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THE SECRETION OF PANCREATIC JUICE IN RESPONSE TO STIMULATION OF THE VAGUS NERVES IN THE PIG

By J. C. D. HICKSON
From the Physiological Laboratory, Cambridge

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SUMMARY

1. The secretory response to stimulation of the vagus nerves has been examined in the pig and compared with that in the dog under similar experimental conditions.

2. In the pig, stimulation of the vagus nerves caused a profuse flow of pancreatic juice with a high content of bicarbonate, in addition to a secretion of digestive enzymes; atropine suppressed the secretion of enzymes but failed to diminish the flow of bicarbonate-rich juice.

3. Intra-arterial injections of acetylcholine closely imitated the effects of stimulation of the nerves, with the difference that both the flow of juice and the secretion of enzymes were abolished together by atropine.

4. Stimulation of the nerves and injections of acetylcholine were effective after resection of the stomach and intestine: these stimuli can therefore act directly on the pancreas, independently of the release of gastrointestinal hormones.

5. In the dog, the pancreas differed from that of the pig in that stimulation of the vagus nerves and injections of acetylcholine acted predominantly on the secretion of enzymes and caused only a sparse flow of juice. Atropine annulled all these effects together.

6. The results show that the vagus nerves can exert a much wider control of the secretion of pancreatic juice in the pig than in the dog. Possible mechanisms for this action are discussed.

INTRODUCTION

Following the discovery of secretin (Bayliss & Starling, 1902), little attention was given to the role of the vagus nerves in the control of the pancreas despite evidence that both nervous and hormonal stimuli were needed for a normal secretion (Babkin & Savitsch, 1908; Bylina, 1911). Mellanby (1925), however, suggested that the vagus nerves regulated the

secretion of enzymes, while secretin controlled the flow of pancreatic juice. This theory found general acceptance (Babkin, 1950; Gregory, 1962), although the discovery of pancreozymin (Harper & Raper, 1943) again raised doubts about the extent of the nervous contribution (Harper, 1959). These ideas were derived mainly from studies on the cat and dog in which stimulation of the vagus nerves causes a negligible or sparse flow of juice, unless potentiated by the simultaneous action of secretin (Grossman, 1962; Brown, Harper & Scratcherd, 1963). The present experiments have been carried out on the pig, in which, in contrast, stimulation of the vagus nerves causes an extremely profuse flow of pancreatic juice in addition to a secretion of enzymes. The administration of atropine abolishes the latter, but leaves the flow of juice undiminished. A preliminary account has already been given (Hickson, 1963) and some of the results have since been confirmed independently (Magee & White, 1965).

METHODS

Landrace pigs of either sex aged 4–12 weeks (weighing 8–20 kg) were used in the majority of experiments. They received a proprietary weaning diet and substitute sow's milk. Dogs (weighing 6–21 kg) were fed tinned meat and biscuit. Food but not water was withdrawn for 24 hr before experiments.

Operative procedure in pigs. Anaesthesia was induced with ethyl chloride and maintained with ether after cannulation of the trachea. A solution of α-chloralose (Koch-Light Laboratories), 10 mg/ml. in 'normal saline' (0·9 % w/v, NaCl solution) was slowly injected through a polyethylene catheter inserted into a femoral or jugular vein, to a final dose of 70 mg/kg body wt., and the administration of ether discontinued. A mixture 5 % CO_2 and 95 % O_2 was given at 1–2 l./min through the side arm of the tracheal cannula, or the inlet of the respiratory pump when this was in use.

The splanchnic nerves were cut. The stomach, duodenum and pancreas were exposed through a mid-line incision. The stomach contained only traces of solid food 24 hr after feeding, but a variable quantity of gas and bile was removed through a wide-bore needle; the pylorus was ligated without obstructing the gastro-epiploic vessels; in some experiments the stomach was cannulated for the collection of gastric secretion. The common bile duct was cannulated and drained with polyethylene tubing. The pancreatic duct was cannulated where it entered the duodenum; standard polyethylene tubing, 1·0 mm internal diameter (Portex Poly 52), was used for this purpose in all experiments.

Partial evisceration. The pyloric antrum, small intestine and in some cases the entire stomach, small and large intestines were resected, taking care to minimize damage to the pancreatico-duodenal blood vessels, and the vagus nerves at the cardia.

Injections. Intravenous injections were given through the cannula inserted for the administration of chloralose. Intra-arterial injections were made directly into the coeliac artery through a polyethylene catheter in the splenic artery.

Dogs. A similar routine was followed in experiments on dogs; the accessory pancreatic duct was ligated.

Stimulation of nerves. The thorax was opened by the removal of sectors from the

posterior ribs of either side, the phrenic nerves cut and the lungs ventilated with a Starling 'Ideal' pump. The dorsal and ventral vagus nerves were cut between ligatures below the oesophageal plexus; the peripheral ends were stimulated with bipolar platinum electrodes, or fluid electrodes based on the design of Porter & Allamon (1936). Square-wave shocks of controlled frequency, strength and duration were delivered from a transistor-operated stimulator.

In some pigs the coeliac branch of the left abdominal vagus nerve was sectioned below the diaphragm close to the cardia, all visible branches to the stomach cut and the peripheral end stimulated through a fluid electrode. A substantial proportion of the vagal innervation of the pancreas could thus be stimulated without pneumothorax. The cervical vagus nerves were cut routinely before dissection of the thoracic or abdominal nerve trunks.

Solutions for injection. Acetylcholine chloride (Roche Products; Koch-Light Laboratories), atrophine sulphate (British Drug Houses), decamethonium iodide (Fluka AG), hexamethonium bromide (May and Baker) and secretin (Boots Pure Drug Co.) were dissolved in normal saline. Figures given for the weights of drugs injected in the form of salts refer to the salt and not to the active principle or free base. The manufacturer's assay of the potency of secretin was accepted and doses are cited in the units of Crick, Harper & Raper (1949).

Records and collection of samples. The flow of pancreatic juice was measured with a photoelectric drop counter; the free end of the polyethylene pancreatic cannula was cut square and smeared with silicone grease to provide a standard nozzle for drop formation. Individual drops were recorded electromagnetically on a kymograph or a multi-channel pen recorder. Timed samples of pancreatic juice were collected in tared or graduated glass tubes and stored at 4° C until analysed for enzymic activity. Samples for inorganic analysis were collected under liquid paraffin in borosilicate tubes.

Arterial blood pressure was measured with a mercury manometer or Statham P23Db pressure transducer connected to a cannula in a carotid or femoral artery. Contractions of the stomach were recorded in a few experiments with a water manometer connected to a gastric cannula.

The pancreas was removed and weighed after experiments. The regression of pancreatic weight, Y g, on body weight, X, in the range 8·5–36·7 kg, was calculated from these data by the equation: $Y = 34{\cdot}73 + b(X - 18{\cdot}62)$ using the method of least squares ($n = 81$; $b = 1{\cdot}43 \pm 0{\cdot}15$ S.E.; $r = 0{\cdot}74$); pancreatic weight was estimated from this equation in eight pigs. A similar equation was derived for pancreatic weight in dogs: $Y = 21{\cdot}68 + b(X - 10{\cdot}35)$; ($X = 6{\cdot}6$–$21{\cdot}0$ kg; $n = 12$, $b = 1{\cdot}43 \pm 0{\cdot}26$ S.E.; $r = 0{\cdot}87$), and used to estimate pancreatic weight in five of the dogs.

