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THE ACTIONS OF PANCREOZYMIN IN PANCREAS SLICES AND THE ROLE OF PHOSPHOLIPIDS IN ENZYME SECRETION

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In previous papers it was shown that pancreas slices actively secrete digestive enzymes (amylase, lipase and ribonuclease) when incubated in the presence of cholinergic agents such as acetylcholine and carbamylcholine (Hokin, 1951*a*; Schucher & Hokin, 1954). It was further shown that the stimulation of enzyme secretion was associated with a marked stimulation of the incorporation of ^{32}P into the ether-soluble phospholipids (Hokin & Hokin, 1953, 1954, 1955*b*). The data in these earlier papers were not sufficient to prove that the phospholipid effect plays a role in the secretion of enzymes, for although the predominant effect of cholinergic agents in the pancreas is the secretion of enzymes, in some species there may be some stimulation of water and bicarbonate secretion (see Babkin, 1950). Fortunately, the existence of two separate hormones—pancreozymin, which stimulates exclusively enzyme secretion, and secretin, which stimulates only water and bicarbonate secretion (Harper & Vass, 1941; Harper & MacKay, 1948; Davies, Harper & MacKay, 1949; Jorpes & Mutt, 1954), has enabled the question to be solved in a most satisfactory manner.

The work reported here shows that the secretion of amylase by pancreatic tissue can be elicited *in vitro* in response to the hormone pancreozymin, and that under these conditions the incorporation of ^{32}P into the ether-soluble phospholipids is stimulated in essentially the same manner as was found previously with cholinergic drugs. The degree of stimulation is the same with either pancreozymin or acetylcholine, and the reaction is shown in each case to involve turnover of phosphate in preformed phospholipids and not synthesis *de novo* of the whole phospholipid molecule. Secretin has no effect on the incorporation of ^{32}P into the phospholipids under these conditions. The phospholipid effect does not seem therefore to be related to water and ion secretion, nor is it a side-effect encountered only with cholinergic drugs. It is concluded that the process of active extrusion of enzyme from the cell involves the turnover of phosphate in the phospholipids of the pancreas.

METHODS

Normal fed pigeons were used. Pancreas slices were prepared as previously reported (Hokin & Hokin, 1953), except that no drugs were administered to the animals before they were killed. Approximately 100 mg of tissue were added to each vessel; the final volume of the medium was 3 ml. Slices were submitted to preliminary incubation for 15 min and then transferred to the experimental vessels as described previously (Hokin & Hokin, 1953). Incubations were carried out in a Warburg bath at 39° C, the duration of incubation being 2 hr except where otherwise indicated. In experiments in which O_2 uptake was measured, a modified (Hokin, 1951 *a*) medium III of Krebs (1950) was used, with O_2 as the gas phase; in the other experiments the medium was the bicarbonate saline of Krebs & Henseleit (1932) with 5% CO_2 + 95% O_2 as the gas phase.

Samples of highly purified pancreozymin and secretin were very kindly supplied by Dr Erik Jorpes, who has reported on the method of preparation and standardization (Jorpes & Mutt, 1953 *a*, *b*, 1954, 1955). The hormone preparations used here were assayed in Dr Jorpes's laboratory. 0·2 mg of the pancreozymin preparation was found to give a strong stimulation of enzyme secretion in the cat. The secretin preparation had a potency of 11,000 cat units/mg, the cat unit being defined as that amount of secretin which will yield a quantity of pancreatic juice containing 0·1 ml. of 0·1 N-$NaHCO_3$. The hormone preparations were dissolved in water and the solutions were either used immediately or stored for not more than 6 days at 4° C.

