FORMATION OF PROTEIN IN THE PANCREAS

By MARIE M. DALY and A. E. MIRSKY

(From the Laboratories of The Rockefeller Institute for Medical Research)

(Received for publication, July 3, 1952)

The exocrine part of the pancreas is highly specialized for the synthesis and secretion of protein. In this organ the investigator can initiate synthesis, for when he stimulates the gland to secrete, the protein thereby lost is restored by synthetic activity of the cells; and once the full supply of protein has been formed the cells become relatively quiescent. It is obviously a great convenience for the study of protein synthesis to be able to control conditions so that the cells in which it occurs are either actively synthesizing or, on the whole, at rest. Another advantage the investigator of protein synthesis finds in the pancreas is that many of the proteins synthesized—trypsinogen, chymotrypsinogen, carboxypeptidase, amylase, ribonuclease, and desoxyribonuclease—have been isolated. Synthesis in the pancreas is not complicated by the occurrence of cell multiplication. In many glands, the mammary for example, cell disintegration occurs during secretion, and synthesis of protein depends upon formation of new cells. Persistence of cells throughout cycles of secretion and synthesis, as in the pancreas, means that protein synthesis can be studied apart from cell multiplication and growth.

The cycle of synthesis and secretion in the pancreas and many factors both of a cytological and chemical nature were first thoroughly studied by Heidenhain and the present investigators have frequently reexamined with admiration his classical paper (1). In recent years there have been surprisingly few studies on the chemical aspects of synthesis and secretion in the pancreas. Some recent interesting contributions are those of Hokin (2, 3).

In our experiments, we have used the pancreas of the white mouse. The mouse pancreas is small and diffuse but to compensate for these disadvantages it should be noted that in relation to body weight the mouse pancreas is much bigger than is the pancreas of larger animals and that by using many animals for each experiment, as is possible with such a small animal, individual differences are averaged.

In a study of protein synthesis in the pancreas it is necessary to determine the quantity of enzyme protein that is synthesized for secretion and the time required for this protein to reappear in the cells after secretion. To form an estimate of the quantity of enzyme protein that is made to be secreted, enzymatic activities in the pancreas were measured at a time when they were

at their maxima. For those enzymes which have been isolated as crystalline proteins the quantity of protein responsible for the measured activity can be computed. This was done for trypsinogen, chymotrypsinogen, carboxypeptidase, amylase, and ribonuclease. The activity of lipase was determined, but the quantity of "lipase protein" could not be derived from this measurement because lipase has not yet been isolated. The results of these assays are that the dry, fat-free pancreas contains 10 per cent of trypsinogen plus chymotrypsinogen (evenly divided between the two), 5 per cent of amylase, 2 per cent of carboxypeptidase, and 0.2 per cent of ribonuclease—17.2 per cent in all. The quantity of lipase is probably of the same order as that of each of the other enzymes—protease and amylase—since its substrate, fat, is present in food in large quantity. The total enzyme protein is, therefore, close to 20 per cent, probably a low estimate because unknown enzymes are not considered. The only reservation that should be made about this estimate is that whereas assays were made on mouse pancreas, the crystalline enzymes used as standards were derived from beef and swine pancreas. The estimate of 20 per cent for the total secretory material elaborated by a pancreas exocrine cell is certainly no more than an estimate that would be made by microscopic observation of cells packed with zymogen granules, the granules which contain the enzymes here considered.

Stimulation of Pancreatic Secretion by Feeding or Pilocarpine.—When the pancreas is caused to secrete by the administration of pilocarpine or by feeding, activities of the enzymes contained in it first drop considerably compared with a fasting control and then gradually rise again, usually to a higher level than in the control animals. In such an experiment a large group of mice under standard conditions was used and animals were killed at various times, the organs of between five and ten being taken for each determination. It required 3 to 6 hours for the pancreas to recover full activity after a low point had been reached due to the injection of pilocarpine. In similar experiments using pilocarpine, Hirsch found that full recovery of mature zymogen granules required about 10 hours (4). This difference in recovery time may be due to minor variations in experimental conditions. Recovery of full enzymatic activity after feeding requires a longer period of time because the stimulus of food persists longer than that of pilocarpine.

