A Cytochemical Study on the Pancreas of the Guinea Pig

VI. Release of Enzymes and Ribonucleic Acid from Ribonucleoprotein Particles*

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ABSTRACT

Ribonucleoprotein (RNP)[1] particles isolated by DOC treatment from pancreatic microsomes have a RNA content of 35 to 45 per cent of their dry weight. In the analytical ultracentrifuge about 85 per cent of the material has a sedimentation coefficient of $\sim$85 S. These particles contain amylase, RNase, and trypsin-activatable proteolytic activities which cannot be washed off or detached by incubation in 0.44 M sucrose. The enzymes are released, however, by incubation in the presence of low concentrations of ATP, PP, or EDTA, and high concentrations of IP and AMP. At the same time, and at the same concentrations, $\sim$80 per cent of the RNA and $\sim$25 per cent of the protein of the particles becomes also non-sedimentable. The simultaneous addition of Mg^{++} to the incubation medium prevents these losses. This finding, together with the observation that all the Mg^{++} of the particles is released by the same agents, makes it likely that Mg^{++} holds the particles together, and that its removal by the chelators used causes the particles to disintegrate. These findings are discussed in relation to the molecular structure of the RNP particles.

INTRODUCTION

The ribonucleoprotein (RNP) particles of the cytoplasm have been an object of considerable interest during the last few years. Many attempts were made to isolate them from various sources and to characterize them morphologically, biochemically, and physicochemically (*cf.* 2–5). The

[1] Abbreviations used are: RNP, ribonucleoprotein; RNA, ribonucleic acid; DOC, Na deoxycholate; GTP and ATP, guanosine and adenosinetriphosphate; PP, inorganic pyrophosphate; IP, inorganic phosphate; GMP and AMP, guanosine and adenosinemonophosphate; DNP, 2,4-dinitrophenol; TCA, trichloroacetic acid; TAPase, trypsin-activatable proteolytic activity; RNase, ribonuclease; EDTA, ethylenediaminotetracetate.

work carried out in our laboratory (6–11) has dealt with the morphology of the particles *in situ* and their fate during current cell fractionation procedures. It has established the existence of two types of RNP particles: one free in the cytoplasmic matrix, the other attached to the membrane of the endoplasmic reticulum, which becomes the microsomal membrane after tissue homogenization. It has shown that satisfactory preparations of both attached and free particles can be obtained, and has explored chemical and metabolic differences between the two (10, 11). More recently we have demonstrated that RNP particles exhibit enzymatic activities and, in the case of chymotrypsinogen, we have brought forward evidence which strongly suggests that this activity is due to nascent enzyme still attached to its site of synthesis (12).

In this report we describe conditions under which enzymes (amylase, proteases, ribonuclease) and RNA are released from RNP particles *in vitro* and we discuss the bearing of these findings upon

current views about the structure of RNP particles.

Experimental

Isolation of RNP Particles.—The pancreases of young (300 to 400 gm.) guinea pigs, usually starved for ∼48 hours, were removed under ether anesthesia either without previous refeeding (starved animals) or 1 hour after a meal of cabbage (fed animals). In each experiment a 1:10 (w/v) homogenate was prepared in 0.88 M sucrose (7) with glandular tissue pooled from 4 to 6 animals. The homogenization and all subsequent operations were carried through at 0°-4°C. The homogenate was centrifuged for 30 minutes at 25,000 g and the ensuing supernatant (mitochondrial supernatant) was spun for 60 minutes at 105,000 g. The microsomal pellets thereby obtained were resuspended in 0.88 M sucrose and enough of the latter and of a 3 per cent solution of DOC (pH 7.5-7.8) were added to bring up the volume of the suspension to that of the original homogenate and to obtain a final detergent concentration of 0.1 per cent. The cleared microsomal suspension was then centrifuged for 40 minutes at 105,000 g to sediment intracisternal granules and residual microsomal vesicles (9). The corresponding supernatant yielded, upon further centrifugation for 120 minutes at 105,000 g, clean translucent pellets of pale amber color, whose surface was washed with sucrose to remove all loose material. In the electron microscope, the pellets proved to consist almost exclusively of dense particles ∼150 A in diameter (7, 9). Since these particles are morphologically similar to those attached to the surface of microsomal vesicles (endoplasmic reticulum *in situ* (3)) we assume that the preparation consists of RNP particles detached from the microsomes as a result of the DOC treatment (*cf.* 6, 7).