Chemical analysis

Amylase. Routine determinations of the amylase content of the juice were made in duplicate by the following modification of the colorimetric method of Noelting & Bernfield (1948).

The substrate solution contained 2% (w/v) soluble starch (A.R. grade, Hopkins and Williams) dissolved in 0·02 M sodium phosphate buffer (pH 7·05 at 18° C),with 0·0067 M sodium chloride. 0·1 ml. of juice was diluted with 3·9 ml. of this buffer and 0·1 ml. diluted juice mixed with 10 ml. substrate at 38° C; 'substrate blanks' contained 0·1 ml. buffer, added to 10 ml. substrate. After 5 min incubation at 38° C, 1·0 ml. from each sample was pipetted rapidly into 5·0 ml. colorimetric reagent. This contained 2·0 g 3,5-dinitrosalicylic acid, 60 ml. 2·0 N sodium hydroxide, 60 g sodium potassium tartrate, made up to 500 ml. with distilled water. A standard solution of maltose (bacteriological sugar, Hopkins and Williams), 14 mg/ml., was prepared in

buffer, serial dilutions made from this, and 1·0 ml. volumes of each added to 5·0 ml. colorimetric reagent. 'Reagent blanks' contained 1·0 ml. buffer added to 5·0 ml. reagent. All assay, standard and blank tubes were then heated together for 10 min in boiling water, cooled and 1·0 ml. from each diluted with 10 ml. water. The optical densities of the diluted solutions were read against the 'reagent blank' at 520 mμ in a Spekker absorptiometer (Hilger and Watts). A standard graph relating maltose concentration to optical density was plotted; the amount of reducing sugar liberated from the substrate during incubation with pancreatic juice was determined from this. A unit of amylase activity linearly related to concentration in the juice was calculated from the following equation (Willstätter, Waldschmidt-Leitz & Hesse, 1923; Lagerlöf, 1942):

$$K = \frac{1}{t} \log_{10} \frac{a}{a-m}$$

(a = maximum amount of reducing sugar yielded by substrate; m = amount released during incubation; t = incubation time, min). Willstätter *et al.* (1923) gave a value of a = 0·75 mg/mg starch: this was adjusted to 15 mg/10 ml. for a 2% starch solution. One amylase unit is defined as 'the activity of a sample of pancreatic juice of which 0·1 ml. gives a value of K = 0·010 under the procedure and conditions specified for the assay'. With very active samples, 0·05 ml. juice was diluted with 3·95 ml. buffer before incubation. Amylase activities are quoted in u./ml. undiluted juice.

The activity of lipase and proteolytic enzymes was estimated in a few cases by the methods of Nachlas & Seligman (1949) and Anson (1939) respectively.

The mean rate of secretion (output) of amylase in each sample was calculated in terms of the weight of the pancreas. Analysis of results from pigs showed that pancreatic weight (20–55 g) was correlated with the volume (r = 0·81, $P < 0·01$; n = 14), and the amylase output (r = 0·57, $P < 0·05$; n = 14) of 10 min samples of juice obtained by standard, maximal stimulation of the vagus nerves. With secretin (0·1 u./kg) the 10 min volume was also correlated with pancreatic weight (r = 0·71, $P < 0·01$; n = 12). A significant correlation of the flow and bicarbonate secretion with weight of pancreas has been reported in dogs, although the relationship for enzyme output stimulated by pancreozymin in this case failed to reach the 5% level of significance (Hansky, Tiscornia, Dreiling & Janowitz, 1963).

Inorganic analyses. Total carbon dioxide was estimated as bicarbonate (m-equiv/l.) by the micro-diffusion method of Conway (1950), or with a Beckman GC Blood Gas Accessory and GC-2A Gas Chromatograph. Chloride was determined potentiometrically (Sanderson, 1952) and sodium and potassium estimated by flame photometry. The pH of juice was measured at 38° C in an EIL blood pH meter.

Statistical treatment. Where appropriate, experimental data were analysed statistically by the methods of Snedecor & Cochran (1967).

RESULTS

The flow of pancreatic juice in the pig

The resting secretion

A sparse flow of pancreatic juice occurred in a majority of preparations under resting conditions, without any specific experimental stimulus to the gland. The mean rate with 95% confidence limits was 0·48 ± 0·16 μl./g gland.min after section of the vagus nerves (twenty observations) and 0·39 ± 0·12 μl./g gland.min with the vagus nerves intact (eighteen observations).

The influence of the vagus nerves

Stimulation in the thorax. Stimulation of the peripheral ends of the vagus nerves caused a remarkably profuse flow of pancreatic juice. The peak flow obtained in response to stimulation of both infra-cardiac nerves at a frequency of 10 c/s at maximal strength for a period of 5 min approached the rate of secretion elicited by a nearly maximal dose of secretin (0·5 u./kg) given as a single intravenous injection (Fig. 1).

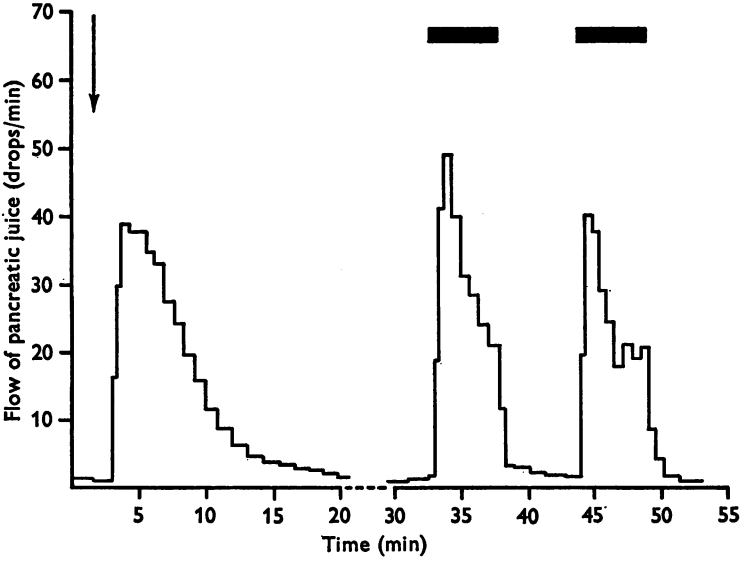

Fig. 1. Pig. Flow of pancreatic juice in response to stimulation of the vagus nerves and the injection of secretin. Ordinate: flow (drops/min) calculated from the interval between drops or groups of drops of juice measured from experimental records. Abscissa: time (min). Key: ↓ I.V. injection of secretin (0·5 u./kg); ■ maximal stimulation of both infra-cardiac vagus nerves in the thorax (10 c/s, fluid electrodes).

The time course of this secretory response to stimulation showed certain characteristic features. There was a long latent period of 30–45 sec before the flow of juice first appeared, during which the resting flow sometimes, but not always, slowed or even halted altogether. Afterwards the flow of juice rose abruptly to a maximum and then declined to a plateau, which was usually well maintained until stimulation ceased; the mean flow with 95 % confidence limits (sixteen observations) was $30{\cdot}3 \pm 4{\cdot}3$ μl./g gland.min during maximal stimulation. The flow then returned rapidly to the resting rate, unlike the more gradual recovery of the response to the intravenous injection of secretin (Fig. 1).

