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The Synthesis and Secretion of Amylase by Pigeon Pancreas *in vitro*

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In this investigation a new approach has been made to the study of protein synthesis in animal tissues *in vitro*. An incorporation of labelled amino-acids into proteins has been shown by previous workers to occur in tissue homogenates and slices, and this has been regarded as evidence of protein synthesis *in vitro*. However, no net increase in proteins has so far been reported.* In planning the present experiments, it was thought that the chance of detecting a net increase of a protein would be good if digestive glands were used as the experimental material. These glands are equipped to synthesize enzymes, i.e. proteins, at rapid rates, and many of these enzymes can be quantitatively determined.

The first series of experiments on the formation of pepsin by gastric mucosa proved unsuccessful. The formation of amylase by pigeon pancreas slices was next studied, and this proved a suitable system. Under appropriate conditions the amylase activity increased by over 100% on aerobic incubation.

Part of this work has been communicated to the Biochemical Society (Hokin, 1950).

EXPERIMENTAL

Media. The following media were used: (1) the bicarbonate saline of Krebs & Henseleit (1932), gassed with either 5% CO_2 + 95% O_2 or 5% CO_2 + 95% N_2; (2) medium III (without the organic constituents) of Krebs (1950), gassed with O_2; (3) phosphate saline of Krebs & Eggleston (1940), gassed with O_2; (4) sheep serum (inactivated by heating at 60° for 2 hr.), gassed with 5% CO_2 + 95% O_2; the heat treatment destroyed the serum amylase activity. NaOH (0·2 ml. of a 10% (w/v) solution) was placed in the centre well when medium 2 or 3 was used. All media contained 0·2% (w/v) glucose. In some experiments either 'supplemented casein hydrolysate' or a mixture of amino-acids was added. The supplemented casein hydrolysate consisted of acid-hydrolysed casein prepared by the method of McIlwain & Hughes (1944) with 1·5 parts of L-tryptophan added per 100 parts of original casein. The amino-acid mixture was added to the media to give a concentration of 20 mg./100 ml. of L-arginine, L-aspartic acid, L-citrulline, L-cystine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-tryptophan, L-tyrosine and L-valine, and 40 mg./100 ml. of DL-serine and DL-threonine.

When the effects of secretory stimulants were studied, carbamylcholine, pilocarpine or acetylcholine with eserine were either placed in the main compartment of the manometer flask initially or tipped from the side arm after 30 min. incubation. The latter procedure was followed when the effect of secretory stimulants on tissue respiration was studied.

Preparation and incubation of pigeon pancreas. Pigeon pancreas has the advantage over the pancreas of many other species of being relatively homogeneous; it contains very little adipose tissue, and its consistency lends itself well to the slicing technique.

The following procedure was found to give good results and was adopted for the preparation of the tissue. Pigeons were provided with an abundant supply of 'pigeon corn'. About 45 min. to 1 hr. prior to killing 0·09–0·15 mg. of carbamylcholine was administered intramuscularly. Salivation usually began within 5 min., and prostration occasionally occurred. The birds were killed by decapitation, the abdomen was immediately plucked and a substernal incision was made. The duodenal loop, which contains the pancreas, was exteriorized and both lobes of the pancreas were dissected from the duodenum with a pair of sharp scissors. The pancreas and duodenum of pigeons receiving carbamylcholine were usually hyperaemic. Care was taken to avoid puncturing the duodenum in order to prevent contamination of the tissue by the duodenal contents.

The lobes were carefully freed of connective tissue, and slices approximately 0·5 mm. thick were made with the slicer of Stadie & Riggs (1944). The base of the slicer was chilled before use. Unless otherwise specified, the tissue was placed immediately after slicing in a covered crystallizing dish lying in chipped ice. Wetted filter paper was kept in the dish to maintain a high humidity. When sufficient slices had been prepared, they were weighed on a torsion balance and placed in conical manometer flasks (18–26 ml. volume) containing

* Since this paper was submitted it has come to the author's attention that Peters & Anfinsen (1950) have reported a net synthesis of albumin by chicken-liver slices.

the media. Since the amylase activity per mg. dry weight of either lobe of the pancreas agreed within the experimental error (average percentage error between duplicate slices from nineteen pigeons, 5 %, extreme range 25 %) slices from both lobes could be used, but usually only slices from the large lobe were taken. From 35 to 75 mg. (wet wt.) of tissue were placed in each flask. In each experiment unincubated weighed slices were dried in an oven overnight at approximately 105°, and the ratio of the dry weight to the wet weight (about 0·23) was thus obtained. From this ratio the initial dry weights of the incubated slices were calculated.