It will be noted that in this fractionation procedure the "base" of both the microsomal fraction and its intermediary subfraction were "cut" higher than in previous work (7, 9). The modification was introduced to free these preparations, as much as possible, of heavy contaminants, *i.e.* mitochondria in the microsomal fraction, and intracisternal granules and microsomal membranes in the attached RNP particles.

In certain experiments one or two postmicrosomal fractions were obtained by centrifuging the original microsomal supernatant for ∼16 hours at 105,000 g. The surface of the ensuing pellets was washed with sucrose to remove all loose material. We assume that these pellets represent the free RNP particles of the cytoplasmic matrix (7, 11).

Incubation Procedure.—To follow enzyme release, RNP particles derived from 0.2 to 0.5 gm. wet pancreatic tissue were needed. The amount was increased to 0.5 to 1.0 gm. tissue equivalent when protein, RNA,

and Mg^{++} losses were investigated. Particles in the required amount were resuspended in 0.88 M sucrose to a final volume of 1 ml. To this suspension was added 1 ml. water (controls) or 1 ml. of the reagent to be tested (see Tables II to VI) previously neutralized to pH 7.0-7.1, and the mixture was incubated at 35°C. for 30 minutes. At the end of the incubation, each mixture was carefully decanted into a plastic centrifuge tube which was filled up to 10 ml. with 2 successive 4 ml. rinses of the incubation vessel. The tubes were then immediately centrifuged for 90 minutes at 105,000 g under refrigeration and the ensuing supernatants and pellets separated for comparative determinations. Ninety minutes was chosen as centrifugation time because at this time ∼90 per cent of the protein, RNA, and enzymatic activity of the controls were already in the sediment. Each pellet was taken up in 10 ml. water for enzymatic assay.

Enzymatic Assays.—Trypsin-activatable proteolytic activity (*cf.* 8) (TAPase) was determined by Kunitz's procedure which measures the summated activities of the trypsinogen and chymotrypsinogen present in the preparations.

Ribonuclease (RNase) was assayed by de Duve's procedure (*cf.* 8).

Amylase activity was determined by the following modification of the method of Meyers *et al.* (13). Soluble starch was dissolved with heat in 0.02 M NaCl in 0.02 M phosphate buffer (pH 6.9) to obtain a 1 per cent slightly opalescent substrate solution. The reagent was prepared by dissolving 1 gm. of 3,5-dinitrosalicylic acid and 30 gm. of sodium potassium tartrate in 20 ml. 1 N NaOH, with water subsequently added to a final volume of 100 ml. A suitable aliquot (usually 1 ml.) of the resuspended pellet or supernatant to be tested was incubated for 30 minutes at 35°C. with 1.0 ml. of the starch solution in a total volume of 2 ml. After the incubation, appropriate aliquots were withdrawn, mixed with 2 ml. reagent in a total volume of 3 ml., and subsequently heated for exactly 5 minutes at 100°C. for color development. Seven ml. of water were then added and the color read after 5 minutes at 530 mμ. Since the undigested starch also gives some color with the reagent, suitable amounts of a 0.5 per cent starch solution were added to all reaction tubes, controls and maltose standards included, to have approximately the same amount of starch in each tube. Under these conditions the maltose standard gave a linear ΔE_{530}/weight curve from 0 to 0.8 mg. and the ΔE_{530} was proportional to the amount of tissue used.

To check the proportionality of the reactions, all enzymatic assays (TAPase, RNase, and amylase) were carried out with two different concentrations of RNP particles or derived preparations.

