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PANCREATIC ENZYME RESPONSE TO SECRETIN AND CHOLECYSTOKININ-PANCREOZYMIN IN THE RAT*

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SUMMARY

1. The report describes a new technique for collecting pancreatic juice in anaesthetized rats. The technique, which involves perfusion of the duodenum, is particularly suitable for analysing the characteristics of pancreatic enzyme secretion. To ensure stable secretory conditions, the rats were kept at 30° C.

2. Different combinations of secretin and cholecystokinin-pancreozymin have been given by continuous intravenous infusion and the patterns of secretion of amylase and trypsin have been defined.

3. Maximal secretion of the pancreatic enzymes was observed with 60 IU/kg.hr CCK-PZ (amylase) or 120 CU/kg.hr (trypsin) combined with 0·5 CU/kg.hr (secretin).

4. Pancreatic enzyme secretion in response to submaximal stimulation with CCK-PZ was potentiated by secretin.

5. Supramaximal stimulation with CCK-PZ resulted in significantly less secretion of pancreatic enzymes than in response to maximal stimulation.

6. The pancreatic secretion of enzymes was poorly sustained during constant-rate stimulation with intravenous hormones, at all dose rates.

7. The significance and possible mechanisms of the biphasic pattern of enzyme secretion in response to increasing doses of stimulant hormones and the fall-off in enzyme secretion during constant-rate stimulation are discussed.

INTRODUCTION

A combination of hormones is released into the blood stream from the small intestine in response to the presence of gastric contents within the intestinal lumen. The two most important of the small intestinal hor-

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mones (secretin (Bayliss & Starling, 1902) and cholecystokinin-pancreozymin (CCK-PZ) (Ivy & Oldberg, 1928; Harper & Raper, 1943)) have been known for many years, but although the pancreatic response to the individual hormones has been studied in detail, little is known about the effect on the pancreas of combinations of the two hormones (Brown, Harper & Scratcherd, 1967; Konturek, Redecki, Mikos & Thor, 1971, cats; Henriksen & Worning, 1967; Henriksen, 1968; Meyer, Spingola & Grossman 1971, dogs; Wormsley, 1969, man).

The purpose of the present study has been to define some consequences of hormonal interactions by considering in detail aspects of the response to combinations of different doses of the two hormones using the pancreatic secretion of enzymes in the rat as criterion of hormonal action on the pancreas.

METHODS

The experiments were performed following a 40 hr fast on male Wistar rats (290–400 g) which had previously been kept on diet 41 (Bruce & Parkes, 1949). During the fast the animals were kept in wire-bottomed cages to prevent coprophagy. The animals were allowed free access to water.

The rats were anaesthetized with urethane (0·6 ml./100 g body wt. of a solution containing 250 g/l.) by I.M. injection at 07.15 hr. Experiments were started 3 hr later, as suggested by Heatley (1968*a*, *b*). During the experiments the body temperature of the rat was maintained at 30° C by means of a heated table (Fig. 1). The rats were kept at a constant temperature of 30° C because Ghosh & Schild (1958) had demonstrated maximal stability of gastric secretory response at this level of body temperature. We confirmed, in preliminary studies, that at 37° C the general state of the rats deteriorated rapidly, with failing respiration and circulation, so that

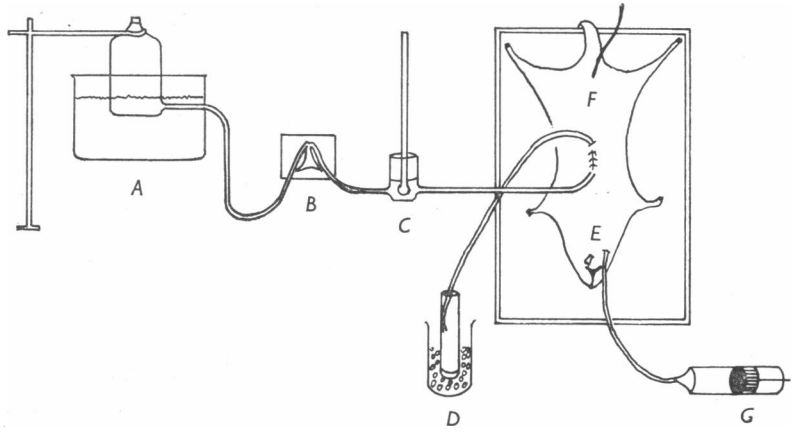

Fig. 1. Schematic representation of perfusion technique. *A*, reservoir of saline perfusate, in water bath; *B*, peristaltic pump; *C*, monitor of temperature of saline; *D*, vessel for collection of effluent perfusate, surrounded by ice; *E*, tracheal cannula; *F*, rectal temperature probe; *G*, syringe pump for infusion into jugular vein.