Stimulation was usually applied for periods of 4–6 min but the flow of juice was also well maintained when maximal stimulation was continued for as long as 10 min. Stimulation of either the dorsal or ventral nerves alone usually caused a similar flow of juice, although in some instances one nerve was more effective than the other. The combined effect of maximal stimulation of both nerves together, however, was usually less than the arithmetic sum of the separate responses to each one alone. The flow of juice due to stimulation of one thoracic or cervical vagus nerve increased during superimposed stimulation of the contralateral nerve, but the effect only appeared after a typically long latent period, during which no inhibition of the established flow of juice was detected.

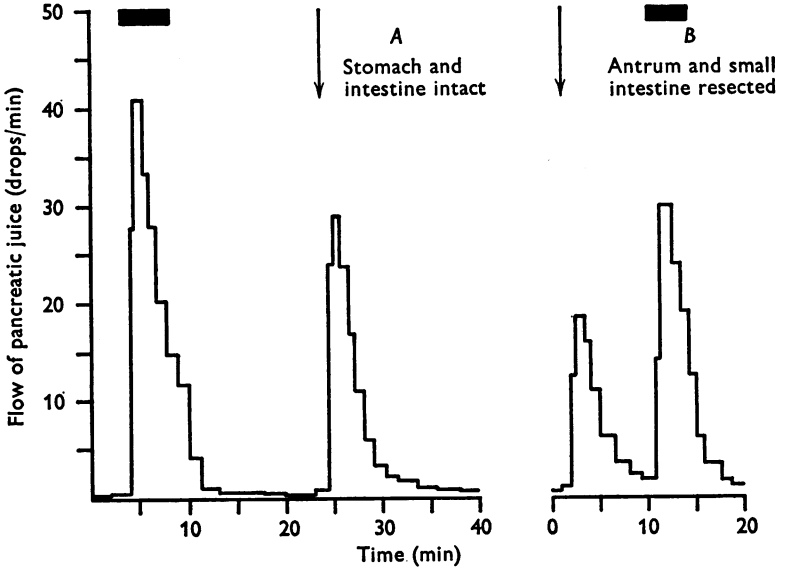

Fig. 2. Pig. Flow of pancreatic juice in response to nerve stimulation and to secretin, (*A*) before, (*B*) after resection of the pyloric antrum and small intestine. Co-ordinates as in Fig. 1. Key: ↓ I.V. injection of secretin (0·1 u./kg); ■ maximal stimulation of thoracic vagus nerves (5 c/s, fluid electrodes).

Stimulation in the abdomen. Maximal stimulation at 10 c/s of the coeliac branch of the dorsal vagus nerve caused a flow of pancreatic juice which was only slightly less than that due to stimulation of both vagus nerves in the thorax. The presence of other pathways in the abdomen for the remaining vagal fibres to the pancreas was indicated by the persistence of a secretory response to stimulation in the neck or thorax after the coeliac branch had been cut. The effects of stimulation of the coeliac branch were

chiefly restricted to the pancreas: in particular the strong contractions of the stomach and increased motility of the duodenum and secretion of gastric juice which normally followed stimulation in the thorax did not occur in this case.

Stimulation after partial evisceration. Stimulation of the vagus nerves in

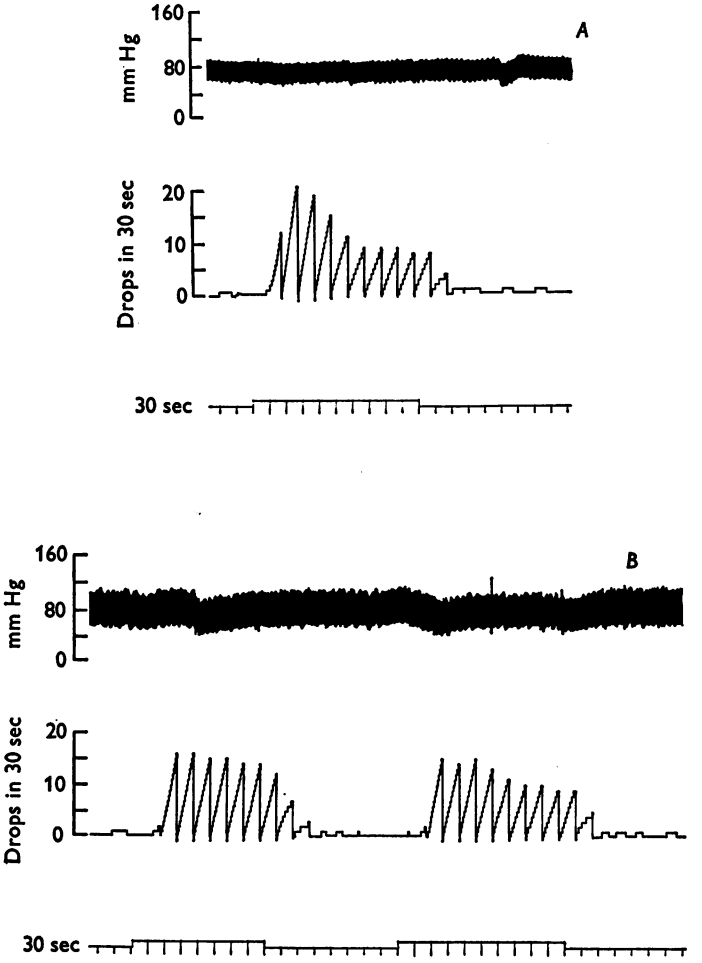

Fig. 3. Pig. Flow of pancreatic juice after removal of entire stomach, small and large intestines. Polygraph records from above down: arterial blood pressure (mm Hg); drops of pancreatic juice; time and event marker (30 sec, displaced upwards during stimulation). (*A*) Both thoracic vagus nerves stimulated (10 c/sec, 20 V, fluid electrodes); 2·3 ml. juice collected during stimulation. (*B*) Same preparation after I.V. injection of atropine (0·5 mg/kg). Dorsal vagus nerve stimulated alone (10 c/s, 15 V, Pt bipolar electrodes). Secretin (0·1 u./kg) injected once only, approximately 1 hr before (*A*), 2 hr before (*B*).

the thorax or abdomen still caused a flow of pancreatic juice after the pyloric antrum and small intestine had been removed (Fig. 2).

Since Edkins (1906) reported that the cardiac glandular mucosa in the pig contained gastrin, the experiments were repeated after the resection of the entire stomach, small and large intestines. A profuse flow of juice with the same latency and time course could still be elicited on stimulation of the vagus nerves in these preparations as in those with the gastro-intestinal canal intact (Fig. 3). Similar responses were obtained repeatedly over 2–3 hr in one of these preparations which had not received secretin at any time during the experiment.

