PROTEIN SYNTHESIS IN RAT PANCREAS

II. CHANGES IN THE INTRACELLUAR DISTRIBUTION OF PANCREATIC AMYLASE DURING THE SECRETORY CYCLE

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In a recent study[1] of the intracellular distribution of amylase in rat pancreas, it was shown that a large part of the amylase activity is found in association with the microsome and supernatant fluid fractions, and that the distribution of activity between these two fractions in the active secreting pancreas differs from that in the resting gland. The present report concerns a more detailed study of the changes observed both in the intracellular distribution of amylase and in the biochemical properties of the microsome and supernatant fluid fractions during a complete secretory cycle.

MATERIALS AND METHODS

A single shipment of 24 rats was used for this study; they were all male rats of the Sprague-Dawley strain, weighing about 350 g. They were starved overnight, in order to minimize secretion of digestive enzymes; under these conditions the acinar cells accumulate digestive enzymes and then become relatively quiescent with respect to synthesis of new enzyme protein[2]. On the morning of the experiment, four animals were chosen at random from each of four cages, to serve as the "starved controls". Each of the other animals received 10 mg of pilocarpine-hydrochloride in 1 cc of isotonic saline, by intraperitoneal injection, in order to stimulate the secretion of pancreatic enzymes. The first four animals injected constituted the first experimental group; these animals were killed about 1 hour later. Members of the subsequent experimental groups of 4 rats were picked at random for sacrifice at approximately 2 h, 4 h, 6 h, and 8 h after the injection of pilocarpine, as indicated on the graph.

In each case, the pancreas tissue was rinsed in isotonic sucrose, dissected free from extraneous fat and connective tissue, pressed through a plastic mincer, and homogenized in 0.88 M sucrose, in a concentration of 12% by weight. The homogenate was strained through a fine stainless steel screen to remove as much as possible of the remaining masses of connective tissue and whole cells. The homogenate was centrifuged at 600 $\times$ g for 10 min to remove the nuclei and cell debris; the supernatant fluid was then centrifuged at 18,000 $\times$ g for 15 min to sediment the secretory granules and mitochondria together. This pellet was saved, to permit visual estimation of the relative changes in quantity of the secretory granule material during secretion. The supernatant fluid from the second centrifugation, which contained the microsome material and soluble proteins, was carefully removed with a pipette, diluted to reduce the concentration of sucrose to 0.25 M (thus facilitating the sedimentation of more microsome material than can be obtained from 0.88 M sucrose[1]), and sedimented at 105,000 $\times$ g for 60 min. Aliquots of the unfractionated homogenates, the microsome material sedimented at 105,000 $\times$ g, and the final supernatant fluids were assayed for amylase activity by the method of MEYER *et al.*[3], and were analyzed for protein-nitrogen, ribose nucleic acid (RNA), and phospholipid-phosphorus, by the methods used previously[1].

RESULTS AND DISCUSSION

The changes in the intracellular distribution of amylase activity that occurred during the secretory cycle are shown in Fig. 1. The curve for the secretory granules was

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obtained by subtracting the sum of the activities recovered in the microsome and supernatant fluid fractions from the total activity of the unfractionated homogenate.

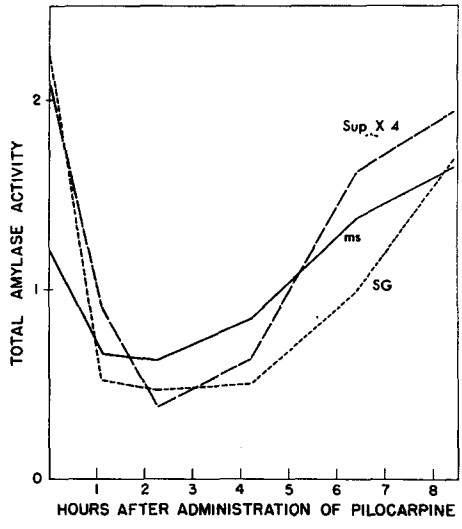

Fig. 1. Amylase distribution following administration of pilocarpine. Sup = Supernatant fluid; ms = microsome fraction; S.G. = "secretory granules" (see text). Units of amylase for each fraction = mg maltose $\cdot 10^4$ produced in 30 min/mg DNA in unfractionated homogenate. Note that the values obtained for the supernatant fluid were multiplied by 4, to facilitate comparison with the other cell fractions.