Approximately 10 μc of ^{32}P as NaH_2PO_4 were added to each vessel. The specific activity of the glycerol-1-^{14}C was 1 μc/μmole; 2·5 μmole were added to each vessel. The amount of amylase synthesized and the amount of amylase secreted were assayed as previously described (Hokin, 1951 *a*, *b*). Amylase activities are expressed as units of Smith & Roe (1949) per mg of the initial wet weight of the tissue. The specific activities of the acid-soluble phosphate esters were determined as described previously (Hokin & Hokin, 1953). The ether-soluble phospholipids were extracted and purified as described (Hokin & Hokin, 1953), except that all evaporations were carried out on a water bath at 55° C under nitrogen. Glycerophosphate was isolated by paper electrophoresis from alkaline hydrolysates of the total phospholipids (Hokin & Hokin, 1954). All ^{32}P specific activities are expressed as counts/min/μg P corrected to an initial specific activity of 100,000 counts/min/μg P for the inorganic P of the medium.

RESULTS

The stimulation by pancreozymin of enzyme secretion and the incorporation of ^{32}P into the phospholipids in pigeon pancreas slices

In pigeon pancreas slices pancreozymin stimulated both the secretion (active extrusion) of amylase from the cells into the medium and the incorporation of ^{32}P into the ether-soluble phospholipids. Within the effective range, these effects increased with increasing concentrations of pancreozymin (Table 1). With this preparation of pancreozymin the minimum effective concentration for stimulating enzyme secretion and the incorporation of ^{32}P into the phospholipids was between 1 and 10 μg/ml.: the maximum effective concentration for the phospholipid effect was between 100 and 200 μg/ml. As was the case with acetylcholine, the concentration of pancreozymin which gave maximal enzyme secretion was lower than that necessary to produce maximal stimulation of the incorporation of ^{32}P into the phospholipids. It should be emphasized, however, that the stimulation of enzyme secretion by pancreozymin was always accompanied by some increase in the incorporation of ^{32}P into the

phospholipids. We previously reported (Hokin & Hokin, 1954) that enzyme secretion could be stimulated half-maximally by a concentration of carbamylcholine sufficiently low to produce no 'significant' effect on the incorporation of ^{32}P into the phospholipids. Actually, there was a 43% increase in the incorporation of ^{32}P into the phospholipids when enzyme secretion was stimulated

TABLE 1. Effect of increasing concentrations of pancreozymin on amylase secretion and the incorporation of ^{32}P into the ether-soluble phospholipids in slices of pigeon pancreas

	Expt. 1		Expt. 2	
Pancreozymin (μg/ml.)	Amylase in medium (units/mg fresh wt. tissue)	Specific activity of phospholipids (counts/min/μg P)	Amylase in medium (units/mg fresh wt. tissue)	Specific activity of phospholipids (counts/min/μg P)
0	8·6	71	3·9	84
0·01	8·3	68	—	—
0·1	8·8	66	—	—
1	8·6	63	—	—
10	10·7	82	5·9	135
50	—	—	7·1	520
100	13·4	1065	7·4	1150
200	—	—	5·7	1400

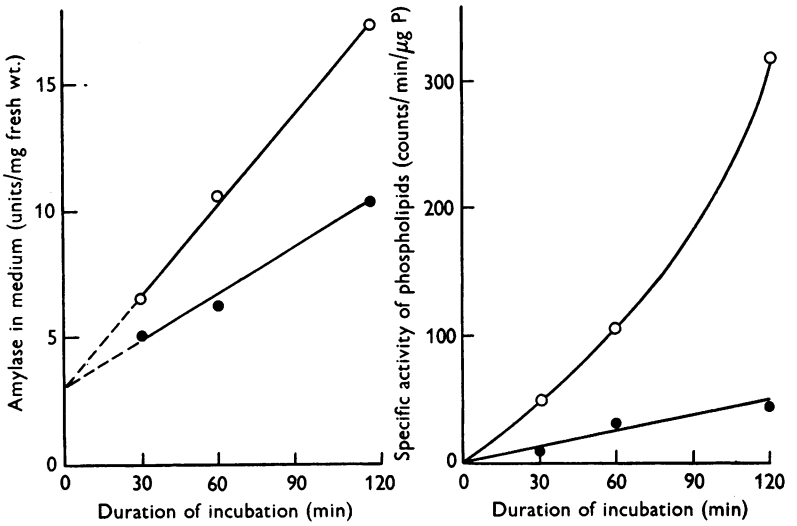

Fig. 1. Effects of pancreozymin on the rate of secretion of amylase and the rate of incorporation of ^{32}P into the ether-soluble phospholipids of pigeon pancreas slices. Solid circles, control; open circles, pancreozymin (100 μg/ml.).

