EARLY CHANGES IN PANCREAS AUTOLYSIS

R. L. HOLTZER, M.S., AND J. L. VAN LANCKER, M.D.*

From the Department of Pathology and Primate Laboratory, University of Wisconsin, Madison, Wis.

The rupture of particles containing acid hydrolytic enzymes constitutes the earliest measured alteration in the course of liver autolysis.[1] The purpose of the present report is to investigate the fate of hydrolytic enzymes in the course of pancreas autolysis. The latter organ differs from the liver in two ways in respect to its hydrolytic enzymes: first, a large proportion of these enzymes is contained in the zymogen granule; second, the acid phosphatase and deoxyribonuclease are contained in a granule with osmotic properties and response to Triton X-100 similar to those of liver lysosomes. These cannot, however, be separated from the bulk of the mitochondrial fraction by differential centrifugation.[2] The differences in the intracellular distribution of the hydrolytic enzymes in the two organs suggest that differences in the mechanisms of autolysis may also exist. Thus, the release of amylase, ribonuclease and acid phosphatase from their respective granules was investigated in the course of pancreas autolysis.

METHODS

Male Swiss albino mice were used for these experiments. The autolysis, homogenization procedures and enzymatic assays, nitrogen and phosphorus determinations have all been described previously.[1] In some experiments, instead of measuring free enzyme activity in pancreas homogenate,[1] the activities of the enzymes were measured in 3 cell fractions. The latter were prepared on the basis of previous tissue fractionation studies of mouse pancreas in which 9 cytoplasmic fractions were isolated.[2] The results of these experiments are summarized in Text-figure 1. Thus, a distribution spectrum of pancreatic cytoplasmic organelles was obtained by preparing several cytoplasmic pellets by differential centrifugation. Each pellet was characterized by biochemical analysis [2] and electron microscopy.[3] The biochemical analyses comprised the determinations of such basic components as nitrogen and ribonucleic acid (RNA). Also investigated were "markers" specific for given organelles, namely deoxyribonucleic acid (DNA) for the nucleus, cytochrome oxidase for mitochondria, amylase for zymogen granules, and acid phosphatase for small mitochondria.

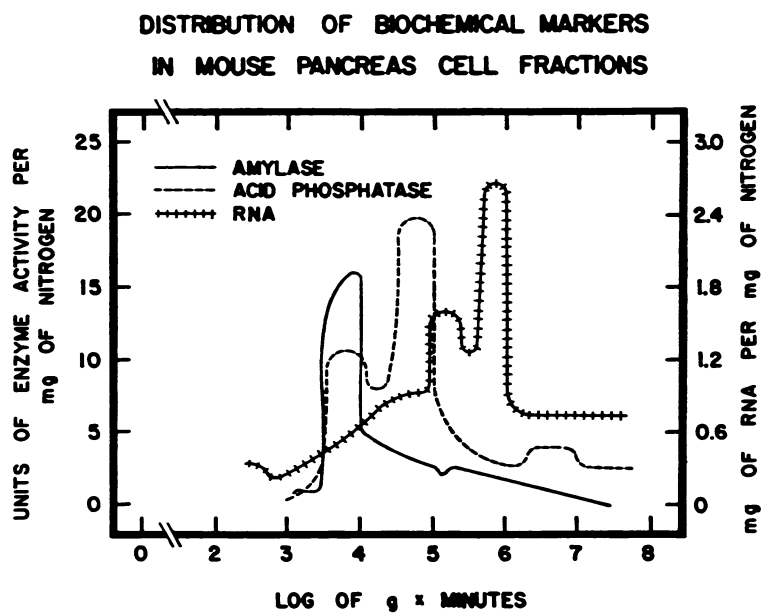
On the basis of quantitative studies of this nature, it was possible to prepare clean pellets characterized mainly by high specific activity in a given compound. Electron microscopic examination of the pellets usually demonstrated the presence of single organelles. For example, the pellet with the highest specific amylase activity mainly contained zymogen granules, while the pellet richest in RNA mainly contained the

This study was aided by grant C 3873 from the United States Public Health Service.
Accepted for publication, October 2, 1961.