pancreatic secretory responses could not be interpreted. On the other hand, at 30° C the state of the rats remained satisfactory for the 4 hr during which the rats were studied in the present investigation. A glass cannula was placed in the trachea (Fig. 1, part *E*). A polyethylene tube (i.d. 2 mm) coming from a reservoir and passing through a peristaltic pump (Fig. 1, part *B*) was introduced though an incision in the pylorus, about 1 cm proximal to the pyloro-duodenal junction and sited in the proximal duodenum. The pylorus was tied off around the tube. A second tube was inserted into the duodenum 4·5 cm distal to the first one and the duodenum was ligated. The duodenum was perfused with 0·15 M sodium chloride at a flow rate of 7·5 ml./15 min. The saline, which came from the reservoir, was heated in a water-bath (Fig. 1, part *A*) to a temperature of 30° C, continuously monitored with a thermometer (Fig. 1, part *C*) and kept at 30° C. The perfusates were collected on ice in 15 min batches (Fig. 1, part *D*).

Schedule of Hormone Infusion

The hormones, from the GIH Laboratory, Karolinska Institutet, Stockholm, Sweden, were infused I.V. in 0·15 M sodium chloride after basal secretion had been collected for four successive 15 min periods. Intravenous administration was effected through a small polyethylene catheter (0·63 mm I.D.) in a jugular vein at a rate of 1·65 ml./hr, by means of an infusion pump. The dose schedules comprised:

(1) CCK-PZ alone (30 Ivy units (IU/kg.hr)).

(2) Secretin alone (120 Clinical units (CU)/kg.hr)).

(3) A range of doses of cholecystokinin-pancreozymin (CCK-PZ): 7·5, 15, 30, 60, 120, 240 IU/kg.hr together with a constant low dose of secretin (0·5 CU/kg.hr).

(4) A range of doses of CCK-PZ: 15, 60, 120 IU/kg.hr with a constant larger dose of secretin (60 CU/kg.hr).

(5) A range of doses of secretin: 0·5, 4, 30, 120, 240 CU/kg.hr with a constant dose of CCK-PZ (30 IU/kg.hr).

Only one dose combination was used in each animal.

Each dose schedule lasted for 3 hr and was studied in three animals.

Analysis of variance was used to assess significance of differences between doses and hormones.

Analysis of duodenal perfusate

1. *Trypsin*

As a result of detailed preliminary studies to determine optimal conditions for measurement of enzyme activities (submitted for publication), freshly recovered non-activated duodenal perfusates were immediately activated with enterokinase (enteropeptidase) and then mixed with an equal amount of glycerol (Analar) (Lagerlöf, 1942).

2 ml. of the neat perfusate was activated by incubating for 60 min at 25° C with 2 ml. 0·05 M Tris-buffer (pH 7·9), containing 50 µl. of an enterokinase solution (16 g/l.) (Miles Laboratories, Inc., Kankakee, USA). The enterokinase solution was freshly prepared before use. The activated samples were mixed with 4 ml. glycerol for storage. The glycerolated samples were kept at −20° C until enzyme estimations were carried out. The technique of storage resembles that used for enzymically active duodenal aspirate obtained from human subjects. As in the case of the latter, no fall-off in the activity of trypsin or amylase occurred in the period of 2–3 weeks when the duodenal perfusates were stored mixed with glycerol.

Tryptic activity was measured by the titrimetic method of Haverback, Dyce, Gutentag & Montgomery (1963) using tosyl–arginine–methylester (TAME) as substrate. The reaction vessels were provided with a constant flow (35 ml./min) of

nitrogen (Ballantyne, 1968). Tryptic activity is expressed as trypsin, μg/ml. Trypsin standards prepared from twice crystallized bovine trypsin (Sigma Chemical Co., London) were estimated each day.