The manometer flasks were kept in a tray of ice before they were attached to manometers. From 35 to 45 min. elapsed from the time of killing until all the vessels were placed in the bath. The tissues were incubated for 2 hr. at 40° unless otherwise specified. In some of the experiments gas exchanges were recorded.

Treatment of tissues and media after incubation. Immediately after incubation the vessels were returned to a tray of ice. The slices were removed from the vessels with forceps, drained on Whatman no. 50 filter paper, placed in a small mortar and ground with Calais sand and 2 drops of water for 1·5 min. At the end of this time the mixture had the consistency of a creamy paste. Water (5 ml. minus 2 drops) was then added, and the mixture was ground for another 1·5 min. The mixture was then centrifuged for 15 min. Portions (1 ml.) of the supernatant and the medium were each diluted by adding to 10, 15 or 20 ml. water. The degree of dilution was determined by the expected enzyme activity. The same dilution was used in any one series of experiments. These solutions were either immediately assayed or stored in the refrigerator overnight and assayed on the next day. No change in enzyme activity was observed when the diluted enzyme solutions were stored in the refrigerator for periods up to 5 days.

Methods of assay. Amylase was assayed by the method of Smith & Roe (1949). Since Lintner's soluble starch (Merck) was not initially available, the following modifications of the original procedure were used. A 3 % (w/v) solution of Analar soluble starch (obtained from Hopkins and Williams Ltd., 17 St Cross Street, London, E.C. 1) was used instead of the 1·2 % (w/v) solution of Lintner's soluble starch (Merck) recommended by Smith & Roe (1949). The use of Analar starch in a higher concentration was made possible because its solutions had a lower viscosity than those of Lintner's soluble starch (Merck). The quantity of I_2-KI solution was four times that recommended by Smith & Roe (1949). The range of the method was increased by the use of higher starch and I_2 concentrations. However, when a second batch of Analar starch was found to differ from the first by a markedly higher viscosity, this brand of starch was abandoned. Lintner's soluble starch (Merck), which had then become available (obtained from Merck & Co., Rahway, N.J., U.S.A.), was used according to the specifications of Smith & Roe (1949).

By assaying several enzyme solutions with the modified method and the method of Smith & Roe (1949), a correction factor (0·87) was obtained to convert the units of the modified method into the units of the method of Smith & Roe (1949).

The approximate maximal amylase activity from each series was determined in a preliminary assay. The time of incubation in the final assay was adjusted accordingly, so that the range of the method was not exceeded. This procedure was permissible because the rate of starch digestion was constant over the maximum period tested (30 min.). The starch-I_2 colours were read in a Beckman spectrophotometer at 620 mμ.

The enzyme activities of the medium and tissue were expressed in units of Smith & Roe (1949) per mg. initial dry weight of tissue. The sum of the medium and tissue amylase activities was referred to as the 'total amylase activity'. It was found that in duplicate experiments the partition of amylase activity between tissue and medium varied more widely than the total amylase activity.

RESULTS

Reliability of amylase assay in slices. When freshly prepared slices of varying weight from different parts of the pancreas were extracted in the same amount of water, the amylase activities found per mg. dry weight agreed within 4 % (Table 1). This shows that amylase was completely extracted and was evenly distributed in the pancreas.

Table 1. *Variations in amylase activity of unincubated slices from a single pancreas*

(No carbamylcholine given.)