Chemical Determinations.—For RNA and protein determinations, the pelleted preparations were resus-

pended in cold, distilled water and mixed with enough cold 100 per cent TCA to obtain a final TCA concentration of 10 per cent. Supernatant preparations were directly mixed with enough cold 100 per cent TCA to reach the same final concentration of acid. The precipitated nucleoproteins were sedimented, washed, their RNA extracted with hot TCA, and their protein defatted as already described (6, 14). The amount of RNA was determined by the orcinol method (15) using a purified yeast RNA as a standard. Total nitrogen in the defatted protein was measured by nesslerization (16) after digestion, using ammonium sulfate as a standard. Protein amounts were calculated by multiplying total nitrogen figures by 6.25.

A scaled-down modification of the method of Orange and Rhein (17) was used to determine Mg^{++} in the cold 10 per cent TCA extract of the precipitated nucleoproteins (cold acid-soluble Mg^{++}) and in the hot 5 per cent TCA extract (RNA extract). For Mg^{++} bound to protein (Kjeldahl digest) the method was further modified as follows: the polyvinyl alcohol was omitted, the color was read within 10 minutes, a standard curve was determined for Mg^{++} in the presence of H_2SO_4, and an H_2SO_4 acid digest was used as a blank. Mg^{++} amounts down to 0.03 μmoles can be accurately determined with this procedure, whereas Ca^{++} gives a negative reaction.

Electron Microscopy.—Pellets of "fresh" and incubated RNP particles were fixed with 1 per cent OsO_4 dissolved in 0.88 M sucrose. To avoid displacement of components within the pellets, the latter were layered with the fixative solution, centrifuged at low speed, and then allowed to fix *in situ* for ∼15 hours at 0°C. After fixation, the hardened pellets were removed from the bottom of the centrifuge tubes, cut into orientable strips, dehydrated, and finally embedded in a mixture (1:3) of methyl and *n*-butyl methacrylate. Thin sections, cut normally to the surface of the pellet, were stained with lead hydroxide (18) and covered with a carbon film (19). They were examined in an RCA, model EM-2B electron microscope, care being taken to survey systematically the entire depth of the pellets.

Ultracentrifugal Analysis.[2]—Fresh particles, or particles spun down after incubation as described above, were gently resuspended in a small volume of a solution containing 100 mM KCl, 0.5 mM Mg Cl₂, and 0.5 mM potassium phosphate at pH 7.0 (*cf.* 20). No washings of the particles were previously done, except for rinsing the surface of the pellets several times with the suspending medium. The suspensions were then examined in the ultracentrifuge at 37,020 R.P.M. at room temperature. Dilutions of the suspensions were made with the suspending medium.
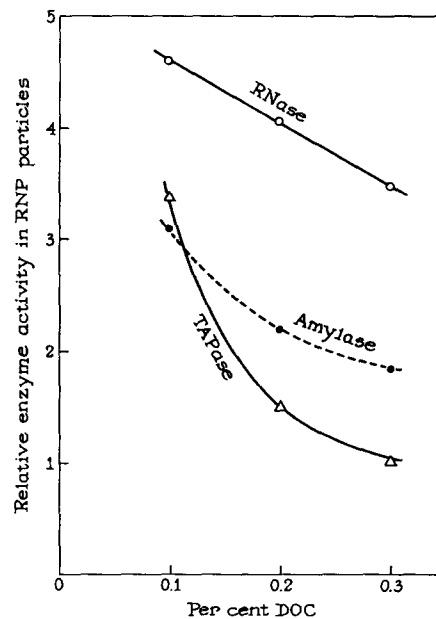
TEXT-FIG. 1. The effect of treating microsomes with increasing concentrations of DOC on the enzymatic activities of subsequently isolated RNP particles.

The guinea pig microsomes were obtained as described. Aliquots of a microsome suspension were treated with varying concentrations of DOC to give final detergent-concentrations of 0.1, 0.2, and 0.3 per cent. The cleared microsome suspensions were first centrifuged at 105,000 g for 40 minutes to eliminate intracisternal granules and membranous residues. The supernatants from this spin were then centrifuged for 2 hours at 105,000 g to obtain the RNP particles. The latter were taken up in 0.88 M sucrose and tested for enzymatic activities as described. The ordinate gives in arbitrary units the amount of enzymatic activity associated with RNP particles isolated from 1 gm. pancreas.