2. *Amylase*

The activity of amylase was assayed with 2,4-dinitro-salicylic acid, using Lintner starch as substrate, according to a method described by Bernfeld & Studer-Pècha (1947). Amylase activity is expressed in terms of the rate of production of maltose during the initial period of the reaction with the starch.

RESULTS

1. *Basal secretion*

The basal secretion of amylase ranged from 2 to 10 m-mole maltose/15 min. 100 g body wt. Basal secretion of trypsin could not be detected with the analytical methods used. The basal secretion persisted unchanged when the control period was extended to 4 hr.

2. *Response to CCK-PZ alone*

The output of amylase and trypsin in response to CCK-PZ 30 IU/kg.hr alone did not differ significantly from the response to a combination of CCK-PZ 30 IU/kg.hr with 0·5 CU secretin/kg.hr (Table 1).

TABLE 1. Hormone response to stimulants

Stimulation (kg.hr) IU or CU		Enzyme output/3 hr.100 g body wt.	
CCK–PZ	Secretin	Trypsin (μg)	Amylase body wt.
7·5	0·5	267	368
—	120	192	110
30	—	1113	870
30	0·5	1051	1082

The results represent the mean values of three experiments for each dose combination.

3. *Response to secretin alone*

Stimulation with secretin 120 CU/kg.hr elicited very little secretion of amylase and trypsin. The output of the enzymes was less than in response to the smallest combined dose of CCK-PZ and secretin (Table 1).

4. *Response to increasing doses of CCK-PZ with constant low dose of secretin*

a, Amylase output. The peak output of amylase was attained during the first 60 min of stimulation (Fig. 2). The secretion of amylase was maintained for up to 1 hr and then decreased rapidly, despite constant rate infusion of stimulant hormones.

The peak and total outputs of amylase increased significantly with increasing doses of CCK-PZ, reaching maximal values with 60 IU/kg.hr and decreasing significantly with the highest dose (Figs. 2 and 3) ($P < 0{\cdot}01$).

The rate at which the peak output of amylase was attained increased with the dose rate of CCK-PZ, being most rapid with CCK-PZ, 240 IU/-kg.hr.

b, *Trypsin output*. The time course of the secretion of trypsin was similar to amylase (Fig. 4). Output of trypsin increased significantly with increasing doses of CCK-PZ to reach maximal levels with 120 IU/kg.hr and decreased significantly ($P < 0{\cdot}01$) with the highest dose of CCK-PZ.