* Senior Fellow of the National Institutes of Health.

membranes and the fine granules of the endoplasmic reticulum (microsomes). Further studies on the nature of amylase binding to zymogen granules and microsomes made it possible to distinguish between the enzyme bound to the former and that bound to the microsomes.[4] Moreover, it was established that the presence of amylase in the microsomal pellet did not result from contamination by zymogen granules.

Investigation of the binding of pancreatic acid phosphatase [2] to its granule yielded results similar to those observed in the liver (lysosomes).[3] Thus, it was suggested that



TEXT-FIGURE 1. The data obtained by preparing 9 cytoplasmic pellets of the pancreatic homogenates have been schematized to show the order of the centrifugal forces (expressed in $g \times$ minutes, according to de Duve and Berthet [5]) needed to sediment fractions with the highest specific activity of amylase (zymogen granule) of acid phosphatase ("lysosome") and the highest concentration of RNA (microsome). The fraction containing the highest specific activity of cytochrome oxidase (mitochondria) was obtained at centrifugal forces intermediate between and somewhat overlapping that needed to sediment the amylase and the acid phosphatase rich fractions.

the pancreatic acid phosphatase, as in the liver, was contained in a granule definitely different from the microsome and possibly different from mitochondria. Since, however, the pancreatic granule was not identified morphologically and tissue fractionation demonstrated that the range of size and density was so wide, it appeared that there was extensive contamination of both mitochondria and microsomes by these granules.

In the present experiment, several of these fractions were pooled in two single pellets in such a way that one contained the bulk of the zymogen granule and the particulate bound acid phosphatase (pellet I), while the other contained the bulk of the microsomes (pellet II). The homogenate was first spun in a Spinco Model L centrifuge equipped with rotor number 40 to yield pellet I (11,400 *g* for 8 minutes); pellet II was obtained by centrifuging the supernatant of pellet I under similar conditions, except that higher centrifugal forces ($1{,}054 \times 10^3$ *g* for 30 minutes) were used. The supernatant of pellet II was also analyzed for enzyme activity because it contained the activity not associated with particles.

RESULTS

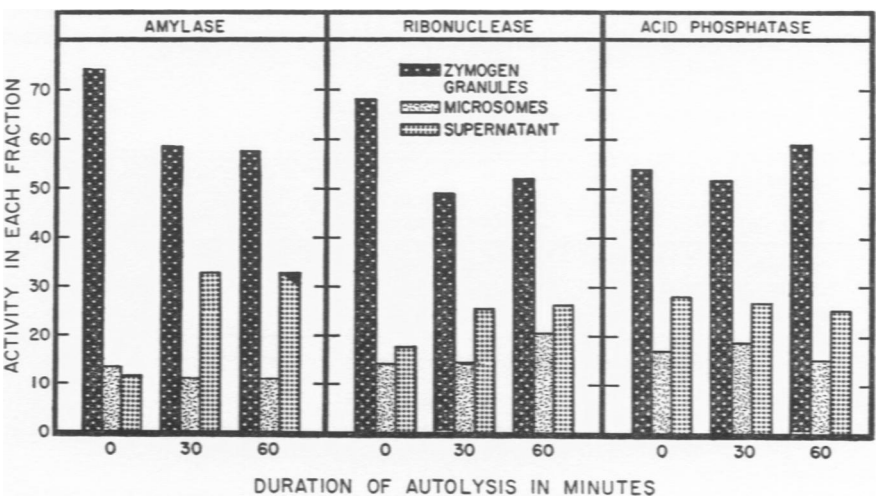
The results shown in Table I and Text-figure 2 indicate that after 30 minutes of autolysis the amylase activity of zymogen granules (pellet I) decreased, while it increased in the supernatant. Similar results