Dry weight of slice (mg.)	Amylase activity (units of Smith & Roe/ mg. dry wt.)
11·0	82
19·8	81
26·2	83
30·2	84

The following substances which were present in the medium of some experiments did not interfere with the amylase assay in the concentrations indicated: carbamylcholine (0·1 mg./100 ml.), acetylcholine (0·1 mg./100 ml.), eserine (0·1 mg./100 ml.), pilocarpine (0·1 mg./100 ml.), supplemented casein hydrolysate (6 mg./100 ml.), bicarbonate saline (1:90 dilution), 2:4-dinitrophenol (10^{-6} M), potassium cyanide (10^{-6} M), sodium iodoacetate (10^{-5} M), serum (1:90 dilution), glucose (2 mg./100 ml.) and the mixture of twenty-one amino-acids (0·2 mg./100 ml. for each amino-acid, except DL-serine and DL-threonine, which were present in 0·4 mg./100 ml.). The concentrations tested were the highest ever present.

Depletion of amylase from the pancreas in vivo. In the beginning of this study the diets of pigeons were not rigidly controlled, nor was carbamylcholine administered prior to killing. Unincubated pancreas slices from sixteen such pigeons gave a mean amylase activity of 160 units/mg. dry wt., range 81–420 units/mg. dry wt. The increase in amylase activity on incubation of such slices was inconsistent. It was thought desirable to use pancreas tissue low in amylase for the study of enzyme synthesis, and attempts were therefore made to deplete the tissue of the enzyme. It is known that the ingestion of food or

cholinergic drugs generally deplete the pancreas of its enzyme stores. Pigeons were therefore provided with a continuous supply of food and were injected with carbamylcholine 45 min. to 1 hr. before killing. The mean amylase level of unincubated pancreas slices from thirteen pigeons was reduced to 53 units/mg. dry wt., range 28–93 units/mg. dry wt. When pancreas slices from these pigeons were incubated under appropriate conditions increases in total amylase activity were consistently observed.

Increase of amylase activity on aerobic incubation. On aerobic incubation in saline containing glucose and supplemented casein hydrolysate the total amylase activity of pancreas slices increased approximately two- to three-fold during 3 hr. of incubation (Table 2). The rate of increase, after an initial lag period during the first 30 min., was fairly constant. Exp. 1 of Table 2 shows that considerable amounts of amylase were discharged into the medium within 30 min. with a concomitant fall in the tissue amylase content. In this experiment the slices were cut dry and kept in the chilled chamber before incubation. In Exp. 2 of Table 2, in which the slices were suspended in chilled basal saline (similar to the saline of Krebs & Henseleit (1932), but containing 0·004 M-HCO_3^-) before incubation, both the amylase of the medium and tissue rose progressively. The initial rapid rise in the amylase content of the medium and the concomitant fall in the tissue amylase in Exp. 1 were thus probably due to the passive discharge of the enzyme resulting from initial tissue damage rather than to active secretion. The suspension of slices in chilled saline prior to incubation is probably a preferable procedure. Most of the experiments reported in this paper were carried out before this was discovered.

Table 2. *Rate of amylase synthesis by pigeon pancreas slices*

(Bicarbonate saline gassed with 5% CO_2+95% O_2; 0·2% glucose; 0·4% supplemented casein hydrolysate.)

Exp. no.	Period of incubation (min.)	Amylase activity (units of Smith & Roe/ mg. initial dry wt.) Medium	Tissue	Total
1	0	0	59	59
	30	16	48	64
	60	21	59	80
	120	31	75	106
	180	31	117	148
2	0	0	52	52
	30	2	55	57
	60	12	70	82
	120	17	131	148
	180	27	153	180

Effect of aerobic and anaerobic incubation on total amylase activity. An increase in total amylase activity upon incubation does not necessarily prove that a complete synthesis of amylase has occurred. An activation of a closely related precursor, analogous to the conversion of trypsinogen to trypsin, might account for such an effect. If this were the case, the increase in total amylase activity might be expected to occur anaerobically, since it is known that the activation of zymogens does not require an external source of energy (Northrop, 1948). The results of aerobic and anaerobic incubation of pancreas slices in saline containing glucose and supplemented casein hydrolysate can be seen in Table 3. There was no increase in total amylase activity when slices were incubated anaerobically, whilst aerobically the total amylase activity rose approximately 100%. These observations support the view that the increase in total amylase activity represents a real synthesis of amylase.