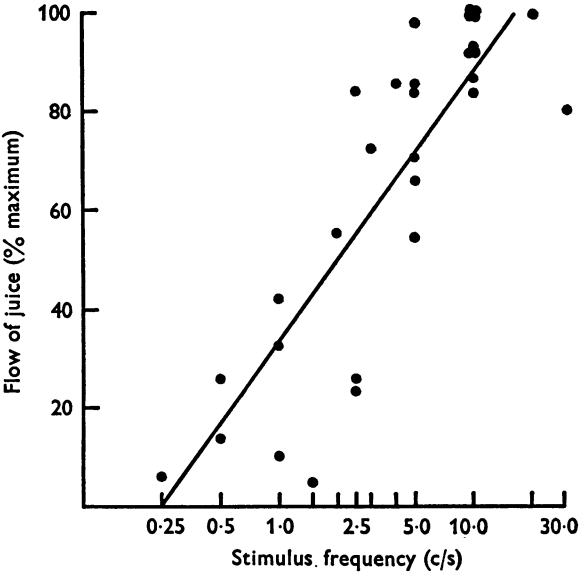

Fig. 4. Relationship between the frequency of stimulation of the vagus nerves and the resulting flow of pancreatic juice in the pig. Ordinate: rate of flow (% maximum attained in each preparation, Y). Abscissa: stimulus frequency (c/sec, log scale). Regression line calculated from experimental data: $Y = 65{\cdot}50 + b\,(X - 1{\cdot}58)$. ($X = 10.\log_{10}$ frequency; $b \pm$ S.E. $= 56{\cdot}21 \pm 6{\cdot}33$ % per tenfold increase in frequency; correlation coefficient, $r = 0{\cdot}85$; $P < 0{\cdot}01$; thirty observations from five pigs.)

The effects of both nervous and hormonal stimulation were, however, similarly reduced after evisceration (Fig. 2). Thus in three preparations the 10 min volume of juice in response to stimulation at 5 c/s during the first 5 min of the collection period fell to an average of 61 % of the previous values; that due to injections of secretin (0·1 u./kg) to an average of 65% of the previous amount. In addition, the first samples of juice collected after the evisceration was completed often contained increased amounts

of mucus. These changes may possibly have been due to injury and vascular impairment caused during the preparatory dissection.

The frequency and strength of stimulation. The rate of flow of pancreatic juice depended within limits on both the frequency and the strength of stimulation of the vagus nerves. With stimuli of constant supramaximal strength, a detectable increase occurred in the resting rate of flow with very low frequencies (0·25–0·5 c/s), while a maximal secretion was elicited at 10–20 c/s. A significant linear relationship could be demonstrated between these limits (Fig. 4): an analysis of the data in Fig. 4 showed that 73 % of the variation in the secretory rate was attributable to changes in the frequency of stimulation ($P < 0{\cdot}01$).

At very low frequencies, the latent period usually exceeded the 30–45 sec characteristic of the maximal response, but if the pancreas had been active shortly beforehand the delay was shortened towards the normal and the following secretory response was slightly enhanced. A threshold strength of stimulation necessary to initiate a flow of pancreatic juice could be defined for each preparation using stimuli of constant maximal frequency (10–20 c/s). Values of approximately 3 V were obtained with bipolar platinum electrodes, slightly more with the fluid electrodes. Above threshold the rate of secretion increased with the strength of stimulation up to a maximum of 10–15 V with platinum electrodes, 15–20 V with fluid electrodes.

The composition of pancreatic juice

Stimulation of the vagus nerves caused substantial changes in the concentration of both the digestive enzymes and the inorganic anions of the juice.

Principal inorganic ions

The concentrations of chloride and 'total carbon dioxide' in the juice were characteristically related to the rate of secretion (Fig. 5). The 'total carbon dioxide', expressed as the concentration of bicarbonate, rose from 20–30 m-equiv/l. in resting juice to a maximum of 130–160 m-equiv/l. in juice secreted at 10–20 μl./g gland.min, with no further change at higher rates of flow. The concentration of chloride altered reciprocally, from approximately 130 m-equiv/l. in resting juice to 18–24 m-equiv/l. above 10–20 μl./g gland.min. Thus the total concentration of the principal anions remained constant at all rates of flow (Table 1) and the changes in their individual concentrations at increasing rates of secretion were the same as those reported in other species (Janowitz, 1967). In the pig, however, these changes occurred generally, whether the flow of juice was increased by stimulation of the vagus nerves or by secretin (Fig. 5*A*, *B*). The small

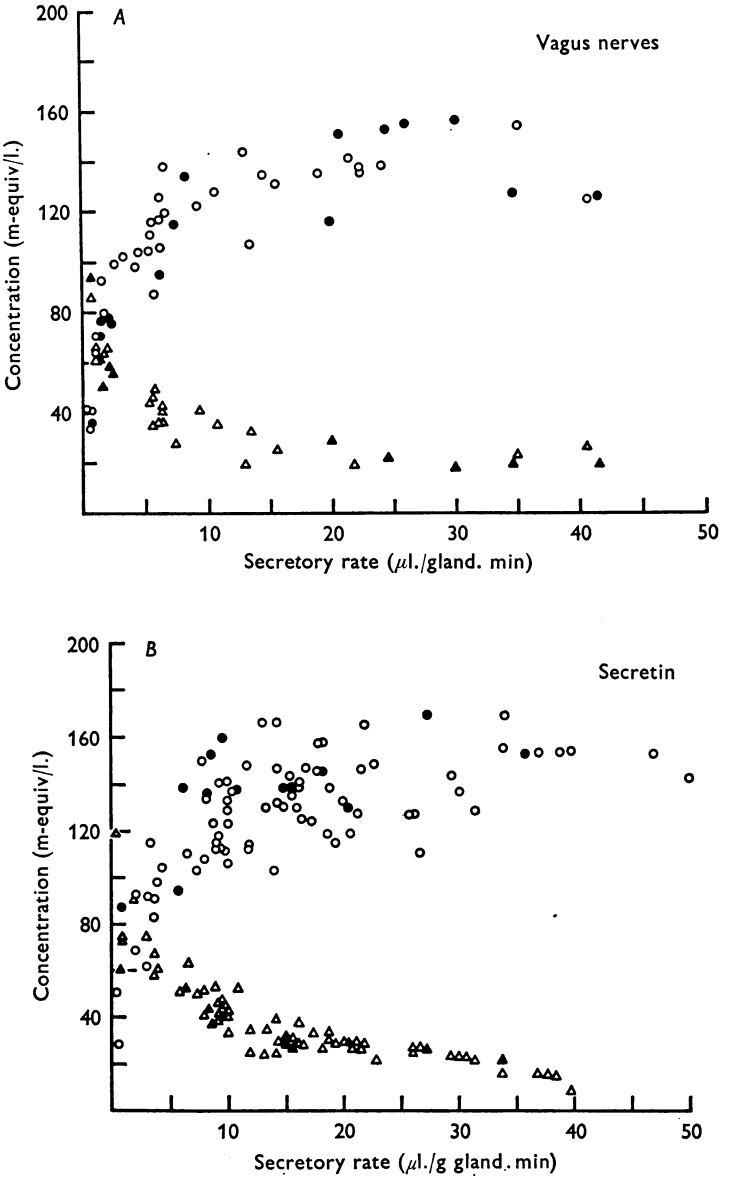

Fig. 5. Pig. Concentration of bicarbonate (○) and chloride (△) ions in pancreatic juice at different rates of secretion. Ordinates: concentration m-equiv/l.). Abscissae: secretory rate (μl./g gland.min). Flow of juice maintained in (*A*) by stimulation of the vagus nerves at selected frequencies (five pigs), in (*B*) by graded I.V. infusions of secretin (eight pigs). ○, △ samples before, ●, ▲ samples after, atropine (0·5 mg/kg).

difference between the mean total concentration of anions in 'secretin' juice and 'vagus' juice in Table 1 was statistically significant ($P < 0.01$), possibly because of the much greater content of protein in 'vagus' juice.

