it might be argued that they alone are responsible for

the increased oxygen consumption during secretion and that it is this increase in oxygen consumption which should be used to calculate the metabolically available carbon dioxide. Recalculating on this basis, the increased carbon dioxide produced in response to secretin stimulation would only account for some 7 % of the secreted bicarbonate.

Whichever figures are taken for probable carbon dioxide production by the gland, it is clear that metabolic carbon dioxide alone is quite insufficient as a source of pancreatic juice bicarbonate. From this consideration and from the observations described in this paper, it is concluded that the major part of pancreatic juice bicarbonate (perhaps 95 %) is derived from bicarbonate circulating in the perfusate. It is assumed that a similar situation exists in the intact animal.

If circulating bicarbonate acts as the major source of juice bicarbonate, in what form is it transported from the perfusate, or plasma, into the pancreatic cell? The simplest explanation would postulate active transport of bicarbonate across the contra-luminal cell membrane. If this were the case, it is difficult to understand the significance of intracellular carbonic anhydrase (Becker, 1962) if, as we have suggested, metabolic carbon dioxide is of little importance as a source of secreted bicarbonate. In the perfused cat pancreas, acetazolamide inhibits pancreatic secretion, just as it does in the intact gland (Case *et al.* 1969*a*). Although acetazolamide is rather a specific carbonic anhydrase inhibitor, it can affect sodium secretion indirectly, perhaps by changing the intracellular pH (Slegers & Moons, 1968). Furthermore, there have been suggestions (Parsons, 1956; Kinney & Code, 1964; Kitahara & Imamura, 1966) that acetazolamide may inhibit chloride transport by mechanisms other than interference with carbonic anhydrase. Kitahara, Fox & Hogben (1967) have offered proof of this supposition by demonstrating that active chloride transport across frog cornea can be inhibited by acetazolamide, despite the complete absence of carbonic anhydrase in this tissue. Such an explanation for the role of acetazolamide seems unlikely in the pancreas where the presence of chloride ions is not an essential factor in the secretion of bicarbonate (Case *et al.* 1968*b*, 1969*b*).

Clearly it is possible that inhibition of pancreatic secretion by acetazolamide could occur independently of an action on carbonic anhydrase. The observations on pH and P_{CO_2} of the effluent from the perfused gland allow a more credible function to be attributed to carbonic anhydrase. The fall in pH observed during secretion is compatible with the active removal of bicarbonate from the perfusate and its passage into the cell (Brodsky & Schilb, 1967), but the concomitant rise in P_{CO_2} indicates that hydrogen ions pass from the cell into the perfusate, where they act on bicarbonate to liberate carbon dioxide. This carbon dioxide could then diffuse into the

secreting cells to be rehydrated, partly under the influence of carbonic anhydrase, as viewed by Maren (1967). The outward movement of hydrogen ions might be linked with the inward movement of sodium in an exchange process across the contra-luminal cell membrane. This hypothesis is in accord with the views of Davies (1949) who suggested that the primary reaction in the pancreatic cell might be the separation of hydrogen and hydroxyl ions from water, the former leaving the cell in exchange for sodium ions and reacting with bicarbonate to produce carbon dioxide, which, in turn, would re-enter the cell and combine with the hydroxyl ions to form bicarbonate. This combination would then create the necessary diffusion gradient for the continued entry of carbon dioxide into the cell.

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