Secretion of amylase. Experiments were carried out to test whether the amylase activity of the medium would be increased in the presence of the secretory stimulant, carbamylcholine. The results are included in Table 3. Aerobically, but not anaerobically, carbamylcholine increased the fraction of amylase in the medium, indicating that the

Table 3. *Synthesis and secretion of amylase under aerobic and anaerobic conditions*

(Bicarbonate saline; 0·2% glucose; 0·4% supplemented casein hydrolysate; phosphorus in centre well when gas phase 5% CO_2+95% N_2.)

Period of incubation (hr.)	Carbamylcholine (mg./100 ml.)	Gas phase	Amylase activity (units of Smith & Roe/ mg. initial dry wt.) Medium	Tissue	Total
0	—	—	0	35	35
0	—	—	0	39	39
2	0	5% CO_2+95% N_2	10	17	27
2	0	5% CO_2+95% N_2	11	22	33
2	1	5% CO_2+95% N_2	10	21	31
2	1	5% CO_2+95% N_2	8	28	36
2	0	5% CO_2+95% O_2	19	57	76
2	0	5% CO_2+95% O_2	19	55	74
2	1	5% CO_2+95% O_2	28	41	69
2	1	5% CO_2+95% O_2	30	43	73

Table 4. *Effect of metabolic inhibitors on synthesis and secretion of amylase*

(Bicarbonate saline; 0·2% glucose; 0·4% supplemented casein hydrolysate; phosphorus in centre well when gas phase 5% CO_2 + 95% N_2.)

Period of incubation (hr.)	Inhibitor added (M concn.)	Gas phase	Amylase activity (units of Smith & Roe/ mg. initial dry wt.)		
			Medium	Tissue	Total
0	—	—	0	44	44
0	—	—	0	39	39
2	None	5% CO_2 + 95% N_2	10	32	42
2	None	5% CO_2 + 95% N_2	10	29	39
2	2:4-Dinitrophenol (10^{-5})	5% CO_2 + 95% O_2	17	37	54
2	2:4-Dinitrophenol (10^{-4})	5% CO_2 + 95% O_2	10	20	30
2	KCN (10^{-5})	5% CO_2 + 95% O_2	17	42	59
2	KCN (10^{-4})	5% CO_2 + 95% O_2	10	30	40
2	Sodium iodoacetate (10^{-4})	5% CO_2 + 95% O_2	14	27	41
2	Sodium iodoacetate (10^{-3})	5% CO_2 + 95% O_2	20	14	34
2	None	5% CO_2 + 95% O_2	20	73	93
2	None	5% CO_2 + 95% O_2	32	68	100

drug had stimulated amylase secretion *in vitro*. Even in the absence of carbamylcholine the amylase activity of the medium was higher in aerobic than in anaerobic experiments, which suggests that 'spontaneous' secretion took place.

Since carbamylcholine had no stimulating effect anaerobically it is probable that the amylase that was present in the medium of anaerobic experiments was due to tissue disintegration rather than secretion.

The 'spontaneous' secretion which occurred aerobically without addition of carbamylcholine may have been due in part to the retention by the pancreas of the carbamylcholine given *in vivo*, but it is noteworthy that it was also observed in experiments in which no carbamylcholine was given prior to killing.

Under the conditions of these experiments the rate of synthesis of amylase was not appreciably affected by carbamylcholine (Table 3). This may have been due to the fact that the glands used in these experiments were largely depleted of their amylase. Under these conditions one would expect the rate of synthesis to be already maximal. It is likely that under some conditions the stimulation of secretion increases the synthesis of enzymes (Langstroth, McRae & Komarov, 1939).