In connection with the large changes in enzyme activity occurring during the cycle of secretion and synthesis it was of interest to know whether changes in the various enzymes were concurrent. In most experiments estimates of protease, amylase, and lipase activities were made and in one experiment carboxypeptidase activity was also determined. Although changes were observed in the relative activities of these enzymes, on the whole the great cyclical variations in activity came at about the same time for the different enzymes.

Once this had been found, amylase activity, being the easiest and most precise to measure, was often the only one determined.[1]

When the decline and subsequent rise in pancreatic enzyme activity caused by feeding and pilocarpine injection are compared it may be said that pilocarpine produced more uniform and reproducible changes, but that the magnitude of decline and subsequent rise in activity were in some experiments considerably greater after feeding.

TABLE I

Enzyme Activities and Nucleic Acid Contents of Mouse Pancreas after Pilocarpine Stimulation

	Amylase	Protease	Lipase	Carboxypeptidase	DNA	RNA
					per cent	*per cent*
Control	225	247	2.73		2.22	12.4
½ hr.	225	113	1.79		2.61	12.9
1 ½ hrs.	219	147	2.16		2.44	12.9
6 "	432	461	8.96		2.49	11.6
$\frac{\text{Minimum activity}}{\text{Maximum activity}}$, *per cent*.....	50.6	24.5	20.0			
Control	333	143	2.76	17.3	2.31	11.3
1 ½ hrs.	172	96	0.80	10.6	2.43	12.5
3 "	443	190	2.67	23.9	2.27	10.7
6 "	468	163	4.47		2.44	10.8
13 "	450	280	2.81	28.1	2.41	11.2
$\frac{\text{Minimum activity}}{\text{Maximum activity}}$, *per cent*.....	36.8	34.3	17.9	37.7		
Control	388				2.49	13.2
½ hr.	296				2.19	13.8
1 ½ hrs.	272					
6 "	459					
$\frac{\text{Minimum activity}}{\text{Maximum activity}}$, *per cent*.....	64.5					

Control animals were fasted for the duration of the experiment; animals receiving pilocarpine were killed at the indicated intervals after the last injection.

[1] In a previous paper (5) it was reported that fetal calf pancreas lacked the secretory enzymes of the mature gland. The examination of other samples indicated that this observation was in error because we found large amounts of protease and lipase though relatively small amounts of amylase.

Some typical experiments in which pilocarpine was used to stimulate secretion are summarized in Table I; those in which feeding was used are summarized in Table II.

1. In all experiments, irrespective of stimulus, the cyclical nature of secretion and enzyme synthesis is apparent. In the case of pilocarpine stimulation, minimal enzymatic activity was reached within 1½ hours after the last injection; in the case of feeding, it occurred in 3 to 5 hours.

TABLE II

Enzyme Activities and Nucleic Acid Contents of Mouse Pancreas Stimulated to Secretion by Feeding

	Amylase	Protease	Lipase	DNA	RNA
				per cent	*per cent*
Control	846	112	6.76	2.58	11.4
½ hr.	519	184	4.18	2.77	12.1
1 "	510	124	5.22	2.61	12.0
2 hrs.	210	59	1.27	2.88	12.3
3 "	120	12	0.91	2.76	12.2
4 "	84	12	0.66	2.70	12.6
5 "	129	45	1.23	2.85	11.4
$\frac{\text{Minimum activity}}{\text{Maximum activity}}$, *per cent*.....	9.9	6.5	9.8		
Control	684				
3 ½ hrs.	441				
$\frac{\text{Minimum activity}}{\text{Maximum activity}}$, *per cent*.....	64.5				

All animals were first fasted for 24 hours. Those killed immediately were designated controls; the rest were fed and killed at the indicated intervals after feeding.

