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WATER AND ELECTROLYTE SECRETION BY THE PERFUSED PANCREAS OF THE CAT

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SUMMARY

1. A technique is described for perfusing the isolated cat pancreas with saline solutions.

2. Single doses of secretin, although present in the perfusate for only a short time, caused a prolonged flow of pancreatic juice.

3. In response to continuous secretin infusion, the preparation secreted for up to 6 hr a juice which was similar to that obtained *in vivo*, with the exception that the bicarbonate concentration decreased and the chloride concentration increased with time, even when the rate of secretion remained constant.

4. The osmolalities of perfusate and secretion were identical over a range of 450 m-osmoles/kg, but the electrolyte concentration of the secretion was always slightly higher than that of the perfusate. Variations from perfusate isosmolality produced inverse changes in the secretion rate, over the range from 600 m-osmoles/kg, at which secretion ceased, to 150 m-osmoles/kg, at which the rate was highest. At perfusate osmolalities below 150 m-osmoles/kg secretion rapidly declined.

5. Reduction in perfusate sodium chloride concentration, isosmolality being maintained with sucrose, caused a fall in secretion rate, but the sodium concentration of the juice remained constant until perfusate sodium concentration was reduced to about 70 m-equiv/l. Below this level it declined and sucrose was detected in the juice in quantities almost sufficient to account for the equiosmolality of juice and perfusate.

6. Two hypotheses about the mechanism of water and electrolyte secretion by the pancreas are presented.

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INTRODUCTION

In the intact animal the osmolality of pancreatic juice and blood plasma are the same, the concentrations of sodium and potassium are approximately equal in the two fluids, and alterations in plasma sodium, potassium or total osmolality are rapidly reflected in the juice (Ball, 1930). As the scope for investigating the mechanism of ionic secretion is limited in the intact animal, a method has been developed of perfusing the isolated pancreas of the cat with saline solutions so that the gland can be exposed to a wide range of osmotic pressure gradients and electrolyte concentrations.

Although studies have often been made on saline-perfused organs in the investigation of secretory processes (see Renkin, 1962), little use has been made of the technique in the study of pancreatic secretion. Babkin & Starling (1926) do not seem to have applied their method of perfusing the dog's pancreas in conjunction with a heart–lung preparation, and Goldstein (1928) recorded only the volume and enzyme content of the secretion from the perfused gland. More recently Nardi, Greep, Chambers, McCrae & Skinner (1963) investigated the actions of secretin and pancreozymin on the canine pancreas, perfused *in situ* with whole blood, and reported that the electrolyte concentrations in the juice were within the range of those observed in unanaesthetized dogs with pancreatic fistulae. A different approach to the *in vitro* study of pancreatic secretion has been described by Rothman (1964) and Rothman & Brooks (1965*a*, *b*, 1966) who were able to maintain secretion by the thin pancreas of the rabbit, immersed in a saline-filled organ-bath.

Preliminary accounts of the work described in this paper have already been published (Case, Harper & Scratcherd, 1966*b*, 1967).

METHODS

The experiments were carried out on unfed anaesthetized cats. Anaesthesia was induced with ether, and maintained by intravenous chloralose (37·5 mg/kg) and urethane (450 mg/kg).

The circulation through the isolated pancreas was maintained by pumping saline solution through the coeliac and superior mesenteric arteries and collecting the effluent from a retrograde catheter in the superior mesenteric vein (Fig. 1). To isolate the pancreas the inferior mesenteric artery and vein were ligated, and the superior mesenteric artery and vein tied distal to the inferior pancreaticoduodenal artery, which supplies part of the duodenum and pancreas. This devascularized the gut beyond the portion of duodenum related to the head of the pancreas. The vessels were cut and the devascularized parts of the small and large bowel removed. The spleen was removed after section of its vascular supply close to the hilum, care being taken to avoid interference with the blood supply to the tail of the pancreas from the splenic artery. The blood supply to the stomach was cut off by tying the left and right gastric arteries, the gastric branches of the splenic artery, and the branches of the gastroduodenal artery other than the superior pancreaticoduodenal vessel supplying the

pancreas and duodenum. Finally the vascular branches to the duodenum from the vessels lying between the duodenum and the head of the pancreas were ligated. At this point, when the pancreas had been isolated, apart from its arterial supply from the coeliac and superior mesenteric arteries and its venous drainage to the portal vein, a narrow-bore stainless-steel cannula was inserted into the pancreatic duct. Next a length of polythene tubing, of as wide a bore as practicable and filled with heparinized saline solution, was inserted into the abdominal aorta in the direction of the heart, and tied in place with its tip just distal to the opening of the superior mesenteric artery. The lumbar arteries, which usually leave the aorta