Effects of 2:4-dinitrophenol, cyanide and iodoacetate on synthesis and secretion. Substances which interfere with the generation of usable energy by the cell would be expected to inhibit the synthesis and secretion of amylase. As shown in Table 4, 2:4-dinitrophenol, potassium cyanide and sodium iodoacetate inhibited synthesis over 99% in a concentration of 10^{-4} M. 2:4-Dinitrophenol and potassium cyanide, in a concentration of 10^{-5} M, inhibited synthesis approximately 70%.

Secretion was inhibited 100% by 2:4-dinitrophenol and potassium cyanide in a concentration of 10^{-4} M and approximately 60% by these two drugs in a concentration of 10^{-5} M. Sodium iodoacetate caused marked visible disintegration of the tissue. This, rather than secretion, was probably responsible for the higher medium amylase activities in experiments in which this compound was added (Table 4).

Effect of various media on synthesis of amylase. To study the effects of various media on the synthesis of amylase, slices were incubated in serum and in saline with and without amino-acids (Table 5). The amino-acids were present either as supplemented casein hydrolysate or as the mixture of amino-acids described above. In glucose saline without added amino-acids there was a variable but significant synthesis. Synthesis was greater in serum and greatest in saline containing either supplemented casein hydrolysate or the amino-acid mixture. There was no significant difference between the results using these two sources of amino-acids. Increasing the concentration of supplemented casein hydrolysate from 0·2–0·4% (w/v) had no significant effect on the rate of synthesis. That serum gave lower rates of synthesis than saline media to which amino-acids were added suggests that in serum the amino-acid concentration was the limiting factor. In the saline media, the amino-acid concentration was three to twenty times higher than in serum. The occurrence of some synthesis in saline media to which no amino-acids have been added can be explained by the considerable store of free amino-acids in animal tissues (see Van Slyke & Meyer, 1913).

Table 5 also shows that the substitution of the bicarbonate saline of Krebs & Henseleit (1932) with the modified medium III of Krebs (1950) was without effect on synthesis or secretion. The more physiological concentrations of bicarbonate and carbon dioxide present in the former medium are therefore not essential for synthesis and secretion under the conditions of these experiments.

The comparative effects of various cholinergic drugs on the secretion of amylase. The effects of carbamyl-

Table 5. *Effect of various media on synthesis of amylase*

(0·2% added glucose; for amino-acid mixture see p. 320; in Exp. 1 carbamylcholine tipped into main compartment after 30 min. incubation (final concentration 1 mg./100 ml.).)

Exp. no.	Period of incubation (hr.)	Medium	Gas phase	Amylase activity (units of Smith & Roe/mg. initial dry wt.)		
				Medium	Tissue	Total
1	0	—	—	0	39	39
	0	—	—	0	41	41
	2	Bicarbonate saline	5% CO_2 + 95% O_2	31	26	57
	2	Bicarbonate saline	5% CO_2 + 95% O_2	30	25	55
	2	Medium III (Krebs, 1950)	Oxygen	26	28	54
	2	Medium III (Krebs, 1950)	Oxygen	28	26	54
	2	Sheep serum	5% CO_2 + 95% O_2	24	38	62
	2	Sheep serum	5% CO_2 + 95% O_2	25	43	68
	2	Bicarbonate saline with 0·2% casein hydrolysate	5% CO_2 + 95% O_2	32	46	78
	2	Bicarbonate saline with 0·2% casein hydrolysate	5% CO_2 + 95% O_2	40	44	84
	2	Medium III (Krebs, 1950) with 0·2% casein hydrolysate	Oxygen	39	44	83
	2	Medium III (Krebs, 1950) with 0·2% casein hydrolysate	Oxygen	28	48	76
2	0	—	—	0	31	31
	0	—	—	0	29	29
	2	Bicarbonate saline	5% CO_2 + 95% O_2	20	22	42
	2	Bicarbonate saline	5% CO_2 + 95% O_2	20	22	42
	2	Bicarbonate saline with 0·2% casein hydrolysate	5% CO_2 + 95% O_2	26	32	58
	2	Bicarbonate saline with 0·2% casein hydrolysate	5% CO_2 + 95% O_2	32	34	66
	2	Bicarbonate saline with 0·4% casein hydrolysate	5% CO_2 + 95% O_2	31	35	66
	2	Bicarbonate saline with 0·4% casein hydrolysate	5% CO_2 + 95% O_2	28	37	65
3	0	—	—	0	24	24
	0	—	—	0	32	32
	2	Sheep serum	5% CO_2 + 95% O_2	14	40	54
	2	Sheep serum	5% CO_2 + 95% O_2	14	42	56
	2	Bicarbonate saline with 0·4% casein hydrolysate	5% CO_2 + 95% O_2	16	46	62
	2	Bicarbonate saline with 0·4% casein hydrolysate	5% CO_2 + 95% O_2	14	51	65
4	0	—	—	0	77	77
	0	—	—	0	69	69
	2	Bicarbonate saline	5% CO_2 + 95% O_2	36	81	117
	2	Bicarbonate saline	5% CO_2 + 95% O_2	27	99	126
	2	Bicarbonate saline with amino-acid mixture	5% CO_2 + 95% O_2	41	127	168
	2	Bicarbonate saline with amino-acid mixture	5% CO_2 + 95% O_2	32	137	169
	2	Bicarbonate saline with 0·4% casein hydrolysate	5% CO_2 + 95% O_2	32	121	153
	2	Bicarbonate saline with 0·4% casein hydrolysate	5% CO_2 + 95% O_2	38	139	177