TABLE 1. Concentration of principal inorganic ions in the pancreatic juice of the pig secreted at rates of 0·04–41·21 μl./g gland.min in response to stimulation of the vagus nerves and injections of secretin

Samples	Concentration (m-equiv/l.) (means ± 95 % confidence limits)	No. of observations	No. of animals
1. Pancreatic juice			
Anions ($Cl^- + HCO_3^-$)			
(i) Stimulation	148·6 ± 4·6	39	5
(ii) Secretin	157·4 ± 2·9	66	6
Cations Na^+ (all samples)	148·6 ± 1·2	105	5
K^+ (all samples)	5·7 ± 0·2	105	5
2. Plasma			
Cations Na^+	142·3 ± 8·2	9	8
K^+	4·5 ± 0·7	8	8

The concentration of sodium and potassium in pancreatic juice in the pig remained constant within narrow limits, and close to the corresponding values for plasma at all rates of flow (Table 1); pooled values for 'vagus' and 'secretin' juice are given in Table 1.

The juice was weakly alkaline. In a typical experiment, the following values were obtained at 38° C: 'resting' juice, pH 8·16; 'vagus' juice secreted at 23·3 μl./g gland.min, pH 8·21: 'secretin' juice secreted at 29·2 μl./g gland.min, pH 8·22.

The secretion of enzymes

The resting output of amylase from the pancreas was very small and, like the resting flow, was not significantly altered by section of the vagus nerves, under the conditions of the present experiments (Table 2).

Stimulation of the vagus nerves greatly increased the concentration of amylase as well as the rate of flow of the juice and these effects combined to raise the output of the enzyme to a very high value (Fig. 6). In juice secreted at similar rates of flow in response to secretin, however, the concentration of amylase fell to a minimum and its output consequently remained very small (Fig. 6). Mean values for amylase output in a series of such experiments are given in Table 2. These show that the responses were obtained consistently throughout the group, when comparable conditions for stimulation and the collection of samples were used.

The concentration of amylase remained high when the secretory rate was altered by variation of the frequency of stimulation. Thus both the output of amylase and the secretion of fluid were similarly related to the stimulus frequency (Fig. 7). The threshold strength of stimulation was the same for the secretion of amylase and the acceleration of the flow of juice in a given preparation, and both increased together when the strength was increased from threshold to maximum at constant frequency. A submaximal dose of secretin was infused intravenously at a constant rate throughout these experiments, to ensure that any secreted enzyme was effectively cleared from the dead space of the gland. Thus the secretion of fluid and amylase by the pancreas could not be separated by changes in either the frequency or strength of stimulation of the preganglionic fibres of the vagus nerves.

TABLE 2. Output of amylase in pancreatic juice in the pig and dog at rest and in response to maximal stimulation of the thoracic vagus nerves (5–20 c/s; for 4–6 min) and the I.V. administration of secretin

Treatment (10 min samples, collected from onset of stimulation or injection; infusions continued throughout sample collection)	Amylase output (u./g gland.hr) (means ± 95 %) confidence limits)		No. of observations	No. of animals
1. Pigs				
Stimulation	51·95 ± 6·70		35	17
Secretin (injections 0·1–0·5 u./kg; infusions 0·01–0·05 u./kg.min)	0·76 ± 0·20		40	22
Resting juice (20–90 min samples)				
Vagi cut	0·48 ± 0·16	$P > 0·5$	37	18
Vagi intact	0·40 ± 0·14		18	7
2. Dogs				
Stimulation	11·38 ± 3·72		13	6
Secretin (injections 0·2–1·0 u./kg; 0·02–0·10 u./kg.min)	1·55 ± 0·38		26	9

Data were not obtained for resting secretion in dogs.

Lipase and the proteolytic enzymes. The secretion of these enzymes was investigated in only a few experiments, but in each case stimulation of the vagus nerves increased their concentration in the juice and their output from the pancreas varied in the same direction as that of amylase.

Pharmacological analysis

The rapid injection of acetylcholine (0·5–2·0 μg) into the coeliac artery caused a short-lasting secretion of pancreatic juice after a latent period of 30–50 sec, which resembled the response to brief stimulation (30–45 sec) of the vagus nerves (Fig. 8*A*, *B*, and *C*). This juice contained a greatly increased concentration of amylase (Table 3). As the total volume secreted

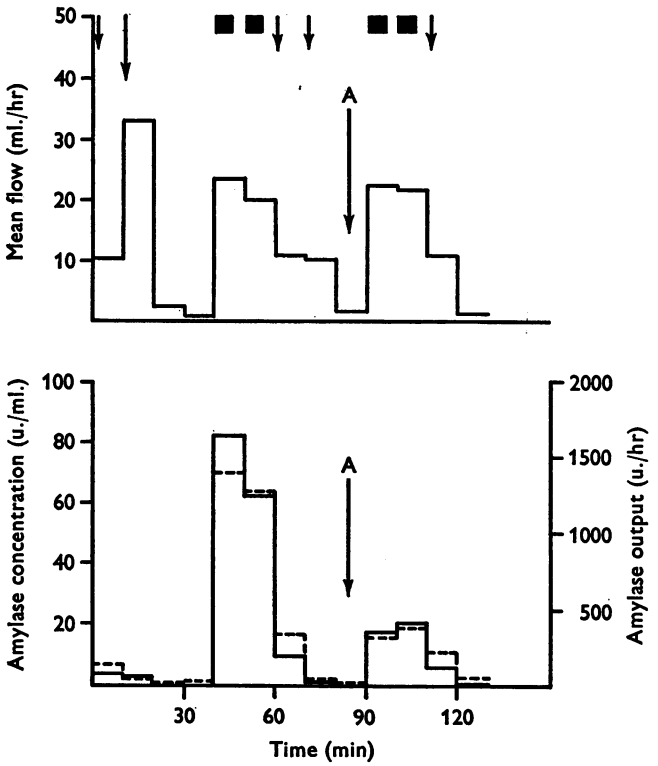

Fig. 6. Pig. Secretion of amylase in pancreatic juice in response to stimulation of the vagus nerves and secretin, before and after the injection of atropine. Ordinates: above, flow calculated from 10 min volumes of juice (ml./hr); below, amylase concentration ------ (u./ml.), amylase output —— (u./hr). Key: ■ maximal stimulation of thoracic vagus nerves (10 c/s); I.V. injections of secretin: short arrows, 0·1 u./kg, long arrow, 0·5 u./kg; A, I.V. injection of atropine (0·5 mg/kg).

in response to such injections was comparatively small and variable, consistent data for the output of amylase comparable to that given for stimulation of the vagus nerves or injections of secretin in Table 2 could not readily be obtained.

The response to acetylcholine could still be elicited after the removal of the stomach and intestine in preparations which had received either no secretin at all, or not more than one submaximal dose more than 1 hr before the test injections of acetylcholine were given (Fig. 8*B*).