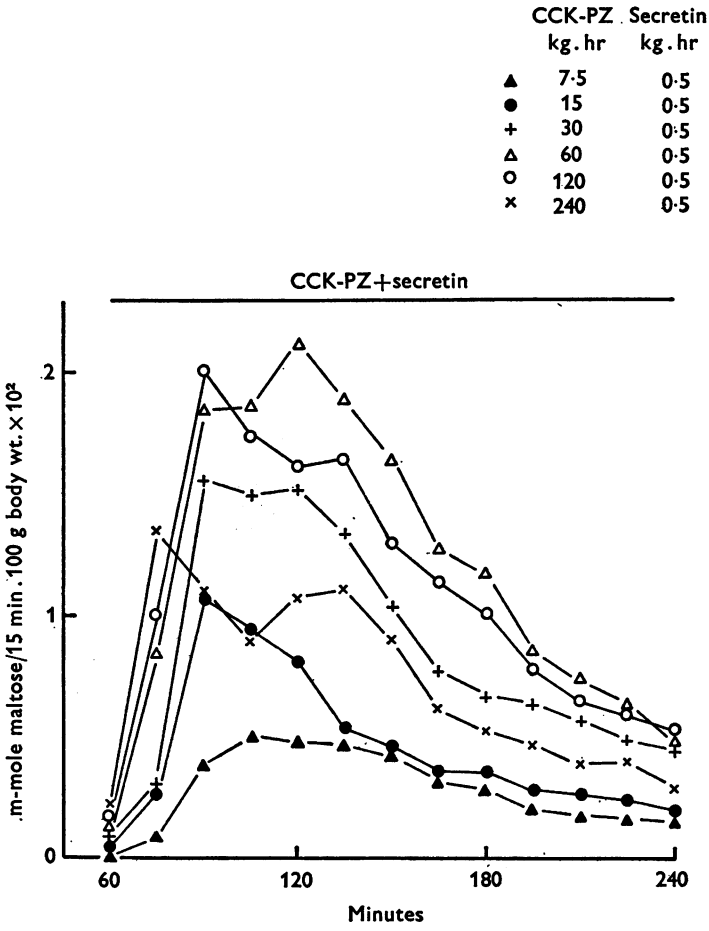

Fig. 2. Time course of secretion of amylase in response to infusion of different dose rates of CCK-PZ with constant dose of secretin (0·5 CU/kg.hr).

Each point represents the mean of values of 15 min outputs from three rats during the course of the 3 hr infusion of hormones. The different dose rates of CCK-PZ are denoted by the tabulated symbols.

5. *Response to increasing doses of CCK-PZ with constant dose of secretin*

a, Amylase output. The output of amylase was maximal with CCK-PZ, 15 IU/kg.hr and significantly ($P < 0{\cdot}01$) exceeded the response to the same dose of CCK-PZ combined with the low dose of secretin (Fig. 5). With increase in the dose rate of CCK-PZ, the output of amylase did not differ from the response to the same dose of CCK-PZ with the low dose of secretin (Fig. 5).

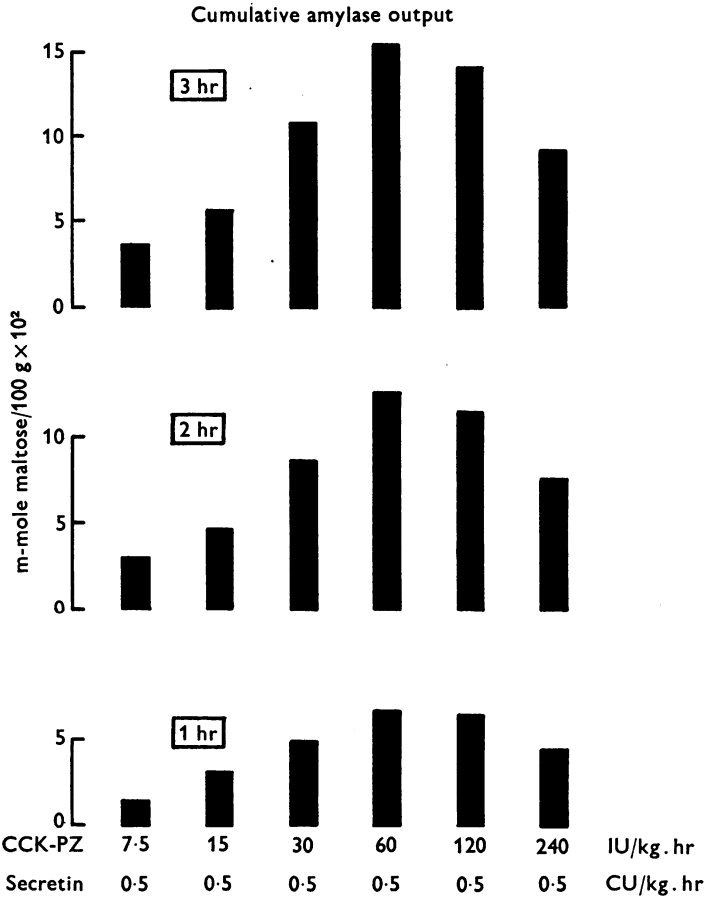

Fig. 3. Cumulative output of amylase in response to different dose rates of CCK-PZ with constant dose of secretin (0·5 CU/kg.hr).

Each vertical bar represents the mean output of three rats during the first, first 2 and all 3 hr of infusion of the dose of CCK-PZ indicated at the foot of the 1 hr column.

The difference between the 3 hr outputs in response to CCK-P2, 7·5, 15, 30 and 60 IU/kg.hr is significant ($P < 0{\cdot}01$). The decrease of CCK-P2 from 120 to 240 IU/kg.hr is significant ($P < 0{\cdot}01$).