2. Variability in pancreatic enzyme concentration among control groups and among those stimulated to secrete must, at least for the present, be attributed to inadequate experimental control of factors pertinent to the process. Full control is difficult in animals in which ultimate stimulation to secretion is governed by a complexity of internal conditions. It is apparent, for example, that the enzyme content of the pancreas is usually less in fasted animals than in those first stimulated to secrete and then allowed to recover, a difference which may be due to a very small but continuous secretion even under conditions of fasting. It is also to the point that stimulation by feeding is more variable in its results than stimulation by pilocarpine; here the cause may be more immediate, for it is difficult to assure the amount of food taken by the mice even when they have been previously fasted.

3. Under optimum conditions for secretory stimulation the extent of enzyme protein loss can be very large. In the most successful feeding experiment here reported, 90 per cent of the total pancreatic enzymes were lost in the course of secretion.

4. From the standpoint of our present objective, the main interest lies not in the magnitude of the changes *per se*, but in the behavior of certain cell components which must somehow be associated with the changes described. These components may be listed as follows: DNA, RNA, soluble nucleotides, and total protein.

Changes in Cell Components during the Cycle of Secretion and Restitution

1. DNA.—At all stages of the pancreatic cycle DNA was determined and used as a base line from which to measure other changes. This can be done because there is good reason to believe that the amount of it per nucleus remains constant during the cycle and since cells remain intact during this period, so too must the total quantity of DNA. The evidence for the constancy of DNA per nucleus is that in the fowl, for which careful determinations have been made, this value is a constant for nuclei of erythrocytes, liver, kidney, spleen, heart, and pancreas (6, 7). If DNA per nucleus is constant for such different cells of an organism it is most likely that it remains constant in each of these cells under different physiological conditions; and, indeed, in the liver the DNA content per cell remains constant under conditions of extreme starvation, when great changes are occurring in many other components of the cell (5). All this evidence for DNA constancy in the pancreas may seem superfluous when it is said that the measured DNA content of pancreas tissue remains fairly constant during the cycle of secretion and synthesis. Nevertheless, without such evidence we could not be sure that this constancy is real, that the apparent constancy is not, in fact, due to actual variability in DNA which is compensated by some other variable.

In Tables I and II some values for DNA contents are given. Although there are considerable fluctuations in the values there is no evidence for a trend which would indicate that the DNA content changes in the course of the cycle of secretion and synthesis.

2. RNA and Soluble Nucleotides.—RNA was determined because of the correlation, to which Brachet and Caspersson have pointed (8, 9), between the concentration of RNA and rate of protein synthesis in many different types of cells. The exocrine part of the pancreas is indeed an excellent example of this correlation. Caspersson and his colleagues, going farther than the evidence given by chemical determinations of RNA, have stated that their microspectrophotometric "investigations of the gland at different time intervals after pilocarpine injection show that the nucleotide concentration in the cell is at a maximum during the '*Stapelstadium*,' and that it decreases with rapid

extrusion (of the secretory material). Nucleotides do not disappear altogether after treatment with big doses but significant amounts always remain at the base of the cell" (10). Chemical determinations of RNA in the whole gland tissue cannot, of course, reveal changes in distribution within the cell, but they can indicate to what extent changes occur in the cell as a whole. We have made RNA determinations before and at various times after pilocarpine injection and feeding, but no significant changes were found either with reference to the DNA content or the dry weight of the tissue.

RNA contents of mouse pancreas are given in Tables I and II in which they may be examined together with DNA contents and enzymatic activities. Here, too, fluctuations are evident, but there is no trend which can be correlated wth the secretory or synthetic activity of the gland. The fluctuations in both RNA and DNA contents can probably be attributed to errors introduced during the removal of the gland from the animals. In the mouse the pancreatic gland

TABLE III

Acid-Soluble Components of Mouse Pancreas in the Cycle of Secretion and Synthesis (Stimulation by Pilocarpine)

	Amylase	Non-protein N μg./mg. tissue	α-Amino N μg./mg. tissue	Acid-soluble nucleotides *E*/mg. tissue
Control	341	8.52	2.82	0.387
½ hr.	199	7.56	2.27	0.349
1 ½ hrs.	228	8.02	2.68	0.365
3 "	624	9.00	2.65	0.362
6 "	592	8.45	2.62	0.374

is quite diffuse and some non-pancreatic tissue may occasionally have been included with the gland.