TABLE I

NITROGEN AND ENZYME ACTIVITIES IN PANCREAS AUTOLYSIS *

Duration of autolysis	Fraction	Amylase ($\times 10^{-5}$) S.A.	Amylase ($\times 10^{-5}$) T.A.	Acid phosphatase S.A.	Acid phosphatase T.A.	Ribonuclease S.A.	Ribonuclease T.A.	Nitrogen (mg.)
0 min.	Total	10.2	118.8	0.27	3.18	5.9	68.7	11.6
30 min.	Homogenate	12.3	151.8	0.28	3.48	6.4	79.1	12.3
60 min.	Homogenate	13.7	142.0	0.31	3.18	6.9	71.0	10.3
0 min.	Large granule	15.6	88.4	0.30	1.71	8.4	47.3	5.7
30 min.	Large granule	16.3	90.4	0.34	1.87	7.1	39.4	5.5
60 min.	Large granule	15.7	81.0	0.36	1.87	7.2	37.2	5.2
0 min.	Small granule	6.6	16.0	0.23	0.56	3.7	9.0	20.7
30 min.	Small granule	5.7	16.5	0.23	0.67	6.8	19.5	23.6
60 min.	Small granule	7.5	15.2	0.25	0.50	7.2	14.7	19.4
0 min.	Soluble	4.1	14.3	0.26	0.91	3.5	12.4	30.2
30 min.	Soluble	11.4	44.9	0.24	0.94	5.1	20.2	31.7
60 min.	Soluble	14.6	45.8	0.26	0.81	6.1	19.1	30.1

* The results are those obtained in a single representative experiment in a series of 4. The data are expressed in units of enzyme activity per mg. of nitrogen (specific activity: S.A.) or as the total number of units present in the system (total activity: T.A.). The amylase activity is expressed as mg. of starch digested after 30 minutes' incubation, the acid phosphatase activity in μM of ortho phosphate liberated after 10 minutes' incubation, and the ribonuclease activity in absorbancy units at 0.26 μ for 1 minute of incubation.



TEXT-FIGURE 2. The activities are expressed in per cent of activity measured at time 0 (= 100 per cent).

were obtained for ribonuclease activity. In contrast, there were no changes in the acid phosphatase activity associated with either pellet or the supernatant. The latter feature differs from previous observations in liver. Therefore, the release of acid phosphatase in liver after autolysis was re-investigated under conditions similar to those used for the pancreas. Liver homogenates were centrifuged in the Spinco Model L apparatus ($3{,}183 \times 10^3\ g \times$ minutes), and acid phosphatase activity was measured in the supernatant and in the total homogenate. The former activity expressed the amount of enzyme put into solution minus

TABLE II

FREE AND TOTAL ACID PHOSPHATASE ACTIVITY IN NORMAL AND AUTOLYZED MOUSE PANCREAS

	Controls	After 1-hour autolysis
Free	8.75 ± 1.2	9.25 ± 1.0
Total *	36.40 ± 9.2	35.0 ± 7.6

The enzyme activity is expressed in μM of phosphorus per pancreas liberated into the incubation mixture after 10 minutes' incubation.

* Mean and standard deviation obtained on 6 individual pancreases. The standard deviation was calculated according to the formula: $\sigma = \sqrt{\Sigma(x - \bar{x})^2/n - 1}$

the fraction absorbed on the sediment. The results were similar to those previously obtained with different methods[1] and are therefore not repeated here. Moreover, when free acid phosphatase activity was measured in pancreas homogenates, under conditions that maintained an intact acid phosphatase granule[2] (Table II), no increase in free acid phosphatase activity was measured, even after autolysis for one hour. The determination of the inorganic phosphorus in both liver and pancreas further confirmed these differences in the course of autolysis (Table

TABLE III

ACID SOLUBLE PHOSPHORUS DURING AUTOLYSIS

Duration of autolysis	Pancreas (mg. P/mg. N.)	Liver (mg. P/mg. N.)
0 min.	9.4 ± 0.49 *	126 ± 5
30 min.	13.4 ± 0.53	324 ± 26
60 min.	15.8 ± 0.77	374 ± 24

* Mean of 3 experiments. The standard deviation was calculated as in Table II.

III; Text-fig. 3). Indeed, the increase in inorganic phosphorus after one hour of autolysis was of much less significance in the pancreas than in the liver.