half-maximally with carbamylcholine (10^{-6} M), but this was not considered significant as compared to the maximal stimulation, which could exceed 1000%. A further analysis of all the experiments has shown that there has never been a case of a definite stimulation of enzyme secretion by either cholinergic agents or by pancreozymin which has not been accompanied by some increase in the incorporation of ^{32}P into the phospholipids.

The rates of secretion of amylase and the incorporation of ^{32}P into the phospholipids during the 2 hr incubation period are illustrated in Fig. 1. Over this period of incubation the rate of enzyme secretion in the presence of pancreozymin was linear, whilst the rate of incorporation of ^{32}P into the phospholipids showed an increase. With acetylcholine the rates of both of these phenomena decreased with time (Hokin & Hokin, 1953), although the cell still had an appreciable enzyme content after incubation. Harper & MacKay (1948) found that the rate of enzyme secretion in response to pancreozymin *in vivo* was linear over a 2 hr period, but that the increased output of enzymes in response to vagal stimulation declined progressively after the

TABLE 2. The incorporation of ^{32}P into the acid-soluble phosphate esters of pigeon pancreas slices in the presence of pancreozymin. Further data from the experiment illustrated in Fig. 1

Duration of incubation (min)	Specific activity of acid-soluble phosphate esters (counts/min/μg P)	
	Control	+Pancreozymin (100 μg/ml.)
30	2210	1980
60	3880	3270
120	5480	5940

TABLE 3. The oxygen uptake of pigeon pancreas slices in the presence of pancreozymin and acetylcholine. The slices were incubated for 30 min before adding the secretory agents

Vessel additions	$\frac{Q_{O_2} \text{ after addition}}{\text{Initial } Q_{O_2}} \times 100$*
None	91
Pancreozymin (75 μg/ml.)	93
Acetylcholine (10^{-5} M) + Eserine (3×10^{-4} M)	85

* The Q_{O_2} values are for the 30 min immediately after adding and are expressed as a percentage of the initial values before adding.

first half-hour. They were unable to attribute any physiological significance to this as they point out that the diminution in enzyme output might have been due either to general deterioration of the animal's condition or to damage to the vagal trunk, although there were no obvious signs of these. In view of the fact that the same picture is also found *in vitro*, it is possible to conclude that the pancreas cells become refractory to cholinergic stimulation if this is prolonged, but that they do not become refractory to pancreozymin under the same conditions.

Respiration and secretory function

In pigeon pancreas slices pancreozymin did not affect the rate of incorporation of ^{32}P into the acid-soluble phosphate esters (Table 2). Nor was the rate of O_2 uptake increased in the presence of either pancreozymin or acetylcholine (Table 3). Previous work, also with pigeon pancreas slices, showed that acetylcholine did not increase the rate of incorporation of ^{32}P into the acid-soluble phosphate esters (Hokin & Hokin, 1953). Deutsch & Raper (1936) and

Davies *et al.* (1949) found that acetylcholine and pancreozymin acted as respiratory stimulants in isolated cat pancreatic tissue. The action of these agents as respiratory stimulants would seem therefore to be species-dependent. Since secretion of enzymes occurs in pancreas slices in response to these agents without any stimulation of respiration, the respiratory stimulation cannot be an essential event in the mechanism of action of these secretory stimulants.

Comparison of the effect of pancreozymin on the incorporation of glycerol-1-^{14}C and ^{32}P into the glycerophosphatides

Acetylcholine stimulated the incorporation of ^{32}P into the glycerophosphatides of pancreas slices over ninefold, but the incorporation of glycerol-1-^{14}C was not increased by more than 20% (Hokin & Hokin, 1954). When pancreozymin was used as the secretory stimulant the same picture was found. In the presence of pancreozymin, glycerol-1-^{14}C incorporation was increased by only 30%, whereas ^{32}P incorporation was increased over fivefold (Table 4).