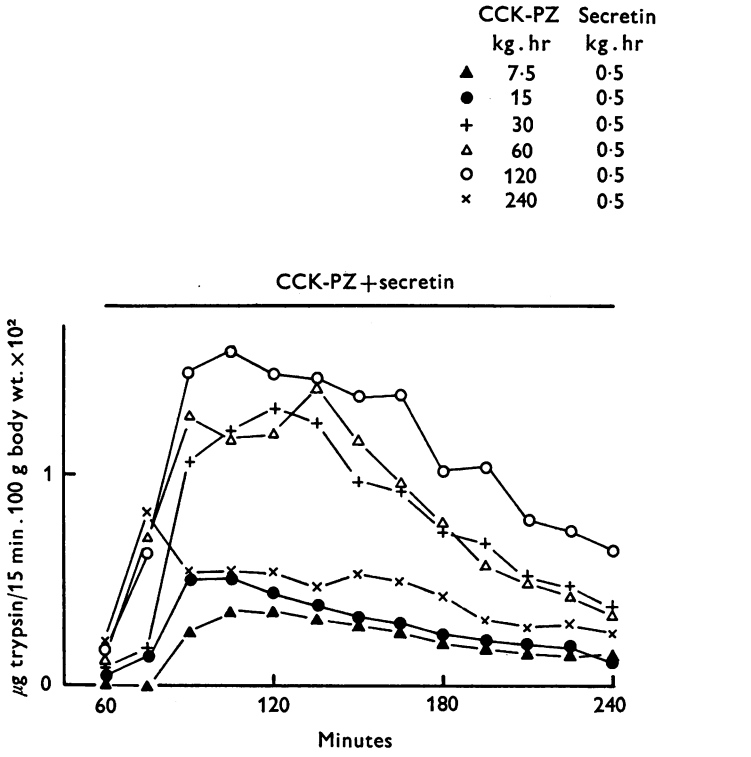

Fig. 4. Time course of secretion of trypsin in response to different dose rates of CCK-PZ with constant dose of secretin (0·5 CU/kg.hr). Meaning of symbols as in Fig. 2.

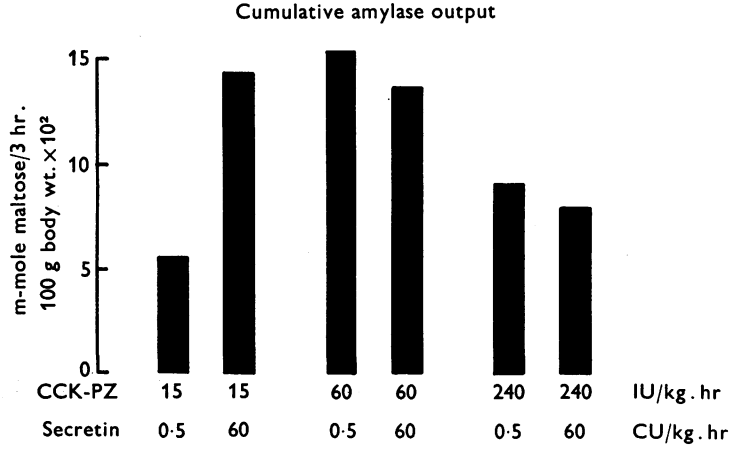

Fig. 5. Cumulative output of amylase in response to different combinations of CCK-PZ and secretin.

Each vertical bar represents the mean 3 hr output of three rats during infusion of the dose rates of CCK-PZ and secretin specified at the foot of each column.

b, *Trypsin output.* The pattern of trypsin secretion resembled that for amylase.

6. *Response to increasing doses of secretin with constant dose of CCK-PZ*

a, *Amylase output.* The secretion of amylase was never maintained for more than 45 min and then decreased (Fig. 6), more rapidly than during stimulation with CCK-PZ and a low dose of secretin.