choline, pilocarpine and acetylcholine with eserine on the secretion of amylase are shown in Table 6.

Weight for weight, carbamylcholine, and acetylcholine with eserine were equally effective in stimulating secretion. Pilocarpine was slightly less effective. Increasing the concentrations of these drugs above 0·1 mg./100 ml. had no greater effect on secretion.

Weight of amylase synthesized. Meyer, Fischer & Bernfeld (1947*a*, *b*) have crystallized amylase from pig pancreas and have assayed its activity by measuring the maltose liberated from starch after digestion with the pure enzyme. Under the conditions of their assay the activity of crystalline amylase was found to be $4{\cdot}0 \times 10^3$ mg. maltose/mg. nitrogen. They found that this preparation of amylase contained 16% nitrogen.

To relate the amylase units of Smith & Roe (1949) to the units of Meyer *et al.* (1947*a*) an extract of pigeon pancreas was assayed by both these methods.

Table 6. *Effect of carbamylcholine, pilocarpine and acetylcholine with eserine on secretion of amylase*

(Bicarbonate saline gassed with 5% CO_2 + 95% O_2; 0·2% glucose; 0·4% supplemented casein hydrolysate.)

Period of incubation (hr.)	Drug	Concentration (mg./100 ml.)	Amylase activity (units of Smith & Roe/mg. initial dry wt.) Medium	Tissue	Total
0	—	—	0	55	55
0	—	—	0	58	58
2	Carbamylcholine	0·1	41	57	98
2	Carbamylcholine	1·0	34	57	91
2	Carbamylcholine	10	40	53	93
2	Pilocarpine	0·1	27	62	89
2	Pilocarpine	1·0	27	54	81
2	Acetylcholine	0·1	38	43	81
	Eserine	0·1			
2	Acetylcholine	1·0	36	47	84
	Eserine	1·0			
2	Acetylcholine	10	36	51	87
	Eserine	10			
2	None	—	23	58	81
2	None	—	23	52	75

The method of Meyer *et al.* (1947*a*) had to be modified because 'Soluble starch Zulkowski' (Merck) was not available. It was replaced by Lintner's soluble starch (Merck). After the period of starch digestion the tubes were promptly plunged into a boiling-water bath for 5 min. The maltose liberated by the amylase was then measured by the method of Miller & Van Slyke (1936), using a standard maltose solution. One unit of Smith & Roe (1949) was found to be equivalent to 0·15 mg. of maltose liberated by the method of Meyer *et al.* (1947*a*). It follows that an average rate of synthesis (20 amylase units of Smith & Roe/mg. dry wt./hr.) would be approximately 5 μg. amylase/mg. dry wt./hr. This calculation assumes that crystalline amylase prepared by Meyer *et al.* (1947*a, b*) is a single enzyme protein. Assuming that α-amylase has a molecular weight of 45,000 (Danielsson, 1947) the rate of amylase synthesis is approximately 10^{-4} μmol./mg. dry wt./hr.