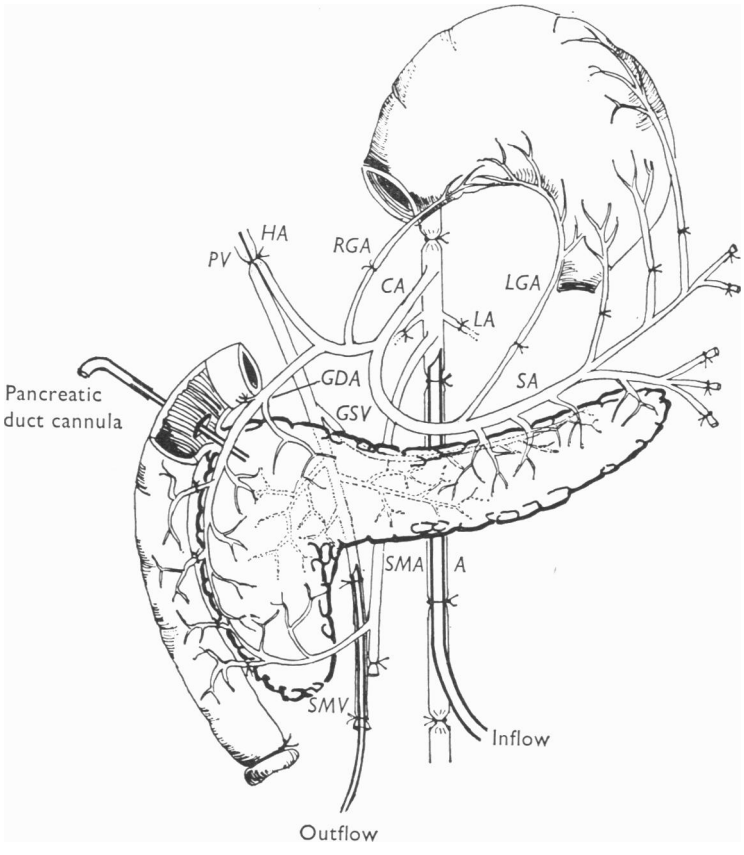

Fig. 1. Vascular supply of the cat pancreas. For clarity, the stomach is shown separated from the duodenum, displaced anteriorly and turned through 180°. *A*, aorta; *CA*, coeliac axis; *GDA*, gastro-duodenal artery; *GSV*, gastro-splenic vein; *HA*, hepatic artery; *LA*, lumbar artery; *LGA*, left gastric artery; *PV*, portal vein; *RGA*, right gastric artery; *SA*, splenic artery; *SMA*, superior mesenteric artery; *SMV*, superior mesenteric vein.

between the tip of the tube and the coeliac axis, were ligated. Polythene tubing was also used to cannulate the superior mesenteric vein. The aorta was then tied above the coeliac axis, and perfusion commenced through the aortic cannula. Finally the portal tract was ligated, thus diverting all the effluent from the pancreas through the cannula in the superior

mesenteric vein. After perfusion was established the animal was killed, but for convenience the pancreas was left *in situ*.

The standard perfusion fluid, isosmolal with cat plasma, had the following composition in m-equiv/l.; NaCl 125, KCl 4·3, $NaHCO_3$ 25, MgCl 1·0, NaH_2PO_4 1·0, $CaCl_2$ 2·5 and glucose 5. Alterations in the composition of the fluid are described at the appropriate points in the Results section. In experiments in which the concentration of sodium chloride was reduced it was found that calcium tended to precipitate from the solution unless phosphate was omitted from its composition. Omission of the phosphate had no effect on the rate of

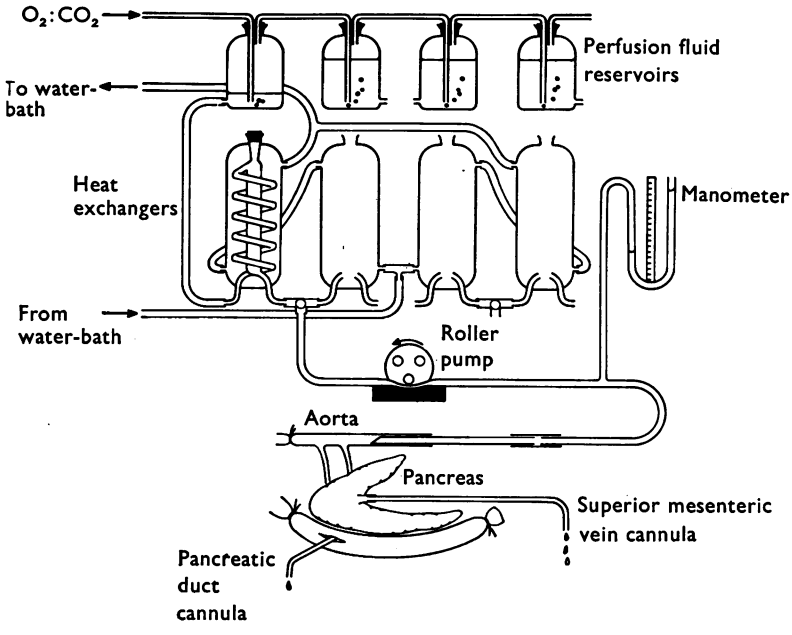

Fig. 2. The perfusion apparatus.

secretion. In a few experiments Dextran B was added to the perfusion fluid to a concentration of 6 %. The fluid was made up on the day of the experiment and filtered through Whatman no. 2 paper before use. Glucose and bicarbonate were weighed out for each experiment, and the other components added from concentrated stock solutions.

Oxygen (95 %) and carbon dioxide (5 %) were bubbled through the reservoir of perfusion fluid, which had a pH of 7·6. From the reservoir the fluid entered a heat-exchange coil maintained at 38° C by circulating water from a thermostatically controlled bath, the temperature of the fluid entering the gland being 33–35° C. The heated solution was delivered by a roller pump to the arterial cannula at a rate of 4–6 ml./min and a pressure of 30 mm Hg. A bank of four reservoirs allowed of rapid changes in the composition of the perfusion fluid (Fig. 2).