TABLE 4. Comparison of the effect of pancreozymin on the incorporation of glycerol-1-^{14}C and ^{32}P into the glycerophosphate of the phospholipids of pigeon pancreas slices

	Specific activity of glycerophosphate of phospholipids* (counts/min/μg P)	
Vessel additions	^{14}C	^{32}P
None	21	56
Pancreozymin (100 μg/ml.)	28	292

* Incubations with glycerol-1-^{14}C and ^{32}P were carried out in separate vessels.

These results confirm that the increased incorporation of ^{32}P is due to an increased turnover of phosphate of the phospholipid and not to synthesis *de novo* of the whole molecule. It was previously shown using acetylcholine that the phosphate turnover was stimulated in phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and phosphoinositide, the latter accounting for 75% of the overall increase in turnover. It was further shown that the incorporation of ethanolamine-2-^{14}C and choline (derived metabolically from the ethanolamine) into phosphatidyl ethanolamine and phosphatidyl choline respectively was increased to the same extent as the incorporation of ^{32}P (Hokin & Hokin, 1955*b*). By analogy, it can be argued that the incorporation of serine and inositol into their respective phospholipids is also increased to the same extent as the ^{32}P incorporation. Thus, the stimulation of enzyme secretion would appear to involve the turnover of phosphate ester moieties in the various phospholipid molecules concerned.

Comparison of the effects of pancreozymin and acetylcholine and the action of atropine

Previous work indicated that the concentration of acetylcholine required to give a maximal phospholipid effect in pigeon pancreas slices was of the order of 10^{-5}M (Hokin & Hokin, 1954). The concentration of the pancreozymin preparation required to give a maximal effect was about 150 μg/ml. Approximately the same percentage stimulation of both enzyme secretion and the incorporation of ^{32}P into the phospholipids was obtained when maximal concentrations of acetylcholine and pancreozymin were compared in the same experiment. Added together in concentrations which were submaximal for

TABLE 5. Effects of pancreozymin and acetylcholine added together in pigeon pancreas slices

Expt.	Vessel additions: Pancreozymin (μg/ml.)	Vessel additions: Acetylcholine* (M concn.)	Amylase in medium (units/mg fresh wt. tissue)	Specific activity of ether-soluble phospholipids (^{32}P) (counts/min/μg P)
1	—	—	6·3	42
	10	—	9·6	142
	—	10^{-7}	—	156
	10	10^{-7}	10·5	280
	50	—	10·7	470
	—	10^{-6}	12·0	514
	50	10^{-6}	11·4	616
	150	—	11·6	676
	—	10^{-5}	12·8	730
2	—	—	9·1	53
	75	—	12·8	285
	—	10^{-6}	13·7	190
	75	10^{-6}	13·3	427
	—	10^{-5}	13·6	487
3	—	—	4·1	73
	150	—	9·3	467
	—	10^{-5}	9·0	572
	150	10^{-5}	9·6	650

* Eserine (3×10^{-4}M) was added with the acetylcholine.

the phospholipid effect, pancreozymin and acetylcholine had an additive effect on the incorporation of ^{32}P into the phospholipids up to the maximal percentage stimulation found with either agent added individually in higher concentrations. The addition of acetylcholine and pancreozymin together, each in a concentration maximal for the phospholipid effect, did not produce any significant increase above the effects observed with either agent alone (Table 5). It seems from these results that the responsiveness of the gland to secretory agents reaches a maximum which is the same for any particular gland, regardless of the method of stimulation.