These experiments indicate that the rupture of zymogen granules, leading to release of amylase and ribonuclease, is one of the first mani-

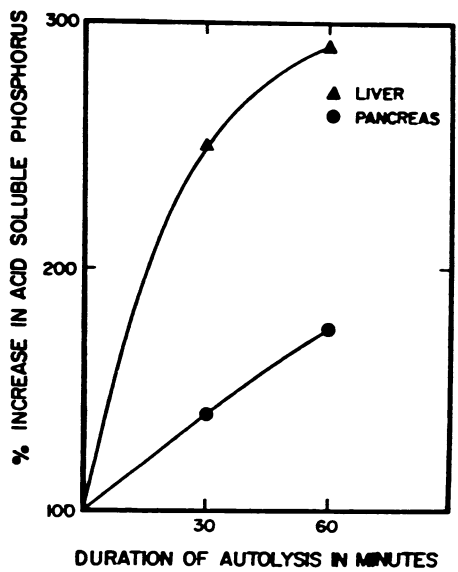
festations of autolysis in mouse pancreas. This is in contrast with previous observations in liver autolysis.[1] Here, rupture of the lysosome, leading to release of acid phosphatase and glucuronidase, was one of the earliest occurrences.

Discussion

It was long ago suspected that the release of hydrolytic enzymes, in particular lipase and trypsin, from zymogen granules was responsible for pancreatic necrosis.[6] The present investigations have shown that one of the first measurable events, if not the *primum movens*, in pancreatic autolysis is the release of both amylase and ribonuclease from the zymogen granules into supernatant fluid. In this respect, pancreas autolysis differs considerably from that in the liver since in the latter instance release of acid phosphatase from lysosomes constitutes one of the earliest manifestations of autolysis. Inasmuch as lipase and trypsinogen are both contained in the same or in similar zymogen granules, it seems safe to assume that they are also released in the course of autolysis.

In contrast, amylase and the ribonuclease associated with microsomes are not released during pancreas autolysis. This is not surprising since previous investigations[4] have shown that the bond between amylase and the microsome is much firmer than that between amylase and the zymogen granule.

The reason for the rupture of zymogen granules in the course of



Text-figure 3. The increase in acid soluble phosphorus 30 and 60 minutes after autolysis is calculated in per cent of acid soluble phosphorus present in normal liver and normal pancreas (100 per cent).

autolysis is not clear. Previous investigations have demonstrated that zymogen granule disruption could be accomplished by mechanical manipulation, osmotic shock, the addition of Triton X-100 or by a basic pH.[4] The present experimental conditions have obviously excluded the first and second of these mechanisms. It is also improbable that alterations of pH were contributory to enzyme release. Microsomes respond to pH alterations [4] in a manner similar to that of zymogen granules, and there was no alteration in the concentration of enzyme in the microsomal fraction, even after one hour of autolysis.

There is no obvious explanation for the failure of pancreatic acid phosphatase release from its granule support. This does, however, suggest that pancreatic acid phosphatase is contained in a more rugged granule than in the liver lysosome. Thus, the pancreatic granules containing acid phosphatase differ from liver lysosomes in at least two ways—namely, by their sedimentation characteristics,[2] and by their resistance to autolysis.

Summary

The release of amylase and ribonuclease from the zymogen granules has been investigated in the course of pancreatic autolysis. Differential centrifugation of the homogenate demonstrated that after 30 minute autolysis, the amylase activity had dropped in the zymogen granule, while it increased in the supernatant. In contrast, no changes occurred in the intracellular distribution of acid phosphatase.

References

1. Van Lancker, J. L., and Holtzer, R. L. The release of acid phosphatase and beta-glucuronidase from cytoplasmic granules in the early course of autolysis. *Am. J. Path.*, 1959, **35**, 563–573.
2. Van Lancker, J. L., and Holtzer, R. L. Tissue fractionation studies of mouse pancreas. Intracellular distribution of nitrogen, deoxyribonucleic acid, ribonucleic acid, amylase, acid phosphatase, deoxyribonuclease, and cytochrome oxidase. *J. Biol. Chem.*, 1959, **234**, 2359–2363.
3. De Duve, C.; Pressman, B. C.; Gianetto, R.; Wattiaux, R., and Appelmans, F. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem. J.*, 1955, **60**, 604–615.
4. Holtzer, R. L.; Swift, H., and van Lancker, J. L. Release of amylase from zymogen granules and microsomes. (In preparation)
5. De Duve, C., and Berthet, J. Reproducibility of differential centrifugation experiments in tissue fractionation. (Letter to the editor) *Nature, London*, 1953, **172**, 1142.
6. Pólya, E. Die Wirkung des Trypsins auf das lebende Pankreas. *Pflügers Arch. ges. Physiol.*, 1908, **121**, 483–507.