Pancreatic secretion was stimulated by administration of secretin prepared by the method of Crick, Harper & Raper (1949). In a few experiments the secretin was given as single rapid injections, but usually it was infused continuously into the arterial cannula by a motor-driven syringe. Samples of juice were collected in weighed Pyrex tubes, which were then reweighed, sealed and stored in the refrigerator. The osmolality of the secretion and of the perfusion fluid were determined on the Fiske osmometer, and their sodium and potassium concentrations measured on an EEL flame photometer. A Natelson microgasometer was

used for bicarbonate estimations by the method of Van Slyke & Neil (1924), and chloride was titrated potentiometrically with 0·05 M or 0·01 M silver nitrate (Sanderson, 1952). Sucrose was estimated by an adaptation of the method of Roe, Epstein & Goldstein (1949). The results are expressed as means ± S.E. of the mean (number of measurements). The term 'isosmolal' indicates solutions of the same osmolal concentration as cat plasma, and 'equiosmolal' describes the identity of osmolal concentrations of pancreatic juice and perfusion fluid over a wide range of osmolalities.

RESULTS

In five animals the response of the pancreas to single injections of secretin, injected rapidly into the arterial cannula, was measured. Over a dose range from 0·01 to 0·15 mg the volume of juice bore an approximately linear relationship to dose (Fig. 3). As in anaesthetized animals, given similar injections into a peripheral vein, the secretion rate reached a maximum in the first two or three drops and thereafter declined. In the perfused pancreas the decline was more gradual, and the total duration of the response much longer than in the intact animal. In all other experiments secretin was administered by continuous infusion into the arterial cannula, at a rate, usually 10 μg/min, somewhat greater than that required to produce a maximal rate of secretion during isosmolal perfusion. The dose was of course much less for the restricted circulation of the perfused gland than that required when secretin was infused into a peripheral systemic vein in an intact preparation.

Pancreatic secretion during isosmolal perfusion. In fifteen experiments the mean concentration of sodium in the juice was 153·3 ± 1·2 and of potassium 4·43 ± 0·075 m-equiv/l. These concentrations were independent of the rate of secretion and were sustained throughout the experiment. In all experiments the sodium concentration in the secretion exceeded that of the perfusion fluid. In eleven experiments the potassium concentration was greater than that of the perfusate and in two it was less; in the remaining two experiments the concentrations were equal. The mean concentrations in the perfusion fluid were 143·7 ± 1·3 for sodium and 4·15 ± 0·07 for potassium. The difference was highly significant for sodium (11·60 m-equiv/l.; $P < 0·001$) but not for potassium (0·28 m-equiv/l.; $P > 0·2$).

The sum of the major anions of the juice, bicarbonate and chloride, remained constant, 163·75 ± 1·7 m-equiv/l. (10). This was significantly greater ($P < 0·05$) than the total anions of the perfusate, and slightly exceeded the sum of the two measured cations of the juice, 159·7 ± 1·2 m-equiv/l. (15). In contrast to the cations the concentration of the anions varied with the rate of secretion. In twenty-eight experiments forty observations were made on the bicarbonate and chloride concentrations

during the first hour of collection, and the results at different flow rates/10 min were grouped as slow (< 0·3 g), fast (> 0·9 g) and intermediate (0·31–0·6 g and 0·61–0·9 g) (Fig. 4). At the fast rate the bicarbonate was

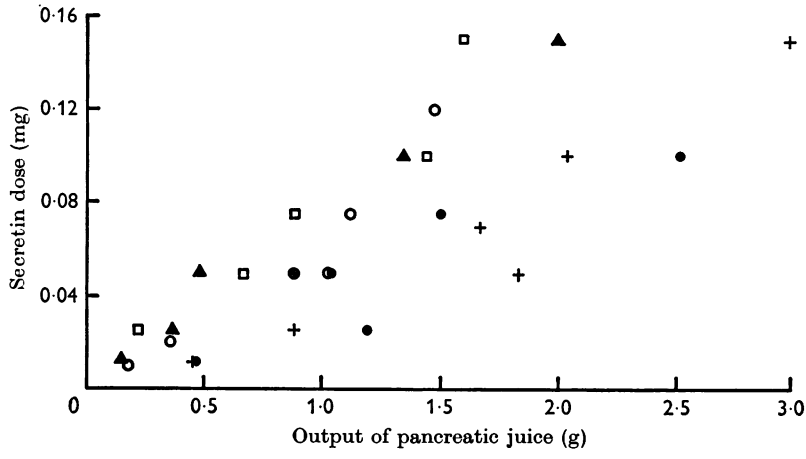

Fig. 3. The output of pancreatic juice from the perfused gland in response to single injections of different doses of secretin, in five experiments, represented by different symbols.

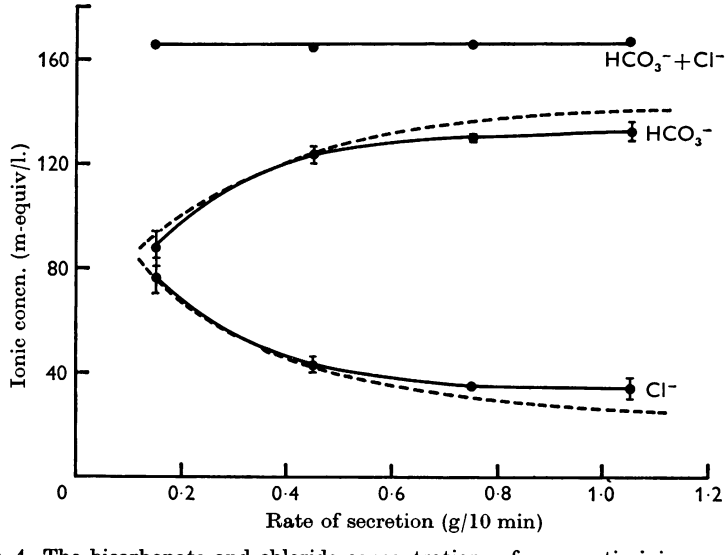

Fig. 4. The bicarbonate and chloride concentrations of pancreatic juice secreted by the perfused pancreas. Observations were made during the first hour of perfusion and were grouped into four secretory rates. The points represent the mean (±S.E.) of each group, which contained, from the slowest rate upwards, 4, 13, 16 and 7 observations respectively. The dashed lines represent the concentrations of bicarbonate and chloride in pancreatic juice secreted by anaesthetized cats (Case *et al.* 1966*a*).