The figures for the secretory granules therefore include activity present in nuclei, mitochondria, and in contaminating whole cells as well as in the secretory granules, but various lines of evidence indicate that the amylase activity derived from the former sources is small[1,10]. As shown in the figure, the most extensive early loss of amylase activity was in the secretory granules; the loss from the supernatant fluid was even more extensive, but the activity reached a minimum somewhat later than did that of the secretory granules. In proportion to the initial activity, the smallest relative loss was in the microsome fraction. Relatively low amylase activities were observed in all three fractions between the first and fourth hours after the injection of pilocarpine. Return toward the values found in the starving pancreas was noted earliest in the microsome and supernatant fluid fractions, between the second and fourth hours, with rapid rises between the fourth and sixth hours. The secretory granule fraction lagged behind the other two in its return toward the resting condition. At 8 hours after injection of pilocarpine, the amylase activity of the microsome fraction was higher than that of the starving controls, while at this time the activities of the secretory granules and supernatant fluid were only approaching the initial values.

The ratios of amylase activity and of RNA and phospholipid-phosphorus to protein-nitrogen in the microsome and supernatant fluid fractions are given in Table I. The ratios of RNA and phospholipid-P to protein-N showed only small ($\pm$ 8%) and random variations throughout the secretory cyle. In contrast, the ratio of amylase to protein-N in the microsome fraction reached a single minimum, at 2 hours, which was less than 40% of the maximum observed at 8 hours, and the relative amylase activity in the supernatant fluid fraction reached a single minimum, also at 2 hours, which was only about 25% of the value observed at 8 hours.

In contrast to the stability of the structural components (protein, RNA, and phospholipid) of the microsome and supernatant fluid fractions during the secretory cycle, the quantity of the secretory granule material changed considerably during the

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TABLE I

RELATIVE CONCENTRATIONS OF CONSTITUENTS IN MICROSOME PELLET AND SUPERNATANT FLUID DURING RESPONSE TO PILOCARPINE

All values represent ratios of given activity or constituent to protein-nitrogen

Hours after injection of pilocarpine	*Microsome pellet*			*Supernatant fluid*		
	*Amylase**	*RNA*	*Phospholipid*	*Amylase**	*RNA*	*Phospholipid*
Starved control	5.7	1.9	0.13	3.3	0.59	0
1	3.9	2.3	0.15	1.9	0.76	0
2	3.8	2.1	0.13	0.97	0.67	0
4	4.8	1.9	0.15	1.3	0.56	0
6	9.3	2.2	0.14	4.3	0.58	0
8	9.9	1.9	0.13	3.9	0.59	0
Av., ± mean deviation		2.0 ± .15	0.14 ± .01		0.63 ± .05	

* mg · 10^4 maltose produced in 30 min per mg protein-nitrogen.