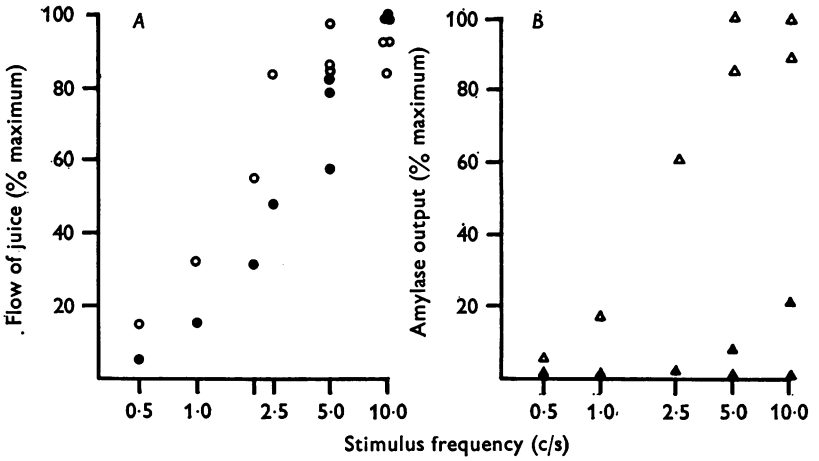

Fig. 7. Pig. Effect of atropine on (*A*) the flow and (*B*) the output of amylase in pancreatic juice on stimulation of the vagus nerves at selected frequencies. Ordinates (*A* and *B*): % maximum response in each preparation. Abscissae: stimulus frequency (c/s, log scale). ○, △ before, ●, ▲ after, I.V. injection of atropine (0·5 mg/kg). Data from three pigs in (*A*); two pigs in (*B*).

Injections of atropine

Atropine did not suppress the profuse flow of pancreatic juice caused by stimulation of the vagus nerves in the pig, whereas the secretion of enzymes was effectively curtailed by the intravenous injection of 0·5–3·0 mg/kg (Fig. 6), which also abolished the effects of parasympathetic stimulation on the heart, and secretion and motility of the stomach. Thus, as shown in Fig. 7, the respective actions of the vagus nerves on the secretion of fluid and amylase by the pancreas of the pig could be dissociated by atropine. The effects of maximal stimulation for periods of 5–6 min during the collection of 10 min samples of juice in a group of comparable preparations are summarized in Table 4. The volume of juice was not significantly altered, whereas highly significant falls, of approximately 82% of the values obtained before the injection of atropine, occurred in both the concentration and output of amylase. A similar atropine-resistant flow of juice containing a greatly reduced concentration of amylase occurred on stimulation of the vagus nerves in eviscerated preparations

(Fig. 3). Like the flow of juice, the related changes in the concentrations of chloride and bicarbonate ions were unaffected by atropine (Fig. 5).

In contrast to the atropine-resistant effects of stimulation of the vagus nerves, all the actions of intra-arterial injections of acetylcholine (0·5–10·0 μg) on the secretion of pancreatic juice were fully abolished by atropine (Fig. 8*A*, *B*).

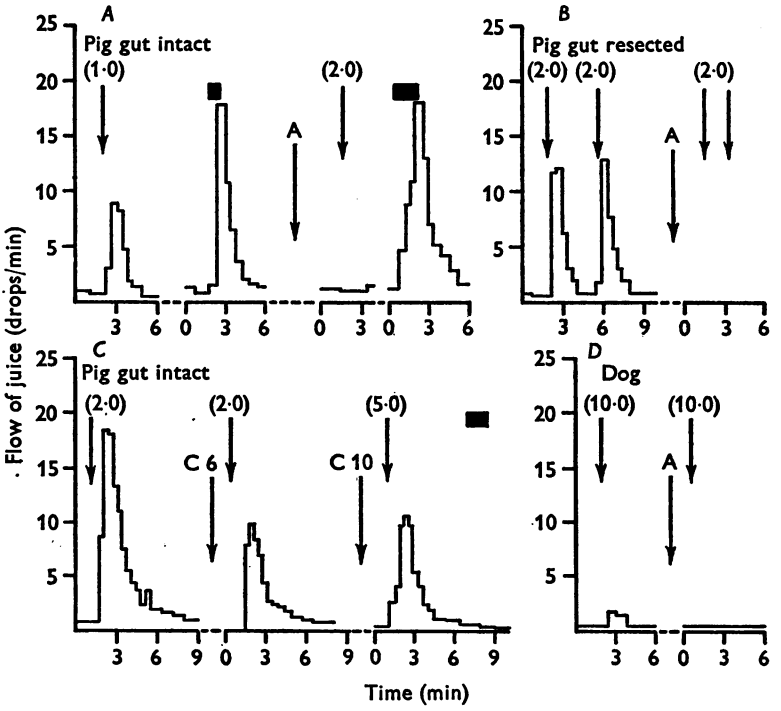

Fig. 8. Flow of pancreatic juice caused by intra-arterial injections of acetylcholine. Co-ordinates as in Fig. 1. Key: ↓ injections of acetylcholine (dose in μg in parentheses); ■ maximal stimulation of vagus nerves (5 c/s); I.V. injections: A, atropine (0·5 mg/kg); C6, hexamethonium (10·0 mg/kg); C10, decamethonium (0·5 mg/kg). (*A*) Pig. Acetylcholine and stimulation before and after atropine. (*B*) Pig. Acetylcholine after resection of stomach and intestines and in absence of injected secretin. (*C*) Pig. Acetylcholine after hexamethonium and decamethonium. (*D*) Dog. Acetylcholine before and after atropine.

Injections of hexamethonium

The response to stimulation of the vagus nerves in the neck, thorax or abdomen was completely abolished by hexamethonium (5–10 mg/kg), whether this was injected alone, or after the prior administration of either atropine or decamethonium (Fig. 9). The simultaneous failure of the effects

of the vagus nerves on the heart and stomach, together with a fall in arterial blood pressure indicated that ganglionic transmission had been blocked throughout the autonomic system in these preparations.

TABLE 3. The effect of I.A. injections of acetylcholine (ACh) on the concentration of amylase in pancreatic juice in the pig and dog*

Treatment	Dose	Mean concentration of amylase ± S.E. (u./ml.) Pig	No. of animals	Dog	No. of animals
Resting juice	—	20·6 ± 3·5	6	31·6 ± 10·9	4
ACh, I.A.	1–10 μg	39·4 ± 4·1	9	72·9 ± 10·2	4
Atropine injected I.V. followed by ACh, I.A.	0·5 mg/kg 1–10 μg	3·6 ± 1·9	4	7·0 ± 0·6	4
Secretion injected or infused I.V.†	See Table 2	2·5 ± 0·6	8	3·2 ± 0·4	4

* Range of body weights: pigs = 12·3–26·1 kg; dogs = 6·0–11·0 kg. Weights of pancreatic tissue: pigs = 25·5–61·0 g; dogs = 14·0–23·0 g.

† Values are for minimum concentration of amylase attained, to avoid error through 'washing-out' of pre-formed secretion.