high (133·3 ± 3·4 m-equiv/l.) and the chloride low (34·3 ± 3·8 m-equiv/l.). At the slow rate the bicarbonate had fallen to 87·8 ± 6·9 m-equiv/l. and the chloride risen to 77·1 ± 6·7 m-equiv/l., figures which were significantly different from those at all other flow rates ($P < 0{\cdot}001$). The concentrations over the whole range differed only slightly from those in the intact cat, in which the inverse relationship of bicarbonate and chloride concentrations has already been described (Case, Harper & Scratcherd, 1966*a*).

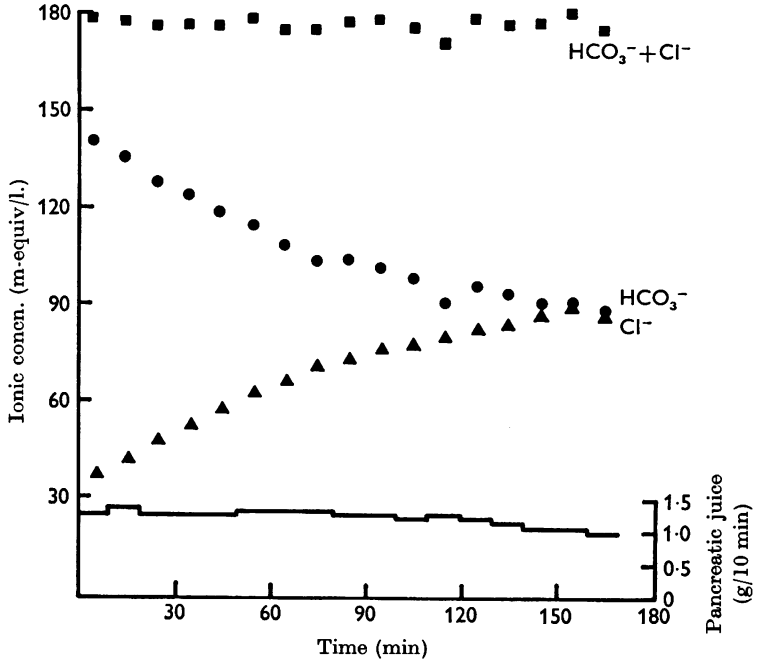

Fig. 5. An example of the variation in the bicarbonate and chloride concentrations of pancreatic juice, secreted at maximal rate.

The secretion of the perfused and intact glands differed in that over a period of several hours of perfusion there was a gradual fall in bicarbonate and increase in chloride concentration, even when the rate of secretion remained constant (Fig. 5). This may be related to the development in the perfused gland of oedema, which can be detected by eye after 1½–2 hr and is marked after 4–5 hr perfusion. In the five experiments in which single rapid injections of secretin were give, Dextran B was added to the perfusate in 6% concentrations in an attempt, only partly successful, to minimize the development of oedema, which could have affected the concentration of secretin reaching the pancreatic cells. It should however be emphasized that, despite the oedema, the bicarbonate concentration in the

juice after 3 hr of perfusion was usually about 4 times that in the perfusate, and even higher in the experiments in which Dextran B was added to the perfusate.

Effect of reduction of sodium chloride concentration and osmolality of the perfusate. If the osmolality of the perfusate was decreased by graded reductions in its sodium chloride content there were corresponding increases in the rate of secretion (Fig. 6). The range over which there was an