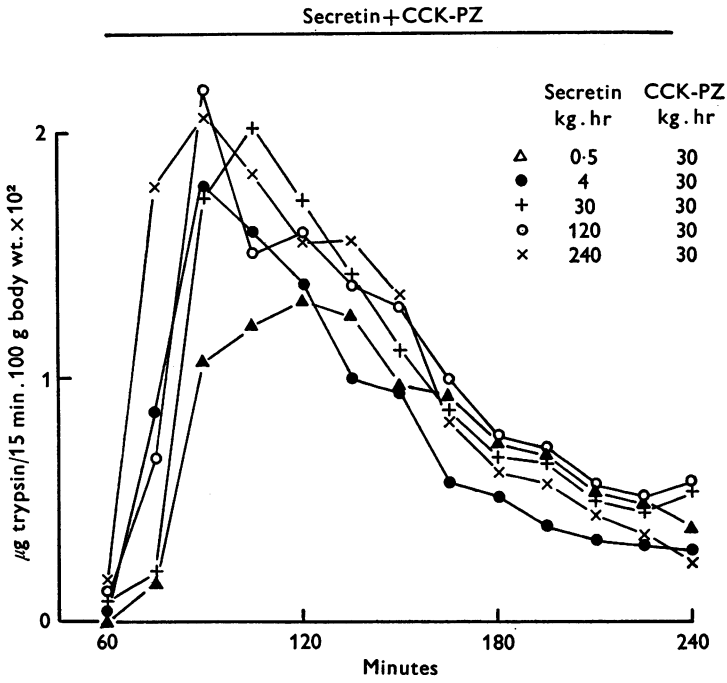

Fig. 6. Time course of secretion of amylase in response to different dose rates of secretin with constant dose of CCK-PZ (30 IU/kg.hr). Meaning of symbols as in Fig. 2.

Peak and total amylase output reached maximal values with 30 CU/kg.hr secretin and did not change significantly with increase in the dose rate of secretin.

b, *Trypsin output.* The time course of the secretion of trypsin resembled that for amylase (Fig. 7). The output of trypsin also attained maximal values in response to 30 CU/kg.hr secretin and remained unchanged with higher dose rates of secretin.

7. *Ratio of amylase to trypsin*

The ratio of amylase to trypsin tended to decrease throughout each 3 hr experiment. During the first hour of study, the ratio tended to be

greater with high intensity of stimulation but the differences did not achieve significance ($P > 0{\cdot}05$).

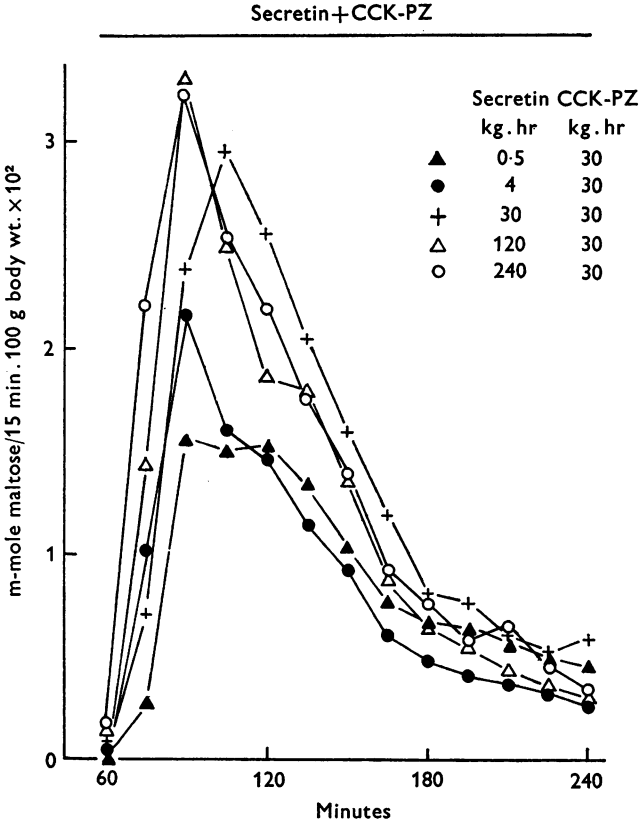

Fig. 7. Time course of secretion of trypsin in response to different dose rates of secretin with constant dose of CCK-PZ (30 IU/kg.hr). Meaning of symbols as in Fig. 2.