TABLE 4. The action of atropine on the secretion of pancreatic juice in response to stimulation of the vagus nerves in the pig*

	Flow of juice† ml./g gland.hr)	Secretion of amylase Concentration (u./ml.)	Output (u./g gland.hr)
(*a*) Mean values ± S.E.			
(i) Before atropine	0·98 ± 0·10	58·25 ± 10·08	51·48 ± 4·52
After atropine	1·04 ± 0·11	11·18 ± 2·37	8·63 ± 2·07
(ii) % change	+6·6 ± 4·6	−82·0 ± 3·4	−83·5 ± 3·5
(*b*) Analysis of difference			
Mean difference ± S.E.	0·06 ± 0·15	47·08 ± 10·35	42·85 ± 4·97
t	0·393	4·549	8·629
Degrees of freedom	14	14	14
Significance	$P > 0·5$; N.S.	$P < 0·001$	$P < 0·001$

* Maximal stimulation of both thoracic vagus nerves (10 c/s) for 5–6 min during collection of 10 min samples of juice. Eight paired observations before and after atropine (0·5 mg/kg injected I.V.), from six pigs.

† Calculated from volume of 10 min samples.

Intra-arterial injections of acetylcholine, however, still caused a secretion of pancreatic juice after the effects of preganglionic stimulation of the vagus nerves had been fully abolished by hexamethonium (Fig. 8*C*). Secretin also continued to elicit a typically profuse flow of juice under these conditions.

Injections of decamethonium

Small doses of decamethonium preferentially block the 'nicotine-like' action of acetylcholine at the motor end-plate of skeletal muscle (Paton & Zaimis, 1952). The injection of 0·1–0·5 mg/kg eliminated contractions of the diaphragm in response to stimulation of the phrenic nerves, but not the flow of juice due to stimulation of the vagus nerves, either before or after the injection of atropine (Fig. 9). However, the peak flow of juice attained during stimulation was usually slightly reduced and the response

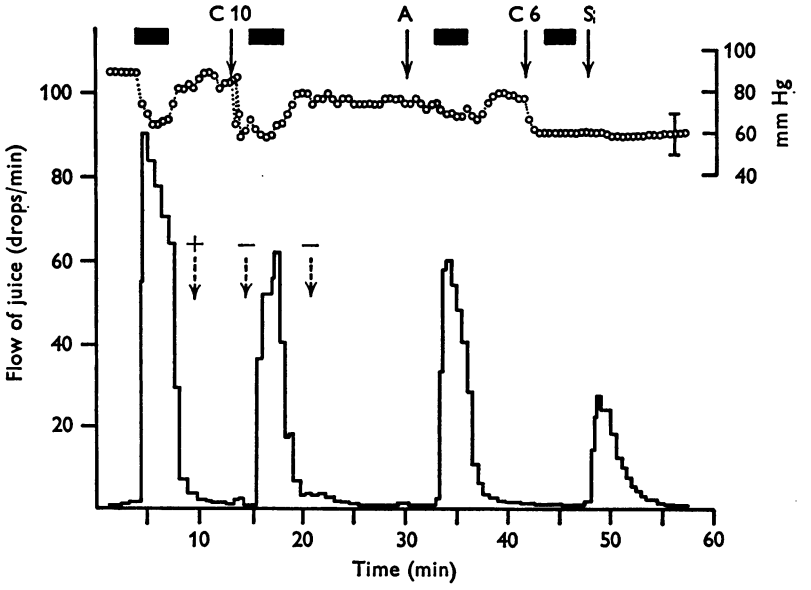

Fig. 9. Pig. Effect of decamethonium and hexamethonium on the flow of pancreatic juice caused by stimulation of the vagus nerves. Key: —— flow of juice (as in Fig. 1); ○ - - - - ○ arterial blood pressure (mm Hg, vertical bar: pulse pressure range); ■ maximal stimulation thoracic vagus nerves (10 c/s); I.V. injections: C10, decamethonium (5·0 mg/kg); A, atropine (0·5 mg/kg); C6, hexamethonium (5·0 mg/kg); S, secretin (0·2 u./kg). Stimulation of right phrenic nerve, ↓; + = contraction of diaphragm; − = contraction abolished.

less well maintained after decamethonium. This attenuation was more pronounced with doses of 1·0–5·0 mg/kg, while still larger doses (5·0–10·0 mg/kg) temporarily abolished the response altogether. This was probably due to the ganglion-blocking action of large doses of decamethonium (Paton & Zaimis, 1952), since it was accompanied by a fall in arterial blood pressure of similar duration. Like hexamethonium, decamethonium did not

abolish the response to intra-arterial injections of small doses of acetylcholine in the absence of atropine (Fig. 8*C*) and did not prevent the normal response to injections of secretin.

The secretion of pancreatic juice in the dog

The copious secretion of pancreatic juice caused by stimulation of the vagus nerves in the present experiments on the pig was in sharp contrast with the characteristically sparse effects of stimulation reported in the cat and dog (Mellanby, 1925; Harper & Vass, 1941; Babkin, 1950; Thomas,

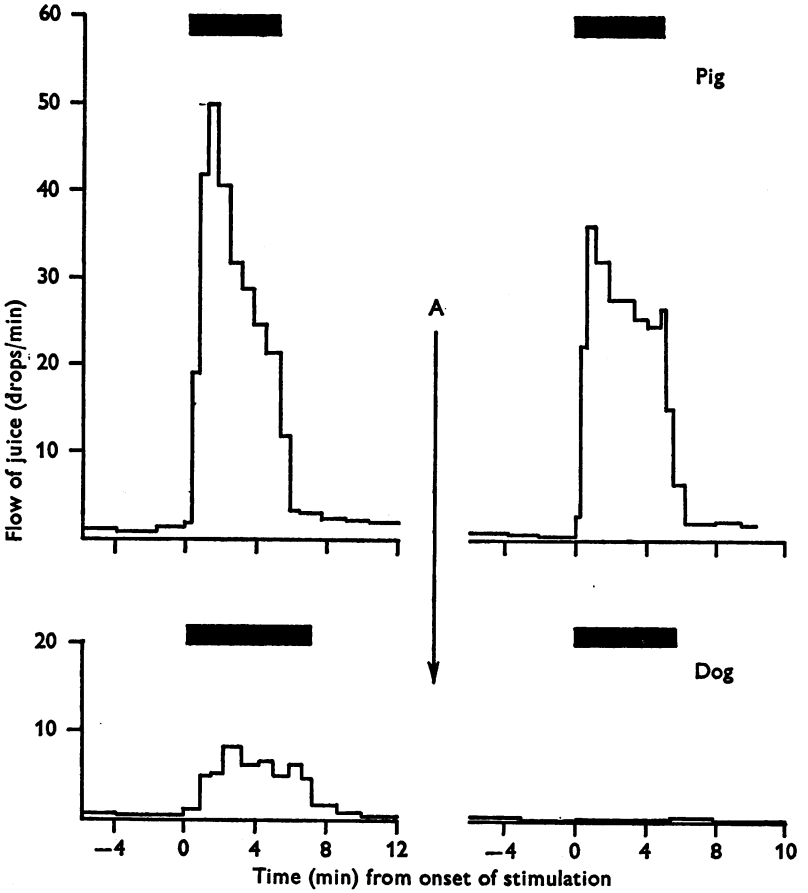

Fig. 10. Comparison of the effects of maximal stimulation of the thoracic vagus nerves (10 c/s) on the flow of pancreatic juice in the pig and dog. Co-ordinates and symbols as in Fig. 1; A, I.V. injection of atropine (0·5 mg/kg). Post mortem weight of pancreas: pig, 26·0 g; dog, 25·5 g.

1950). Some further experiments were therefore made on dogs, so that a direct comparison with the pig could be made under the same conditions.