Harper & Raper (1943) found that the secretory effect of pancreozymin on the pancreas *in vivo* was not affected by doses of atropine sufficient to abolish enzyme secretion brought about by cholinergic stimulation. We have found the same to be true *in vitro* (Table 6). Concentrations of atropine as high as

10^{-4}M did not abolish the secretion of enzymes from slices incubated in the presence of pancreozymin. Nor did atropine have any effect on the stimulation by pancreozymin of ^{32}P incorporation into the phospholipids. The effects of the maximal concentration of acetylcholine (10^{-5}M) on enzyme secretion and on the incorporation of ^{32}P into the phospholipids were completely abolished by 10^{-6}M atropine, which had no effect on the actions of pancreozymin (150μg/ml.) in the same experiment. The failure of atropine to block the stimulatory effects of pancreozymin in pancreas slices rules out the possibility that these stimulatory effects might be due to contamination of the pancreozymin preparation with acetylcholine.

TABLE 6. Comparison of the effects of atropine on the responses of pancreas slices to pancreozymin and acetylcholine

	Vessel additions			Amylase in medium	Specific activity of ether-soluble phospholipids (^{32}P)
Expt.	Pancreozymin (μg/ml.)	Acetylcholine* (M concn.)	Atropine (M concn.)	(units/mg fresh wt. tissue)	(counts/min/μg P)
1	—	—	—	3·4	62
	—	—	10^{-4}	3·1	52
	100	—	—	6·1	326
	100	—	10^{-4}	5·4	342
	100	—	6×10^{-5}	6·4	—
	100	—	3×10^{-5}	5·3	326
2	—	—	—	15·0	98
	150	—	—	21·4	588
	150	—	10^{-6}	20·2	592
	—	10^{-5}	—	21·2	437
	—	10^{-5}	10^{-6}	12·0	100

* Eserine (3×10^{-4}M) was added with the acetylcholine.

Amylase synthesis in vitro *in the presence of pancreozymin or acetylcholine*

Throughout the work reported here and in preceding related work, we have used the term secretion to mean strictly the active extrusion of material across the plasma membrane (see Abercrombie, Hickman & Johnson, 1951). The term has been used by some to mean the overall process of elaboration, separation and discharge of material from the cell. Possibly because of this general type of definition, there has been considerable confusion in the interpretation of studies on secretion. It has often been assumed that changes in the rate of extrusion of enzymes from the pancreas give rise directly to changes in the rate of synthesis of the digestive enzymes. This has, in some cases, been extended to the assumption that secretory stimulants *per se*, both hormonal (as, for instance, elicited by feeding), and cholinergic (e.g. the injection of pilocarpine) give rise to an immediate increase in the rate of synthesis of digestive enzymes in the pancreas. The possibility of studying the synthesis of enzymes by the pancreas *in vitro*, where the total enzyme content of the system can be followed, has enabled more definite conclusions to be reached regarding the relationship between secretory stimulants and enzyme synthesis.

Previous work *in vitro*, using pigeon pancreas slices, showed that there was little direct relationship between the rate of enzyme secretion and the rate of enzyme synthesis under a variety of conditions (Hokin, 1951*a*; Hokin, 1956). Stimulation of the extrusion of enzymes by cholinergic drugs *in vitro* or by the injection of pilocarpine *in vivo* up to 2 hr before killing was never found to produce any increase in amylase synthesis during the incubation period. However, depletion of the mouse pancreas with cholinergic agents *in vivo* was followed after 24 hr by a period in which the rate of amylase synthesis (measured *in vitro*) was somewhat increased (Hokin, 1956). This was, however, a delayed effect suggesting that secretory stimulants *per se* have no stimulatory effect on amylase synthesis.

TABLE 7. Amylase synthesis *in vitro* in pigeon pancreas slices in the presence of pancreozymin or acetylcholine

Vessel additions		Amylase synthesis (units/mg fresh wt. tissue)		
Pancreozymin (μg/ml.)	Acetylcholine* (M concn.)	Expt. 1	Expt. 2	Expt. 3†
—	—	4·9	6·7	17·2
75	—	—	5·1	—
150	—	4·3	7·2	16·0
—	10^{-6}	—	6·0	—
—	10^{-5}	2·2	3·6	12·5
—	10^{-4}	−0·3	—	—
75	10^{-6}	—	7·1	—
150	10^{-5}	3·8	—	—
150	10^{-4}	3·6	—	—

* Eserine (3×10^{-4} M) was added with the acetylcholine.