DISCUSSION

1. *Collection technique*

Almost all studies of pancreatic secretion in the rat have used the method introduced by Colwell (1951) for collecting pancreatic juice. The technique involves tying off, or cannulating, the upper end of the bile duct below the liver and cannulating its lower end, into which drain the pancreatic ductules. The disadvantage of annulation is that collection of juice may be incomplete, due to leakage around the cannula or to partial or complete obstruction to the flow of juice, with back pressure resulting in ductal rupture or pancreatic acinar damage (Colwell, 1951; Heatley, 1968*b*). Interference with flow or collection of the pancreatic juice results

in marked distortion of the secretory pattern, in view of the very small volumes of rat pancreatic secretion (Heatley, 1968*a*).

In order to avoid obstruction to the free flow of pancreatic juice and yet to ensure complete collection, a new technique has been developed, based on the method used for the collection of gastric juice in rats (Ghosh & Schild, 1958; Lai, 1964). Perfusion of the duodenum and collection of the perfusate for analysis obviates the need for operative manipulation of the biliary and pancreatic ductular systems and represents a particularly satisfactory method for analysing the characteristics of the processes involved in the secretion of pancreatic enzymes. That the technique is satisfactory is confirmed by Schmidt, Goebell & Johannson (1972), whose report, published during the course of the present study, utilizes a similar method for collecting rat pancreatic juice.

2. *Stimulation technique*

The study presents the only systematic investigation of the response of the rat pancreas to continuous I.V. infusion of different combinations of the principal small intestinal hormones which are considered at present to be involved in the stimulation of the pancreas. Previous investigations have reported the response of the rat pancreas to rapid I.V. injection of either secretin (Love, 1957; Svatoš & Jelinek, 1957; Grossman, 1958; Lin & Alphin, 1962; Debray, Vaille, de la Tour, Rozé & Souchard, 1962; Ramirez, Hubel & Clifton, 1966; Heatley, 1968*a*; Tachibana, 1971; Dockray, 1972) or cholecystokinin-pancreozymin (CCK-PZ) (Lin & Alphin, 1962; Debray, Vaille, de la Tour, Rozé & Souchard, 1963; Heatley, 1968*b*; Dockray, 1972), although it seems unlikely that rapid transient release of very large amounts of single hormones ever occurs under physiological conditions.

In the present study, only one dose combination of stimulant hormones has been used in each rat, since the secretory state of the rat pancreas changes markedly during the course of continuous stimulation with intravenously infused hormones. It seems probable, therefore, that 'step-dose' methods of assessing the secretory characteristics of the rat pancreas, as employed by Schmidt *et al.* (1972), result in artifactual dose-response curves, similar to those encountered during investigation of gastric secretion (Hirschowitz 1968; Hirschowitz & Sachs, 1969; Emås & Svensson, 1972).

3. *Sensitivity of the rat pancreas to stimulation*

In comparison with canine (Lin & Alphin, 1962; Stening & Grossman, 1969; Konturek, Tasler & Obtulowicz, 1972), feline (Way & Grossman, 1970; Konturek *et al.* 1971) and human (Wormsley 1968, 1969; Ribet,

Pascal & Vaysse, 1968) pancreatic enzyme secretion, much higher doses of both secretin and CCK-PZ are required to elicit maximal pancreatic enzyme secretion in the anaesthetized rat. Anaesthesia has been shown to decrease the volume of secretion in response to secretin in rats (Lin & Alphin, 1962), but it seems probable that the lack of sensitivity of rats to both hormones, demonstrated in the present study, is due mainly to species differences in responsiveness to the hormones derived from the porcine small intestine, since the difference in sensitivity between rats and dogs persists during anaesthesia (Lin & Alphin, 1962) and since the reduction in the amylase response to secretin plus CCK-PZ in dogs during anaesthesia is only 30% (Ben-Ari, Rudick, Kark & Dreiling, 1967).

The present study has shown that in rats secretin potentiates the secretion of enzymes in response to submaximal doses of CCK-PZ, although the enzyme secretory response to maximal or supramaximal doses of CCK-PZ is not increased by secretin, unlike the findings in man (Wormsley, 1969) and dog (Henricksen, 1968; Csendes, Isenberg & Grossman, 1971), both of which species show greater response to the combined hormones than, maximally, to the individual ones.