Because of the claim that the RNA content of the pancreas decreases after the injection of pilocarpine some pancreatic juice secreted by a dog after feeding was obtained through the courtesy of Dr. Franklin Hollander of the Mount Sinai Hospital. No nucleic acid was found in this pancreatic juice.

The possibility remained that even if no change in over-all RNA concentration occurs during secretion and synthesis, there might be changes in concentration of smaller nucleotides soluble in dilute acid. No change was found, however, in the quantity of nucleotides soluble in 2 per cent perchloric acid (Table III). Although no change in the over-all concentration of RNA occurs during secretion and synthesis in the pancreas, there might well be intracellular shifts in its distribution and there are good reasons for supposing that RNA plays a part in both secretion and synthesis.

In the course of determining the RNA content of pancreas tissue, samples from beef and swine, as well as from mice, were analyzed. The concentration

of RNA in the mouse pancreas (12.1 per cent) is considerably higher than it is in either beef (8.2 per cent) or swine (7.8 per cent) pancreas. It may also be noted here that in fetal calf pancreas, in which the exocrine portion is less active than after birth, the RNA content is 5.5 per cent, considerably lower than in the mature animal. This is an exception to the situation in other fetal tissues in which the RNA content is higher than in the adult (11).

When mice are fasted for 40 to 48 hours, a longer period than in the experiments that have been referred to so far in this paper (and a very prolonged fast for a small animal having a high rate of metabolism) the activities of their pancreatic secretory enzymes are reduced to a low level. The results of these experiments are summarized in Table IV. Under these circumstances, the

TABLE IV

Pancreatic Amylase Activity and Nucleic Acid Content of Mice Subjected to a Prolonged Fast and Then Stimulated to Secrete by Feeding

	Amylase	DNA	RNA
		per cent	*per cent*
Control	222	3.32	10.7
2 hrs.	146	3.26	10.9
4 "	120	2.86	10.0
6 "	173	3.21	10.4
8 "	282	3.25	10.3
24 "	281	3.16	10.1
Control	102	2.99	10.9
3 hrs.	171	2.48	10.6
5 "	96	2.70	10.1

All animals were first fasted for 40 to 48 hours. Those killed immediately were designated controls; the rest were fed and killed at the indicated intervals after feeding.

concentration of pancreatic DNA may rise, indicating a drop in the total tissue protein and this rise may persist even after feeding. There is also some indication of a slight decrease in pancreatic RNA under these conditions. A pancreas in this condition behaves differently in several respects from the pancreas of well nourished mice. One difference is that when these fasted mice are fed, there is sometimes, at first, a rise (rather than a fall) in the activities of pancreatic enzymes; another difference is that even after feeding, recovery of the normal level of the secretory enzymes is very slow.

3. Protein Content.—If two sets of data—the changes in enzyme concentration and the degree to which DNA remains constant during the cycle of secretion and synthesis—are considered in relation to each other, an interesting conclusion emerges concerning the process of protein synthesis in the pancreas. DNA determinations were made after lipid extraction and since no significant

change in DNA content was observed, it follows that no change in weight of the lipid-free dry tissue occurred. There was also no change in the amino acids, peptides, or total non-protein nitrogen soluble in trichloroacetic acid (Table III). The conclusion from these measurements is that there does not seem to be a significant change in the total content of pancreatic protein during secretion and synthesis. There are, however, considerable changes in the content of the enzyme proteins that constitute the secretory material. In some experiments the changes in content of these enzymes, as determined by activity measurements, would by themselves be enough to cause a significant inverse alteration in the DNA content, reckoned as per cent of dry weight of tissue, since the absolute amount of DNA remains constant.