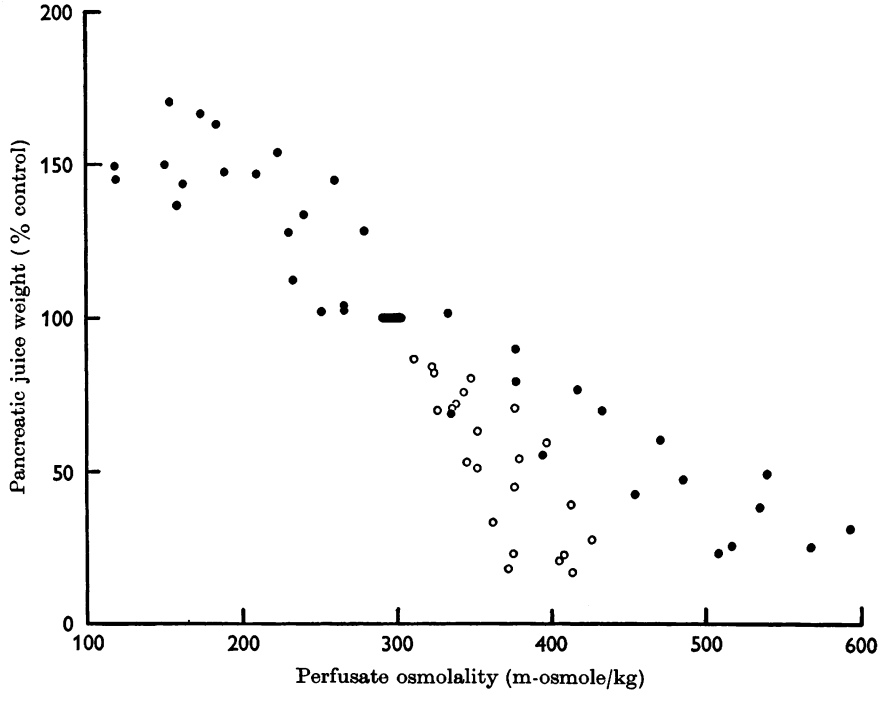

Fig. 6. The relationship of perfusate osmolality and rate of secretion. Secretory rate is expressed as a percentage of that obtained when perfusing with solutions of 300 m-osmole/kg, represented by the bar. Decreases in osmolality were achieved by omission of appropriate amounts of sodium chloride (5 experiments); increases were obtained by addition of either sodium chloride (4 experiments, ●) or sucrose (5 experiments, ○) to the perfusate.

inverse relationship between perfusate osmolality and secretion rate varied in different experiments, but on average in five preparations it was maintained down to a sodium concentration of 75 m-equiv/l. Further reduction of the sodium concentration and osmolality was accompanied by a decrease and eventual cessation of flow. Over the whole range the concentration of sodium in the secretion ran parallel with, but was slightly greater than, that in the perfusate. Although the sum of the measured ions

in the secretion exceeded that of the perfusate, the osmolalities of secretion and perfusate were almost identical (Fig. 7, 8).

Effect of reduction of sodium chloride concentration in an isosmolal perfusate. To dissociate the effects of reduction of sodium chloride and of osmolality, glands were perfused with solutions containing reduced amounts of sodium chloride, but maintained isosmolal by addition of sucrose. In eight experiments the secretion rate decreased in proportion to

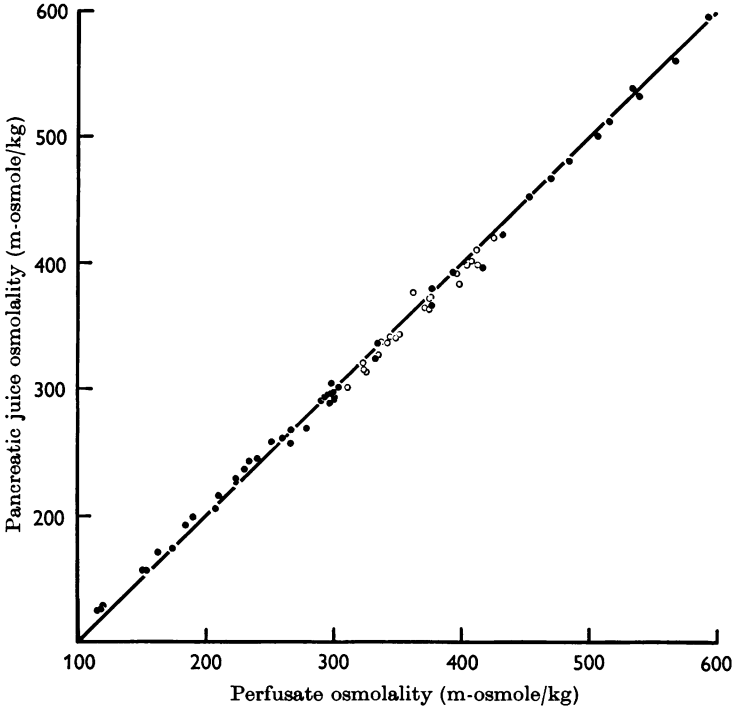

Fig. 7. The relationship of perfusate osmolality and the osmolality of pancreatic juice. The observations are from the same experiments as in Fig. 6. The straight line is the line of 45° slope.

the reduction of the sodium concentration in the perfusate and ceased at concentrations of about 30–40 m-equiv/l. Both the relationship of flow rate and perfusate sodium concentration and the concentration at which secretion ceased appeared to be independent of the initial rate of flow obtained with a perfusate containing the normal (150 m-equiv/l.) concentration of sodium (Fig. 9). Although in these circumstances the rate of secretion decreased, its sodium concentration remained constant at about 160 m-equiv/l. until the perfusate sodium concentration was lowered to 75 m-equiv/l. Below this level, the sodium concentration as well as the

rate of flow of juice declined. Over the whole range of sodium concentrations the potassium concentration of the perfusate was maintained constant. The potassium in the juice, however, showed a steady increase from a concentration of 4·2 m-equiv/l. with a perfusate sodium of 150 m-equiv/l. to a maximum of 12 m-equiv/l. when the sodium had been reduced