Results obtained from a pig and dog of similar size and with approximately the same post mortem weight of pancreatic tissue, are shown in Fig. 10. Two particular features distinguish the response to maximal stimulation in the dog from that in the pig: the maximum flow of juice attained was much smaller and was effectively abolished by atropine (0·5 mg/kg). A long latent period was common to both species in these experiments. The mean rate of secretion (with 95 % confidence limits) of 10 min samples elicited by 4–6 min of maximal stimulation of the thoracic vagus nerves (5–10 c/s) was $4{\cdot}2 \pm 1{\cdot}1$ μl./g gland.min in the dog ($n = 5$) compared with $14{\cdot}3 \pm 1{\cdot}9$ μl./g gland.min in the pig ($n = 21$).

The output of amylase was also less in the dog: Table 2 contains values for both species and shows that the mean output in the dog was approximately 21 % of that in the pig under the same conditions of collection and stimulation. The injection of atropine (0·5 mg/kg) reduced the output in the dog from the value given in Table 2 to $0{\cdot}51 \pm 0{\cdot}51$ u./g gland.hr (mean with 95 % confidence limits; $n = 5$), with comparable maximal stimulation. The amylase output in the dog during stimulation by secretin, however, was almost twice the very low value in the pig (Table 2). While the resting output of amylase may be higher in the dog, this effect could also be due to a less efficient 'washing-out' of pre-formed secretion from the ducts of the gland, which also appears to be less sensitive to secretin in this species (Hickson, 1970).

Intra-arterial injections of acetylcholine caused a transient flow of pancreatic juice with an increased concentration of amylase in the dog as in the pig (Fig. 8*D*; Table 3). The effect was weaker than in the pig, however, and larger doses (up to 10·0 μg) were needed to elicit only a very sparse volume of juice. This was not increased if the dose was raised still further to 10·0–50·0 μg. The much higher concentration of amylase in this juice in the dog compared with the pig may depend on the relatively weak action of acetylcholine on the rate of flow (Table 3). As in the pig, atropine (0·5 mg/kg) abolished all the secretory actions of such doses of acetylcholine on the pancreas of the dog.

DISCUSSION

The two distinctive features of the secretory response of the pancreas in the pig which emerge from the results of the present experiments are, first, that stimulation of the vagus nerves causes a profuse flow of juice and, second, that this is not abolished by the injection of atropine, which nevertheless annuls the effect of stimulation on the secretion of enzymes.

The parasympathetic, secretory nerve supply therefore appears to exert a wider and quantitatively greater influence on the secretion of pancreatic juice in the pig than in the cat or dog.

The flow of pancreatic juice caused by nervous stimulation in the pig was so profuse that in certain respects it resembled the secretion of saliva. Thus the relationship between the frequency of stimulation and the rate of secretion, and the frequency for a maximal response (10–20 c/s) were similar in the salivary glands (Burgen & Emmelin, 1961) and in both tissues intra-arterial injections of acetylcholine closely imitated the secretory effect of stimulation of the parasympathetic nerves. The pancreas differed, however, in the long latent period of the secretory response to either injected acetylcholine or stimulation of the vagus nerves (20–45 sec), which greatly exceeded that in the salivary glands (0·1–2·0 sec) and also outlasted the latency of the vasodilatation in the pancreas (1·0–5·0 sec; Hickson, 1970). This long delay cannot be explained from the present results, but it is unlikely to be due to inhibitory or duct-constrictor fibres in the vagus nerves since any inhibition of the background secretion was capricious in occurrence and variable in extent. Furthermore, a duct musculature or myoepthelial cells were not readily apparent on histological examination of the tissue. A comparably long delay also occurs in the pancreas of the dog, where it has been ascribed to the slow development of the secretory process and not to constriction of the ducts (Gayet & Guillaumie, 1933).

Although the pancreas of the pig appears to be extremely sensitive to secretin (Hickson, 1970), the possibility that the vagus nerves exert their action through the release of this or a similar hormone from the gastrointestinal mucosa can be excluded. The response to stimulation, which remained after complete removal of the stomach and intestine, did not depend on a persistent background of secretin in the blood since the nerves retained their activity over a period of hours in preparations which received no secretin at all, or not more than one submaximal dose (usually 0·1 u./kg) not less than 1 hr before stimulation was first tested. Although the potentiation of the response to infusions of secretin by stimulation of the vagus nerves in the atropinized cat has been attributed to the effects of vasodilatation (Brown *et al.* 1967), such a mechanism could not account for the response in the pig, where the large volume of the secretion, its persistence after evisceration and the improbability that enough secretin would remain active in the blood for sufficiently long to mediate the effects of repeated stimulation, all indicate that the vagus nerves act directly on the secretory cells of the pancreas. This theory is supported by the results of histochemical studies which show that cholinergic nerves and ganglia occur more abundantly in the pancreas of the pig than of the cat or dog

and suggest that the parasympathetic innervation is more prolific in the pig than in the other species (Comline, Hickson & Message, 1964).

The persistence of a profuse flow of pancreatic juice on stimulation of the vagus nerves after the injection of atropine in the pig is a unique effect. In the salivary glands atropine-resistant responses are largely restricted to the vasodilation and only a fraction of the normal response of the secretory cells remains (Barcroft, 1914; MacIntosh & Rawlinson, 1935; Strömblad, 1959). However, like the vasodilatation in the submaxillary gland of the cat (Hilton & Lewis, 1955), the atropine-resistant effects in the pancreas of the pig are unlikely to be due to non-cholinergic fibres in the parasympathetic innervation. A 'nicotine-like' component is also improbable since the effects of either stimulation or injections of acetylcholine were not abolished by decamethonium in a dose which paralysed the diaphragm and since the effects of injections of acetylcholine remained after the response to stimulation of the preganglionic nerves had been blocked by hexamethonium. Conversely, the complete suppression by atropine of all the actions of injected acetylcholine indicates that the flow of juice as well as the secretion of enzymes depends on a 'muscarine-like' action of the transmitter released from the nerve terminals. To explain these effects little can be added to the theory of Dale & Gaddum (1930) which implied the existence of specialized neuro-effector junctions at which an essentially 'muscarine-like' action of the acetylcholine released by nerve impulses was locally protected from the effects of atropine. If this theory is correct many of the characteristics of the secretory response of the pancreas of the pig could be attributed to the development of a particularly close relationship between the terminals of the post-ganglionic parasympathetic nerves and the secretory cells. These also appear to be more sensitive to acetylcholine, since although intra-arterial injections increased the concentration of amylase to very high values in the juice of the dog, their effect on the rate and volume of the secretion was much greater in the pig.

The secretory action of the vagus nerves in the pancreas of the pig can therefore be dissociated by atropine into a typical 'muscarine-like' action on the secretion of enzymes, which may chiefly depend on the diffusion of acetylcholine from the nerve terminals, and an anomalous, atropine-resistant 'secretin-like' action on the flow of juice which may occur predominantly at the neuro-effector junctions. A complete explanation of these effects depends on the identification of the pancreatic cells responsible for the secretion of the enzymes and of the water and inorganic constituents of the juice, which is still uncertain. It is clear, however, from the present experiments that in the pig the vagus nerves contain efferent fibres which can exert a much wider control of the flow of pancreatic juice

than has been found in other species so far. An important implication of these results is that reflex control of pancreatic secretion may be a more prominent feature in the pig than in the cat or dog.

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