In the first experiment described in Table II, for example, the average content of DNA is 2.74 per cent, the fluctuations being well within the experimental error. The decrease in enzymatic activity is equivalent to a loss of enzyme protein representing about 16 per cent of the dry fat-free weight of the tissue. The DNA content should, therefore, increase by 16 per cent or to 3.19 per cent at the lowest point of enzymatic activity if the loss in enzyme protein were not compensated in some way. Since such a percentile change in DNA does not accompany large changes in enzyme content, it would seem that as enzymes are extruded, other protein is formed in the gland relatively rapidly; and this protein, it would appear, is gradually transformed into trypsinogen, chymotrypsinogen, amylase, and the other enzymes that are destined to be secreted.

Synthesis of the rapidly formed protein is probably, at least in part, from amino acids supplied to the cells of the pancreas, for Hokin has shown that isolated slices of pancreas can use amino acids present in the medium for the ultimate synthesis of amylase (2). If synthesis is largely from amino acids, then it would appear that the bulk of the energy required for synthesis of the pancreatic enzyme proteins is utilized in making the rapidly formed protein and that much less energy would be needed for the gradual transformation of this material to the finished products contained in zymogen granules. How many different protein components there are in the rapidly formed protein is not known from the present experiments.

The chemical evidence for a substantial loss in enzyme protein as a result of secretion is paralleled by the microscopic observations of Heidenhain and later workers. They have shown that zymogen granules form a considerable part of the resting cell and that almost all these granules may be extruded during secretion. Although at first glance such extrusion appears to occur in acini throughout the gland, careful observation has shown that even in maximal secretion there are acini here and there which are still well stocked with granules. Nevertheless, the mass of material extruded from the gland is very large, so that, if no change occurs in DNA content, from microscopic observa-

tion it would seem that a considerable quantity of material must fairly soon appear in place of the zymogen granules. Since this replacement material does not consist of lipids or nucleotides, does not possess the characteristic properties of pancreatic enzymes, and is insoluble in protein precipitants, it would appear to be protein and probably a precursor of the pancreatic enzymes.

Formation of enzyme protein in the pancreas as it has just been described —rapid synthesis of protein followed by gradual transformation of this into the characteristic pancreatic enzymes—is a process essentially the same as one outlined in a hypothesis of protein synthesis advanced by Northrop (12). Referring to the protein that appears as a precursor he says, "No experimental evidence for this step exists at present . . . even the existence of such a protein is purely hypothetical." This protein has now perhaps been brought within the reach of experiment.

EXPERIMENTAL

Albino mice of the Swiss and Rockefeller Institute strains were used in these experiments. For studying pilocarpine stimulation of secretion, one group of animals was kept in starvation cages and the other was given four 0.1 ml. injections of a 0.2 per cent solution of pilocarpine-hydrochlroide, 30 minutes apart. All animals were given water but no food in the course of the experiments. The mice were killed by decapitation after slight ether anesthesia at various intervals after the last injection. The pancreas was removed and placed immediately in ice cold acetone, if an acetone powder was to be prepared, or immediately frozen and then lyophilized. The acetone powder was obtained by grinding the tissue briefly with acetone in a Waring blendor, washing 4 times with acetone in the cold, and then drying *in vacuo*. Lyophilized preparations, which were used for the determination of non-protein nitrogen and other more soluble components, were washed several times with petrol ether to remove fat and then dried *in vacuo*.

In the experiments in which secretion was stimulated by feeding, the animals were first fasted in starvation cages for periods of time indicated in the text and tables, but were allowed to have water. The control animals were killed at the end of the fast and the experimental group was given bread and milk. The animals were observed while they were feeding, and when they appeared to have finished, the food was removed. They were then killed at various times after feeding was completed. The tissues were treated as indicated above.

Nucleic Acid Determinations.—The tissue samples were washed first with 3:1 alcohol-ether to remove lipid not already removed by previous treatment and then with cold 2 per cent perchloric acid to remove acid-soluble substances. The nucleic acids were extracted by heating with 10 per cent perchloric acid for 20 minutes at 70°C. DNA and RNA were estimated in aliquots of this extract by the diphenylamine and orcinol reactions, respectively.