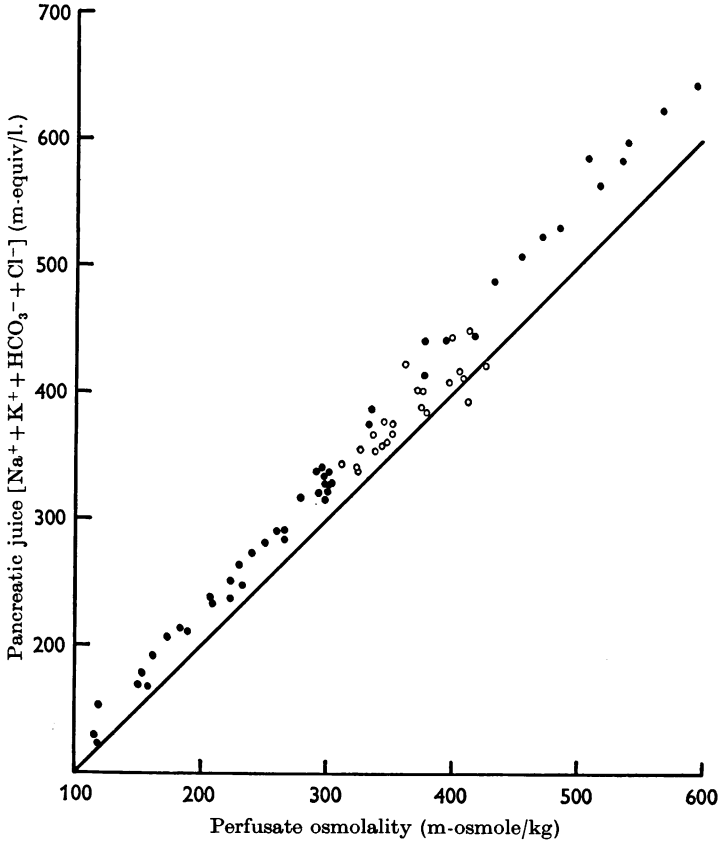

Fig. 8. The relationship of perfusate osmolality and the ionic concentration of pancreatic juice. The observations are from the same experiments as in Fig. 6. The straight line is the line of 45° slope.

to 40 m-equiv/l. (Fig. 10). In these experiments, as in those in which the perfusate osmolality was reduced, the osmolalities of the juice and perfusate were identical. In isosmolal perfusions, when the perfusate sodium concentrations were less than 70 m-equiv/l. and the sum of the measured ions in the juice was reduced, sucrose was found in the juice in quantities almost sufficient to account for its isosmolality with the perfusion fluid (Fig. 10).

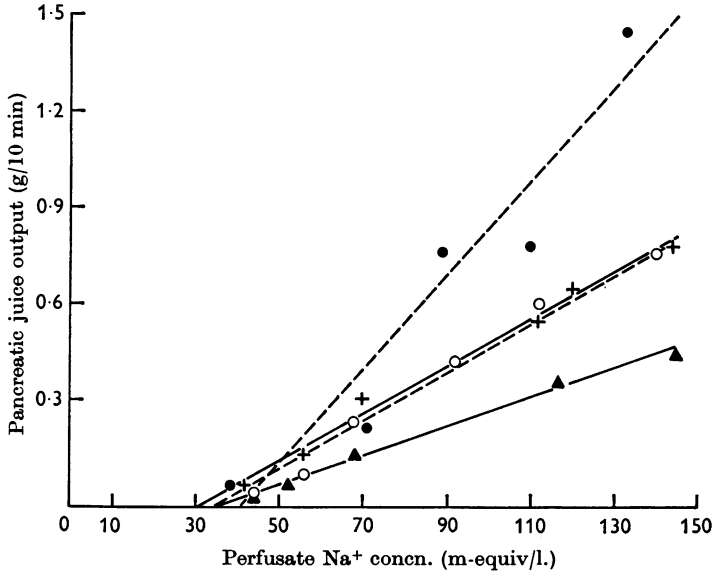

Fig. 9. The effect of lowering the perfusate sodium chloride concentration on the rate of secretion in four experiments. The perfusate was maintained isosmolal by the addition of appropriate amounts of sucrose. The slopes for each experiment are calculated regression lines.

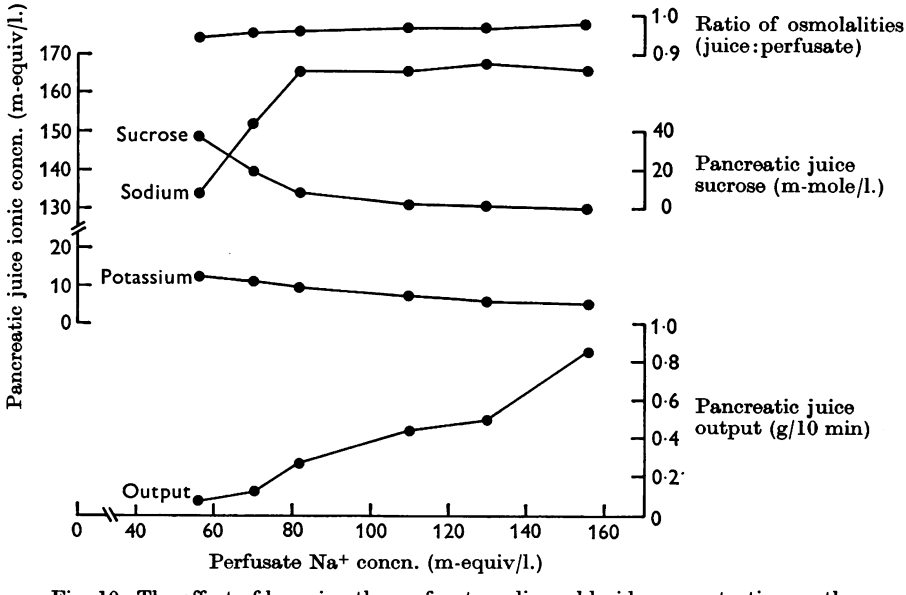

Fig. 10. The effect of lowering the perfusate sodium chloride concentration on the rate of secretion and composition of pancreatic juice. Perfusate isosmolality was maintained by addition of osmotically equivalent amounts of sucrose.