4. *Time course of the enzyme response to combinations of hormones*

The time courses of the responses to all combinations of secretin and CCK-PZ were similar and included a rapid initial increase in the output of enzymes, followed by a short peak response, which was better sustained with high, than with low, doses of CCK-PZ and with CCK-PZ than with secretin. The peak response was always followed by a rapid fall to much lower rates of enzyme secretion. Poorly sustained secretion of enzymes, despite constant rate I.V. infusion of stimulant hormones, has also been observed in cats (Hansson, 1959), dogs (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1969) and man during stimulation with secretin (Sarles, Prezlin, Souville & Figarella, 1966; Wormsley, 1968) or with secretin plus CCK-PZ (Schütz, Andersson & Lagerlöf, 1969). The phenomenon cannot be explained solely, or even mainly, in terms of pancreatic 'exhaustion', since the pattern of enzyme secretion is independent of the magnitude of the hormonal stimulus. Moreover, microscopy of the pancreas during the phase of low secretory response demonstrates the presence of plentiful zymogen granules (to be published). It seems, therefore, that enzyme secretion is inhibited either because the stimulus becomes relatively ineffective or because there is interference with the processes involved in the synthesis or secretion of the enzymes. In this connexion, it is not clear whether the decreased enzyme secretion indicates a direct effect of the small intestinal stimulant hormones, their breakdown products or contaminants, and represents a form of 'tachyphylaxis', due to

inactivation of receptor sites, or is due to secondary hormonal or metabolic effects. It has been demonstrated that the response to CCK-PZ involves the secretion of glucagon (Unger, Ketterer, Dupré & Eisentraut, 1967; Buchanan, Vance, Morgan & Williams, 1968; Fussgänger, Straub, Goberna, Jaros, Schröder, Raptis & Pfeiffer, 1969; Iversen, 1971) and promotes the secretion of other hormones such as calcitonin either directly (Care, Bruce, Boelkins, Kenny, Conaway & Anast, 1971; Cooper, Schwesinger, Ontjes, Mahgoub & Munson, 1972) or indirectly (Avioli, Birge, Scott & Shieber, 1969; Care, Bates & Gitelman, 1970). Both glucagon (Dyck, Rudick, Hoexter & Janowitz, 1969, 1970; Nakajima & Magee, 1970) and calcitonin (Schmidt, Hesch, Hüfner, Paschen & Creutzfeldt, 1971; Minne, Ziegler, Bellwinkel & Hotz, 1972) have been shown to inhibit the secretion of pancreatic enzymes, as have high concentrations of glucose (Robberecht & Christophe, 1971*a*) and low concentrations of calcium (Robberecht & Christophe, 1971*b*; Argent, Case, Fraser & Scratcherd, 1972; Kanno, 1972; Heisler, Fast & Tenenhouse, 1972).

5. *Enzyme secretion in response to increasing dose rates of hormones*

A biphasic pancreatic enzyme response to CCK-PZ has been demonstrated in the present study, since the output of enzymes rises with increasing doses of *CCK-PZ* to reach maximal values with 60 or 120 IU/kg.hr, but decreased significantly with the highest dose of the hormone. A similar biphasic pattern of pancreatic enzyme secretion has been demonstrated in rats in response to injection of CCK-PZ (Debray *et al.* 1963; Leroi, Morisset & Webster, 1971) and acetyl choline (Debray, de la Tour, Vaille, Rosé & Souchard, 1972) and in man in response to secretin (Wormsley, 1968, 1971). It had been postulated that the decrease in enzyme secretion observed with high doses of hormones indicated inhibition of release of enzymes from the pancreas, in view of the post-stimulatory rebound secretion of enzymes (Wormsley, 1968). No rebound increase of enzyme secretion was observed in the present study after stopping the infusion of the high dose rate of CCK-PZ, perhaps because the longer duration of stimulation had more markedly altered the state of enzyme-secreting cells.

Biphasic dose–response patterns appear to be a property of the pancreatic enzyme-secreting cells themselves, since supramaximal decrease in pancreatic enzyme secretion has been described in pancreatic fragments in response to acetyl choline (Hokin, 1968), carbamylcholine (Kulka & Sternlight, 1968; Robberecht & Christophe, 1971*a*, *b*) and to caerulein (Vincent & Baudin, 1972).

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