Non-Protein Nitrogen.—Lyophilized samples of tissue were extracted for 30 minutes in the cold with 5 per cent trichloroacetic acid. The nitrogen contents of the extracts were determined by Kjeldahl digestion followed by nesslerization; the con-

centration of acid-soluble free amino acids and peptides was estimated by the ninhydrin reaction and is expressed as micrograms of α-amino nitrogen per milligram of tissue.

Nucleotides.—An estimation of the relative concentrations of nucleotides of low molecular weight was made by extracting lyophilized tissue in the cold with 2 per cent perchloric acid and then measuring the extinction of the extracts at 260 mμ which was the wave-length of maximum absorption for these extracts.

Enzyme Activities.—The relative enzyme contents of pancreas tissue during the cycle of secretion and synthesis are expressed as activities per unit dry weight of tissue. The estimate of the actual enzyme content of mouse pancreas given in the text was calculated from the activity of isolated crystalline preparations. As crystalline preparations of all the enzymes were not available to us for comparison with pancreas tissue, activity measurements of the tissue were carried out under the same conditions described in the literature for the crystalline products. Of course, such estimations

TABLE V

Reaction of Trypsin and Chymotrypsin with Soybean Inhibitor

Trypsin	Chymotrypsin	Inhibitor	E_{280}
ml.	*ml.*	*ml.*	
0.25	—	—	0.194
—	0.25	—	0.115
0.25	0.25	—	0.285
0.25	0.25	0.05	0.210
0.25	0.25	0.20	0.103
0.25	0.25	0.25	0.105
0.25	0.25	0.30	0.109
0.25	0.25	0.35	0.105

are subject to reservations because of the necessary assumption that the specific activities of the enzymes of mouse pancreas are the same as those of the enzymes used as standards which were derived from beef and swine pancreas.

Assays were made on suspensions of tissue prepared at 0°C. by homogenization; suspensions were used for all activity measurements although amylase and procarboxypeptidase were completely extracted during the homogenization.

(*a*) *Trypsin and Chymotrypsin.*—Suspensions of tissue, 5 mg./ml., in 0.0025 N HCl (final pH 4) were activated by the addition of a small amount of crystalline trypsin—an amount which would contribute less than 10 per cent to the activity of the fully activated suspension. Maximum activation was reached after the suspension had been allowed to stand at 1–2°C. for 2 days. The activity of the suspension at 37.5°C. was determined by the casein digestion method of Kunitz in which the concentration of acid-soluble products is determined by their absorption at 280 mμ (13).

Activity.—Activities were calculated from a standard curve and expressed as the amount of trypsin in micrograms which would be required to produce an extinction equal to that measured for 1 mg. of tissue under identical conditions. The curve

was prepared using a sample of crystalline trypsin (containing $MgSO_4$) kindly furnished by Dr. Kunitz.

By this procedure the combined activities of trypsin and chymotrypsin were measured in most experiments. In order to determine the activities of the two enzymes separately, a procedure utilizing the selective action of the soybean inhibitor on the two proteases was employed. The soybean inhibitor brings about the complete inactivation of trypsin when added in equimolecular amounts, and the amount of trypsin inactivated is directly proportional to the amount of inhibitor used. The amount of chymotrypsin inhibited per unit weight of inhibitor is small compared with that of trypsin, and the reaction is of the reversible type (13). Preliminary experiments on mixtures of crystalline trypsin and chymotrypsin showed that it was possible selectively to inactivate the trypsin and retain chymotrypsin activity. Under the conditions used, the activities of trypsin and chymotrypsin were additive. Equal volumes of a solution containing trypsin and chymotrypsin in 0.0025 N HCl were pipetted into a series of test tubes, increasing amounts of inhibitor were added, and the activity measured by casein digestion. When sufficient inhibitor had been added to combine with the trypsin, a plateau was reached which was equivalent to the activity of the chymotrypsin alone (Table V).