Effect of increasing the osmolality of the perfusate. The effect on the rate and composition of pancreatic secretion of increasing the osmolality of the perfusion fluid was investigated by adding various amounts of sodium chloride or sucrose to the fluid. The rate of secretion decreased in linear fashion when the perfusate osmolality was increased by addition of sodium chloride and the depression was even more marked when sucrose was added to the perfusion fluid (Fig. 6). In these experiments also the osmolality of the secretion mirrored that of the perfusate (Fig. 7). When sucrose was added to the perfusate there was a corresponding increase in the sodium of the juice, so that for every 2 m-moles/l. of sucrose 1 extra m-equiv/l. each of sodium and anion appeared in the juice (Fig. 8).

DISCUSSION

To justify attributing physiological significance to observations on the isolated perfused pancreas one must demonstrate similarities between the behaviour of the intact and the isolated gland. The strongest evidence for according 'physiological status' to the isolated pancreas preparation is the high concentration of bicarbonate in the secreted juice. At high rates of secretion, during the first hour or two of perfusion, the bicarbonate concentration is only a few m-equiv/l. less than in the intact anaesthetized cat, and the inverse relationship of bicarbonate and chloride concentrations over a range of secretion rates is very similar in the isolated and intact preparations.

Although the concentration of bicarbonate in the juice secreted by the isolated gland is several times that in the perfusate after 5 or 6 hr of secretion, it is nevertheless true that the bicarbonate concentration gradually falls and the chloride concentration rises during the progress of an experiment, even when the secretion rate remains high. This evidence of gradual deterioration in the isolated preparation may be related to the development of oedema, which will lengthen the diffusion paths for nutrients entering and metabolites leaving the pancreatic cells. The extent and rate of development of oedema is very variable but it is usually visible to the eye after $1\frac{1}{2}$ hr of perfusion. As it is greatly enhanced by venous obstruction, it is a point of practical importance in making the preparation to avoid any obstruction to the venous outflow from the pancreas. In the intact animal anoxia depresses pancreatic secretion (Hartiala, 1951), and this is equally true of the isolated preparation. Secretion rapidly fails if the perfusion rate is slowed, or oxygenation of the perfusion fluid is omitted. The addition of Dextran B to the perfusion fluid delays both the onset of oedema and the decrease in bicarbonate concentration of the juice.

The pancreas of the cat, whether isolated or in the intact anaesthetized preparation, does not secrete unless secretin is administered. In the isolated pancreas small amounts of secretin have a much more prolonged secretory effect than in the intact animal. Nardi *et al.* (1963) have made a similar observation on the isolated pancreas of the dog, and this may indicate that the *in vivo* inactivation of secretin occurs at some extra-pancreatic site.

Although the volume of juice in response to a constant level of secretin stimulation was increased by reducing the osmolal concentration of the perfusing fluid below that of blood, and decreased by hyperosmolal concentrations, the osmolal concentrations of juice and perfusate were equal over the whole range from 150 to 600 m-osmoles/kg. Nevertheless, over this range the sum of the measured cation ($Na^+ + K^+$) and anion ($HCO_3^- + Cl^-$) concentrations was greater in the juice than in the perfusate. When the gland was perfused with isosmolal saline the sodium concentration in the juice significantly exceeded that in the perfusate by 11·6 m-equiv/l., and a similar relationship held when the osmolality of the perfusate was altered by decreasing or increasing the sodium chloride concentration of the perfusate. If the perfusate was kept at isosmolal concentrations by replacing varying amounts of sodium chloride by sucrose, or made hyperosmolar by addition of sucrose, the sodium concentration in the juice greatly exceeded that in the perfusate.

An excess of sodium in the juice over that in the nutrient fluid was reported by Rothman & Brooks (1965*a*) in the isolated rabbit pancreas, and Ball (1930) found that juice collected from the intact canine pancreas contained a higher concentration of sodium than the plasma. This difference disappeared if the sodium concentrations were expressed as m-equiv/kg plasma water, and Ball concluded that the discrepancy in the blood and juice concentrations was related to the high protein content of plasma. This explanation is not relevant to our results, since protein is absent from the perfusate.

There is an apparent anomaly between the equiosmolality of juice and the excess of measured electrolytes in the juice. In part the excess may represent the glucose and the calcium, magnesium and phosphate ions present in the perfusate, to which the pancreas has been shown to be relatively impermeable (Solomon, 1952). This concept is supported by the observation that sucrose added to the perfusate does not normally appear in the juice, which however contains additional measured electrolytes, sufficient to balance the sucrose exactly and maintain equal osmolality. A similar phenomenon has been observed on the gall-bladder by Diamond (1964). The equality of the osmolal concentrations of juice and perfusate has been determined by measuring the depression of freezing point. To check these measurements by calculation from the chemical composition

of the solutions is impracticable because the osmotic coefficients of the various ions in such mixtures are unknown. It has been shown that the osmotic coefficient of a sodium bicarbonate solution is considerably less at the high juice concentration than at the lower perfusate concentration. The alteration in the osmotic coefficient of a sodium chloride solution between juice and perfusate concentrations is appreciably less (Bayliss, 1959). If these measurements can be applied to mixtures of bicarbonate and chloride, the equiosmolality of juice and perfusate may in part be due to the fact that the juice contains bicarbonate at a higher concentration but a lower osmotic coefficient.

The parallel changes in the osmolal concentrations of perfusate and juice in the isolated pancreas have also been observed in the intact pancreas of anaesthetized animals (De Zilwa, 1904; Ball, 1930; Gilman & Cowgill, 1933). Gilman & Cowgill found that the equiosmolality of blood and pancreatic juice was maintained when they varied the blood concentration over a range of 80 m-osmoles/l. by injections of hypertonic saline or water. We have confirmed their results and extended the range of observations to 450 m-osmoles/kg. Although the pancreas is surprisingly resistant to changes in perfusate osmolality there are limits to its endurance. With increased osmolality of the perfusate the volume of secretion in response to the same level of secretin stimulation progressively decreases and almost ceases at 400 m-osmoles/kg when the osmotic load is increased by addition of sucrose, and at 600 m-osmoles/kg when sodium chloride is added. Osmotic stress produced by reduction of perfusate sodium chloride is accompanied by an increase in the volume of juice down to a concentration of 75 m-equiv/l. sodium. Below this the volume decreases and secretory function fails.