† 0·2 ml. of an amino-acid mixture (4% acid hydrolysed casein + 0·4 mg/ml. L-tryptophan) were added to all vessels in Expt. 3; values given in this experiment are the average of results from duplicate vessels, the duplicates did not differ by more than 10%.

When pancreozymin (75–150 μg/ml.) was used as a secretory stimulant in pigeon pancreas slices there was no significant effect on the amount of amylase synthesized *in vitro*, either in the absence or presence of added amino-acid substrate. 10^{-6} M acetylcholine also had no effect on amylase synthesis in these experiments, but 10^{-5} M and 10^{-4} M acetylcholine produced some inhibition of synthesis (Table 7). (The pigeons used in these experiments were not treated by the procedure followed in previous work on amylase synthesis in which carbamylcholine was injected before killing to deplete the pancreas of enzymes.) The inhibitory action of acetylcholine on amylase synthesis was partially reversed by pancreozymin.

Effects of secretin in pigeon pancreas slices

Secretin, which stimulates the secretion of water and bicarbonate ions by the pancreas, had no effect on amylase secretion or on the incorporation of ^{32}P into the phospholipids of pancreas slices (Table 8). There was some stimulation of O_2 uptake (about 10%) during the 50 min after secretin was added. At the

lower concentrations of secretin there may have been a commensurate stimulation of the incorporation of ^{32}P into the acid-soluble phosphate esters. Secretin has been reported to increase respiration in isolated pancreatic tissue of a variety of species (Gerard & Still, 1933; Kiyohara, 1934; Deutsch & Raper, 1936; Davies *et al.* 1949), although Ågren (1934) was unable to confirm this in rat pancreas.

Although this work cannot be interpreted as showing conclusively that the pigeon pancreas can respond *in vitro* to secretin, it does serve the purpose for which it was designed. Since a highly potent preparation of secretin had no effect on the incorporation of ^{32}P into the phospholipids of the pancreas, the possibility that the effects noted with pancreozymin might be due to contamination with secretin can be excluded, and with it the possibility that the phospholipid effect is linked with water and bicarbonate transport.

TABLE 8. Effects of secretin in pancreas slices. The slices were incubated for 30 min before adding the secretin, then incubated for a further 90 min

Secretin (cat units*/ml.)	$\frac{Q_{O_2} \text{ after adding}\dagger}{\text{Initial } Q_{O_2}} \times 100$	Specific activities (^{32}P) (counts/min/μg P)		Amylase in medium (units/mg fresh wt. tissue)
		Acid-soluble phosphate esters	Ether-soluble phospholipids	
0	101	6880	87	4·0
0·005	107	7140	91	3·5
0·05	113	7180	78	4·1
0·5	112	7430	76	3·5
5	108	6720	67	4·3
50	114	6660	74	3·9

* As defined in the text.

† Q_{O_2} values are for the 50 min following addition and are expressed as a percentage of the initial values before adding.

DISCUSSION

In an earlier paper (Hokin & Hokin, 1954) evidence was presented which was thought to argue against the view that the turnover of phosphate in phospholipids was directly connected with the secretion of enzymes by the pancreas. It was found that, although the administration of pilocarpine or carbamylcholine to mice gave rise to an increased secretion of enzyme and an increased incorporation of ^{32}P into the phospholipids of the pancreas *in vivo*, the incorporation of ^{32}P into the phospholipids of the pancreas of both fed and fasted mice were found to be the same. The results were interpreted as showing that a pancreas which is secreting under physiological conditions (i.e. in response to feeding) does not have a higher rate of incorporation of ^{32}P than has a non-secreting pancreas (i.e. that of the fasting animal). However, in the course of another study we subsequently obtained evidence which indicated that the mouse pancreas secretes digestive enzymes spontaneously, the amount of material secreted over a given period being much the same, irrespective of whether the animal has been allowed to feed continuously or

has been fasted for 24 hr (Hokin, 1956). The finding of an equal rate of incorporation of ^{32}P into the phospholipids of the pancreas of fasted and fed mice is therefore not valid as evidence against a relationship between the phospholipid effect and enzyme secretion.