(*b*) *Lipase and Carboxypeptidase.*—Homogenization of tissue was carried out in 0.9 per cent NaCl at 0°C. The methods developed by Seligman and coworkers (14, 15) using β-naphthylpalmitate-stearate as substrate for lipase (kindly supplied by Dr. Seligman), and carbonaphthoxyphenylalanine as substrate for carboxypeptidase were followed. The methods depend upon the colorimetric determination of the β-naphthol released by enzymatic hydrolysis of the substrates. Lipase was activitated by sodium taurocholate and procarboxypeptidase by trypsin. Incubations were carried out at 15°C. for lipase and 37.5°C. for carboxypeptidase.

Activity.—Lipase—E_{530}/mg. tissue/60 minutes

Carboxypeptidase—E_{540}/mg. tissue/120 minutes

(*c*) *Amylase.*—The procedure followed was that of Meyer *et al.* (16), using soluble starch (Merck) as substrate and is based on the colorimetric determination of liberated reducing sugars by means of their reaction with 3,5-dinitrosalicylate. Incubations were carried out at 37.5°C. in the presence of 0.02 M NaCl and 0.01 M phosphate. Samples were removed periodically, treated with the 3,5-dinitrosalicylate reagent, and extinctions measured at 530 mμ.

Activity.—Milligrams maltose liberated/milligram tissue/30 minutes.

SUMMARY

1. The total known secretory enzyme content of the mouse pancreas has been determined and found to represent about 20 per cent of the weight of the dry, fat-free tissue.

2. The changes in secretory enzyme content that occur during the cycle of secretion and synthesis have been measured.

3. In the course of the cycle no significant changes have been found in DNA or RNA content of pancreatic tissue.

4. Constancy of DNA content, along with other observations, indicates that total protein content of the gland remains substantially unchanged during the cycle of secretion and synthesis. These facts point to the conclusion that following upon the secretion of enzyme protein, synthesis of new protein takes place relatively rapidly in the exocrine cells of the pancreas and this protein is then gradually transformed into the characteristic pancreatic enzyme proteins.

REFERENCES

1. Heidenhain, R., *Arch. ges. Physiol.*, 1875, **10,** 557.
2. Hokin, L. E., *Biochem. J.*, 1951, **48,** 320.
3. Hokin, L. E., *Biochim. et Biophysic. Acta*, 1952, **8,** 225.
4. Hirsch, G. C., *Z. Zellforsch.*, 1932, **15,** 36.
5. Stern, H., Allfrey, V., Mirsky, A. E., and Saetren, H., *J. Gen. Physiol.*, 1952, **35,** 559.
6. Davidson, J. N., Leslie, I., Smellie, R. M. S., and Thomson, R. Y., *Biochem. J.*, 1950, **46,** xl.
7. Mirsky, A. E., and Ris, H., *Nature*, 1949, **163,** 666.
8. Brachet, J., Embryologie Chimique, Paris, Editions Desoer, Masson et cie, 1947.
9. Caspersson, T., Cell Growth and Cell Function; a Cytochemical Study, New York, W. W. Norton, 1950.
10. Caspersson, T., Landström-Hydén, H., and Aquilonius, L., *Chromosoma*, 1941, **2,** 111.
11. Davidson, J. N., *Cold Spring Harbor Symp. Quant. Biol.*, 1947, **12,** 50.
12. Northrop, J. H., Kunitz, M., and Herriott, R. M., Crystalline Enzymes, Columbia Biological Series, No. 12, New York, Columbia University Press, 2nd edition, 1948, 232.
13. Kunitz, M., *J. Gen. Physiol.*, 1947, **30,** 291.
14. Nachlas, M. M., and Seligman, A. M., *J. Biol. Chem.*, 1949, **181,** 343.
15. Ravin, H. A., and Seligman, A. M., *J. Biol. Chem.*, 1951, **190,** 391.
16. Meyer, K. H., Fischer, E. H., and Bernfeld, P., *Helv. Chim. Acta*, 1947, **30,** 64.