Even when the osmolality of the perfusate is maintained constant by addition of sucrose, 75 m-equiv sodium/l. seems to be the lowest perfusate concentration at which a constant sodium concentration can be maintained in the juice. With further reduction the sodium concentration of the juice falls and secretion ceases at sodium perfusate concentrations of 30–40 m-equiv/l. It is interesting, but possibly only a coincidence, that this is slightly below the mean intracellular sodium concentration of pancreatic tissue, 43·6 m-equiv/l. intracellular water (Clark, Harper & Scratcherd, 1961). The progressive increase in the potassium concentration of the juice in these experiments may be an indication of increasing stress on the primary secretory mechanism. The replacement of sodium in the juice by sucrose at perfusate sodium concentrations less than 75 m-equiv/l. was unexpected, as sucrose does not normally appear in the juice. Ussing (1966) has presented evidence of an active inward transport of sucrose by frog skin from an outer hypertonic medium, but Franz & Van

Bruggen (1967) claim that sucrose is confined to the extracellular space and traverses the skin passively by intercellular pathways. Either concept could be used to explain the appearance of sucrose in pancreatic juice. It is possible that when the secretory elements are subjected to extreme changes in external sodium concentration, the permeability of the contraluminal cell membrane is so altered that sucrose may enter and be actively secreted. Alternatively the sucrose may diffuse passively between the secretory or duct cells into the lumen.

To explain the striking parallelism in the perfused pancreas between juice and perfusate osmolality over such a wide range of total osmolal and sodium chloride concentrations two hypotheses may be advanced. The secretory tissue of the pancreas may, over the whole range of perfusate osmolality, produce a fluid of constant composition, isosmolar with cat plasma and containing mostly sodium bicarbonate with possibly small amounts of potassium and chloride. This primary secretion would then be modified by equilibration with the perfusate across the membrane of the duct system. Alternatively the secretory cells may produce a secretion which mirrors the perfusate osmolality without subsequent fluid transfer across the ductal epithelium.

The variations in the electrolyte composition of pancreatic juice with rate of flow *in vivo* can be accounted for on the assumption that the secretory tissue produces a fluid, isosmolar with plasma, which is modified by a process of passive interchange across the pancreatic ducts of chloride from the high concentration in the extracellular fluid and bicarbonate from the duct lumen. The extent of this exchange varies inversely with the rate of flow of juice (Harper, 1968). The inverse relationship between bicarbonate and chloride in the juice at low rates of flow, which we have observed both in the intact gland (Case *et al.* 1966*a*) and in the perfused pancreas, is consistent with this hypothesis.

If, in addition to the concepts of a primary secretion of constant electrolyte concentration and passive interchange across the duct walls, one postulates that the volume of the primary secretion is depressed when the milieu of the secretory cells is altered either by changes in total osmolality or in sodium chloride, it is possible to explain most, but not all, of our experimental findings. The changes in volume of juice can be accounted for by water movement across the ducts resulting from differences between the osmolal concentrations of the constant primary secretion and the varying perfusate. The differences in concentration of bicarbonate and chloride concentrations in the primary secretion and in the perfusate are such as to bring about the required changes in bicarbonate and chloride concentrations in the juice by passive diffusion across the ducts. It is not, however, possible to explain the sustained higher concentration of sodium

in the juice with isosmolal perfusates containing a low sodium concentration, or the replacement of sodium by sucrose in extreme reductions of sodium in an isosmolal perfusate. These observations would require additional postulates of a ductal impermeability to sodium and sucrose, and possibly an active sucrose secretion in certain circumstances.

The alternative hypothesis is that the primary pancreatic secretion, still largely a bicarbonate solution, always has the same osmolality as the perfusate. Changes in volume of the juice do not result from osmotically determined water movement across the ducts but reflect changes in the volume of the primary secretion secondary to alterations in its osmolality. Such a mechanism has already been demonstrated by Diamond (1964), who found that over a wide range of osmolalities the fluid transported across the wall of the isolated rabbit gall-bladder had the same osmolal concentration as that in the lumen of the organ. If the pancreatic secretory elements behave like other biological membranes in which water movement is a passive process secondary to solute transport, the volume of secretion will be determined by the frictional resistance of the cell membrane, which varies directly with changes in the osmolality of the cellular milieu (Diamond, 1966). This concept could explain our observation of the inverse relationship between pancreatic juice volume and perfusate osmolality. The failure of secretion at perfusate concentrations less than 150 m-osmole/kg probably indicates the limit of endurance of the secretory process.

The decrease in the rate of secretion when sodium chloride is replaced by sucrose in an isosmolar perfusate, and the more rapid decline in secretion when perfusate hyperosmolality is attained by addition of sucrose rather than sodium chloride may be explained by the need for an adequate external supply of electrolyte for the primary solute pump. An inadequate supply would depress the primary secretion with a secondary effect on the passive transport of water. On the evidence at present available it is not possible to decide between these two hypotheses, but their validity may be tested by applying to the isolated perfused pancreas preparation the technique of ductal perfusion which we have already used on the intact pancreas (Case *et al.* 1966*a*).

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