The disproportionate effects of cholinergic substances on amylase secretion and the incorporation of ^{32}P into the phospholipids was regarded as another line of evidence that the phospholipid effect might not be concerned with enzyme secretion; the concentration of cholinergic drug required to give a maximal secretion of amylase was in all cases less than the concentration required to give maximal stimulation of ^{32}P incorporation into the phospholipids. The earliest interpretation of our results, that the phospholipid effect was secondary to the secretion of enzymes (Hokin & Hokin, 1953), was accordingly modified to the view that the phospholipid effect may be a direct response to cholinergic drugs (Hokin & Hokin, 1954). Since this disproportionality has now been shown to exist in response to pancreozymin, this conclusion requires further modification. The fact that enzyme secretion elicited by either of these two very different types of stimulus is accompanied by an increased turnover of phosphate in phospholipids, coupled with the fact that secretin does not produce such an effect, indicates that enzyme secretion and the phospholipid effect are in fact directly related. The phospholipid effect appears to be a primary stage in the extrusion of material from the cell, rather than secondary to the passage of material through the plasma membrane, since the effect can be increased by concentrations of the stimulatory agents which no longer elicit further enzyme secretion. Presumably other factors place a limit on the maximum amount of enzyme which can be secreted.

From the evidence discussed here we have formulated the working hypothesis that all active transport of proteins out of the cell involves the breakdown and resynthesis of the glycerophosphate bond in phospholipids. Evidence that amylase secretion in the rabbit parotid gland and mucin secretion in the submaxillary gland, in response to either acetylcholine or adrenaline, are accompanied by the stimulation of ^{32}P incorporation into the phospholipids, has also been obtained in support of this hypothesis. We previously found that acetylcholine stimulated the incorporation of ^{32}P into the phospholipids of brain cortex slices (Hokin & Hokin, 1955*a*, *b*). At the time of publication it was thought that this might be connected with the transport of potassium ions. However, further work using ^{42}K has indicated that this is unlikely. Applying the above hypothesis we are inclined to the view that the simplest explanation of the phospholipid effect in brain cortex is that the secretion of some protein hormone at present of unknown nature is stimulated by acetylcholine.

SUMMARY

1. Pancreozymin stimulates the secretion (active extrusion) of amylase in pigeon pancreas slices; it has no effect on the rate of synthesis of amylase under these conditions.

2. The incorporation of ^{32}P into the ether-soluble phospholipids of the tissue is stimulated by pancreozymin in essentially the same manner as was found previously with cholinergic drugs. The incorporation of glycerol-1-^{14}C is not concomitantly increased, confirming that the phospholipid effect in each case involves turnover of phosphoryl units of the phospholipids and not synthesis *de novo* of the phospholipid molecule.

3. Pancreozymin does not affect the rate of incorporation of ^{32}P into the acid-soluble phosphate esters, nor does it increase the rate of O_2 uptake by pigeon pancreas slices.

4. The responsiveness of the pancreas *in vitro* to secretory agents, as measured by both enzyme secretion and the phospholipid effect, reaches a maximum which is the same for any particular gland regardless of whether the stimulation is effected by the use of pancreozymin, acetylcholine or a combination of the two.

5. Atropine, which abolishes all *in vitro* responses of the pancreas to cholinergic drugs, has no effect on the responses of pancreas slices to pancreozymin.

6. Secretin has no effect on the incorporation of ^{32}P into the phospholipids of pancreas slices.

7. It is concluded that the activation of the splitting and resynthesis of the glycerol-phosphate bond in the phospholipids is a primary stage in the process of active extrusion of enzymes from the cell.

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REFERENCES

ABERCROMBIE, M., HICKMAN, C. J. & JOHNSON, M. L. (1951). *A Dictionary of Biology*, p. 204. Harmondsworth, Middlesex: Penguin Books Ltd.