DISCUSSION

Pancreas slices as a tool for studying protein synthesis. The experiments reported here provide evidence that the increase in total amylase activity upon aerobic incubation of pancreas slices is due to a synthesis of amylase and that this increase can be used as a measure of protein synthesis under controlled conditions. It cannot be concluded, however, that the entire amylase molecule is synthesized directly from amino-acids, since it is possible that the enzyme is formed by the addition of a few amino-acids to a polypeptide or protein precursor.

Many workers have recently studied protein synthesis *in vitro* by measuring the incorporation of labelled amino-acids into the proteins of tissue homogenates and slices (Melchior & Tarver, 1947; Winnick, Friedberg & Greenberg, 1947, 1948; Winnick, Moring-Claesson & Greenberg, 1948; Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1949*a, b*; Anfinsen, Beloff, Hastings & Solomon, 1947; Frantz, Zamecnik, Reese & Stephenson, 1948; Zamecnik, Frantz, Lotfield & Stephenson, 1948; Rutman, Dempster & Tarver, 1949; Simpson & Tarver, 1950). The phenomenon studied by this method is essentially the 'dynamic state' of tissue proteins. The method measures the rates of exchange of free and protein-bound amino-acids, but gives no information about the net synthesis of protein. Furthermore, the proteins studied are those obtained by precipitation with trichloroacetic acid or similar agents, or by boiling. They thus represent a mixture of tissue proteins. The method presented here gives a measure of a net synthesis of a specific protein.

Enzyme secretion in vitro. The experiments in this paper indicate that pigeon-pancreas slices actively secrete amylase *in vitro*. The *in vitro* secretion of enzymes by digestive glands has been observed previously. Davies, Harper & Mackay (1949), by histological studies, have shown the discharge of secretory granules from isolated cat pancreas incubated in saline media containing pancreozymin or acetylcholine with eserine. In preliminary experiments Edwards & Edwards (1949*a, b*) have reported *in vitro* secretion of pepsin by isolated dog stomach. Secretion was apparently increased by acetylcholine, pilocarpine or eserine. Ringer solution was found to be 'rather unsatisfactory' as a nutrient medium (Edwards & Edwards, 1949*b*), and most of the experiments were performed in homologous serum. Edwards & Edwards (1949*a*) did not assay the pepsin of both the medium and tissue to test

whether the 'total peptic activity' (sum of medium and tissue peptic activities) had increased during incubation.

SUMMARY

1. A method is described for measuring the synthesis and secretion of amylase by slices of pigeon pancreas *in vitro*.

2. The amylase content of pancreas tissue can be considerably reduced by abundant feeding of the pigeons and injection of carbamylcholine before killing.

3. An increase in the total amylase activity (sum of medium and tissue amylase activities) occurs when depleted pancreas slices are incubated aerobically in media containing glucose.

4. This increase in total amylase activity requires oxygen and is inhibited by 2:4-dinitrophenol, cyanide and iodoacetate.

5. The increase in total amylase activity is greatest (80–190 %) in saline containing amino-acids, least (40–70 %) in saline without amino-acids and intermediate (60–100 %) in serum. In the presence of amino-acids the increase in total amylase activity corresponds to about 5 μg. amylase/mg. dry wt./hr.

6. In the presence of carbamylcholine, pilocarpine or acetylcholine with eserine, pancreas slices discharge more amylase into the medium than in the absence of these drugs. This discharge of enzyme is dependent on oxygen and is inhibited by 2:4-dinitrophenol and cyanide.

7. The above observations indicate that pigeon pancreas slices synthesize and actively secrete amylase *in vitro*, if respiration is intact.

8. Under the conditions of the experiments synthesis of amylase is not appreciably affected by secretion.

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