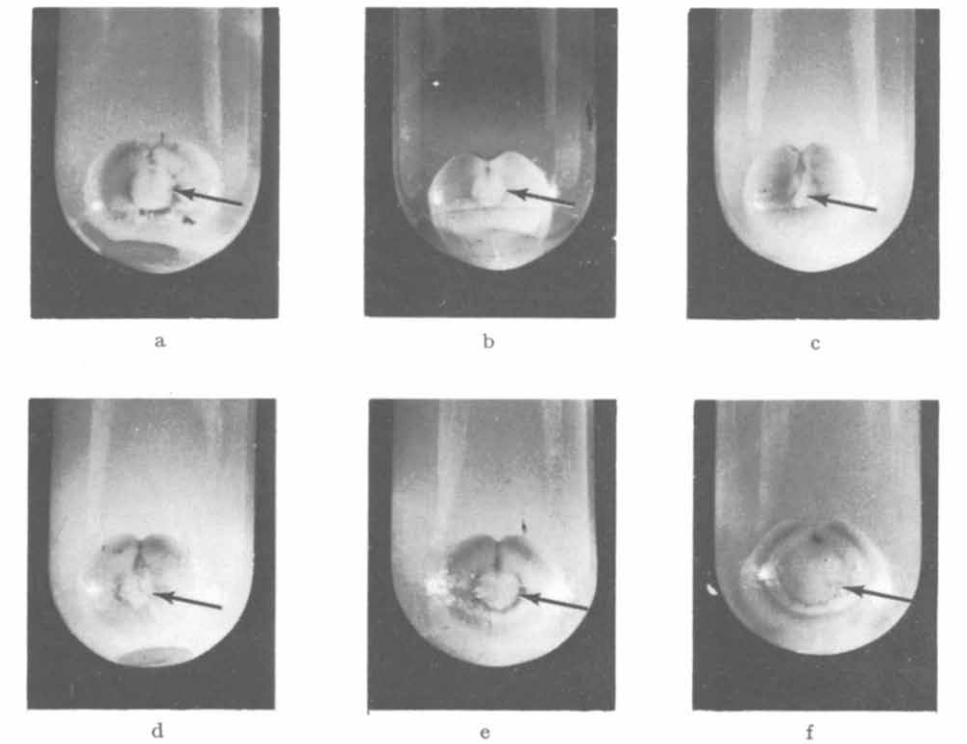

Fig. 2. Pellets obtained from pancreas homogenates by centrifugation at 18,000 × *g* for 15 min after removal of nuclei. The secretory granules form the compact white layer at the bottom of the pellet as indicated by the arrow; the overlying sediments are mitochondrial components. The dark oval area visible at the bottom of several of the tubes is a reinforcing layer on the tube, and is not related to the sediments. a. Starving controls; b. 1 hour after pilocarpine; c. 2 hours after pilocarpine; d. 4 hours after pilocarpine; e. 6 hours after pilocarpine; f. 8 hours after pilocarpine.

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cycle, as indicated by Fig. 2. In this photograph are shown the sediments obtained at 18,000 × *g* from closely similar amounts of pancreas; the white layer at the bottom of the pellet (marked with an arrow) is the secretory granule material. The dark oval area visible at the bottom of several of the tubes is a reinforcement of the tubes and is not related to the sediments. The round white pellet of secretory granule material was rather large and easily seen when obtained from starving pancreas, but its relative size decreased rapidly after injection of pilocarpine, reaching a minimum at 2 hours, when it was reduced to a narrow white slit. Recovery was evident at 4 hours and continued through 6 hours; at 8 hours the pellet was distinctly larger than in the starving controls.

These results taken together suggest that the amylase that is stored in the secretory granules is the first to be secreted after the injection of pilocarpine, as would be expected on the basis of classical histological studies[4]. The "soluble" amylase of the supernatant fraction lags behind that of the secretory granules in the order of secretion, but it is also rapidly lost from the cell. The activity associated with the microsomes is the best conserved. The loss of amylase activity in the secretory granules is associated with a loss of the substance of the secretory granules, in contrast to the losses sustained by the microsomes and supernatant fluid, whose structural components are conserved throughout the cycle. The intracellular origin of the amylase found in the final supernatant fluid fraction is not known; this enzyme protein may have been free in the "cell sap", or it may have been released from the membrane-bound spaces of the cytoplasm during homogenization. These membrane-bound spaces include the endoplasmic reticulum[5,6,7], the Golgi material[7], and the secretory granules themselves [5,7]. The data reinforce the suggestion made earlier in these studies[1] that the new enzyme protein is synthesized in association with the microsomes and then released from the site of synthesis in soluble form, and that the soluble enzyme protein is then condensed, possibly by the Golgi apparatus[9], into the relatively dry secretory granules where it is stored awaiting secretion.

SUMMARY

Changes in the intracellular distribution of amylase and in the biochemical properties of the microsome and supernatant fluid fractions of rat pancreas have been studied during a complete secretory cycle. In general, the amylase activity reached a minimum about two hours after the injection of pilocarpine, returning gradually to normal at about eight hours after the injection. The most extensive early loss of amylase activity occurred in the secretory granules; the smallest relative loss occurred in the microsome fraction. Essentially no change was observed in the protein, ribose nucleic acid or phospholipid of the microsome and supernatant fluid fractions, but extensive changes were observed in the quantity of secretory granule material. Apparently the structural components of the microsome and supernatant fluid fractions are conserved throughout the secretory cycle, while the substance of the secretory granules undergoes marked changes in quantity.

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