ÅGREN, G. (1934). Über die pharmakodynamischen Wirkungen und chemischen Eigenschaften des Secretins. *Skand. Arch. Physiol.* **70**, 10–87.

BABKIN, B. P. (1950). *Secretory Mechanism of the Digestive Glands*, 2nd ed. pp. 858–859. New York: Paul B. Hoeber Inc.

DAVIES, R. E., HARPER, A. A. & MACKAY, I. F. S. (1949). A comparison of the respiratory activity and histological changes in isolated pancreatic tissue. *Amer. J. Physiol.* **157**, 278–282.

DEUTSCH, W. & RAPER, H. S. (1936). Respiration and functional activity. *J. Physiol.* **87**, 275–286.

GERARD, R. W. & STILL, E. U. (1933). Studies on the physiology of secretin. V. The effects on the respiration of the excised pancreas. *Amer. J. Physiol.* **103**, 232–234.

HARPER, A. A. & MACKAY, I. F. S. (1948). The effects of pancreozymin and of vagal nerve stimulation upon the histological appearance of the pancreas. *J. Physiol.* **107**, 89–96.

HARPER, A. A. & RAPER, H. S. (1943). Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of small intestine. *J. Physiol.* **102**, 115–125.

HARPER, A. A. & VASS, C. C. N. (1941). The control of the external secretion of the pancreas in cats. *J. Physiol.* **99**, 415–435.

HOKIN, L. E. (1951*a*). The synthesis and secretion of amylase by pigeon pancreas *in vitro*. *Biochem. J.* **48**, 320–326.

HOKIN, L. E. (1951*b*). Amino-acid requirements of amylase synthesis by pigeon pancreas slices. *Biochem. J.* **50**, 216–220.

HOKIN, L. E. & HOKIN, M. R. (1955*a*). Effects of acetylcholine on phosphate turnover in phospholipids of brain cortex *in vitro*. *Biochim. biophys. acta*, **16**, 229–237.

HOKIN, L. E. & HOKIN, M. R. (1955*b*). Effects of acetylcholine on the turnover of phosphoryl units in individual phospholipids of pancreas slices and brain cortex slices. *Biochim. biophys. acta*, **18**, 102–110.

HOKIN, M. R. (1956). The formation of amylase by mouse pancreas *in vitro*. *J. biol. Chem.* **219**, 77–83.

HOKIN, M. R. & HOKIN, L. E. (1953). Enzyme secretion and the incorporation of ^{32}P into phospholipids of pancreas slices. *J. biol. Chem.* **203**, 967–977.

HOKIN, M. R. & HOKIN, L. E. (1954). Effects of acetylcholine on phospholipids in the pancreas. *J. biol. Chem.* **209**, 549–558.

JORPES, E. & MUTT, V. (1953*a*). New method for the preparation of secretin. *Ark. Kemi*, **6**, 273–276.

JORPES, E. & MUTT, V. (1953*b*). Purification of secretin by freezing out of impurities fron methanolic solution at $-80°$. *Nature, Lond.*, **172**, 124.

JORPES, E. & MUTT, V. (1954). On the action of highly purified preparations of secretin and pancreozymin. *Ark. Kemi*, **7**, 553–559.

JORPES, E. & MUTT, V. (1955). On the strength of secretin. *Ark. Kemi*, **8**, 49–51.

KIYOHARA, K. (1934). Action de la sécrétine sur la respiration des tissues de certains organes. *C.R. Soc. Biol., Paris*, **116**, 1166–1168.

KREBS, H. A. (1950). Body size and tissue respiration. *Biochim. biophys. acta*, **4**, 249–269.

KREBS, H. A. & HENSELEIT, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyl. Z.* **210**, 33–66.

SCHUCHER, R. & HOKIN, L. E. (1954). The synthesis and secretion of lipase and ribonuclease by pigeon pancreas slices. *J. biol. Chem.* **210**, 551–557.

SMITH, B. W. & ROE, J. H. (1949). A photometric method for the determination of α-amylase in blood and urine, with the use of the starch iodine colour. *J. biol. Chem.* **179**, 53–59.