RESULTS

Enzymes Associated with RNP Particles—We have already shown that RNP particles isolated by DOC treatment from pancreatic microsomes have TAPase and RNase activity and that the specific activities of these enzymes are 2 to 3 times higher in the particles than in the whole microsomal fraction (9). The present experiments confirm these findings and extend them to amylase.

Text-fig. 1 indicates that the amount of enzymatic activity decreases as the concentration of DOC used in the preparation of the particles increases, the effect being more pronounced for

TAPase than for amylase and especially RNase activity. It should be mentioned that the yield of RNP particles is not affected in the same way by the detergent: it remains about the same at 0.1 and 0.2 per cent and increases slightly at 0.3 per cent, presumably because more particles are freed from their membrane attachments by the higher DOC concentration. It appears therefore that the detergent strips these enzymes from the RNP particles with a varying degree of efficiency. The most firmly attached seems to be the RNase as already inferred from other data (9). That this difference in binding might have some physiological meaning is indicated by the finding that radioactive RNase appears in the pancreatic juice later than does radioactive trypsinogen and chymotrypsinogen after injection of radioactive cystine into the calf (21). An extrapolation of the curves in Fig. 1 to zero suggests that in situ the particles contain more proteases than RNase and amylase. As isolated by 0.1 per cent DOC treatment, the attached RNP particles account for ∼10 per cent of the RNase and ∼3 per cent of the TAPase activity of the cell. In the preparative procedure adopted for the rest of the work we decided to use the lowest DOC concentration because the population of RNP particles thereby obtained is homogeneous in the electron microscope and in the ultracentrifuge (cf. below) and contains enough enzymatic activity for reliable assays.

For each enzyme tested, the amount of activity recovered in 1 gram tissue equivalent of RNP particles varied as much as 100 per cent from one experiment to another. Part of this variation can be tentatively correlated with the secretory cycle of the gland. Indeed the amounts of amylase activity were consistently 2 to 4 times higher in fed than in starved animals.[3] It appears that in fed animals the RNP particles carry a heavier load of enzymes, an inference in agreement with the view that the synthetic activity of the pancreas is cyclic (cf. 9, 10, 22). Admittedly, more work is needed to establish firmly this correlation and especially to provide a satisfactory explanation for it.

Chemical determinations showed that in these experiments, like in those already reported (7, 11), the attached particles consisted of RNA and protein, the former representing 35 to 45 per cent of the particle mass. If we assume that the guinea

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[3] TAPase and RNase activities were not comparatively tested in starved and fed animals.

pig RNase and chymotrypsinogen have the same specific activity as the corresponding bovine enzymes, and if we transform the entire TAPase activity to chymotrypsinogen weight, we find that 3 to 7 per cent of the protein of the particles is represented by RNase and 3 to 10 per cent by chymotrypsinogen (and trypsinogen). As the RNP particles contain in addition amylase and possibly other digestive enzymes, it follows that these attached enzymes could account for 10 to 20 per cent of the total proteins of the particles.

Release of Enzymes from RNP Particles.—As shown by Keller and Zamecnik (23), GTP is a necessary cofactor for the *in vitro* incorporation of labeled amino acids into microsomal proteins. Since it might be assumed that GTP may act by releasing the newly synthesized protein from its site of synthesis—thus making the latter available for renewed and continued production—we decided to test the effect of the triphosphate on the enzymatic activities of the pancreatic RNP particles. As already shown (12), there are reasons to believe that the enzymes in question are newly synthesized proteins still attached to their site of synthesis.

We found that not only GTP, but also ATP and PP, release—at relatively low concentrations—RNase, TAPase, and amylase activities from these particles. Table I shows that in the case of RNase and TAPase 80 to 90 per cent of the original activity of the RNP particles is recovered in the incubating medium plus resedimented particles after incubation in H_2O, ATP, or PP, the incubating medium accounting for most of what is recovered (∼80 per cent) in the case of ATP and PP treatment. For amylase the situation is different. The summated activity of the resedimented particles and their supernatant exceeds by 30 to 50 per cent the activity of the original preparation. The finding cannot be explained by (a) increased availability of the enzyme upon release; (b) the presence of an inhibitor in the particles; or (c) ATP or PP activation in the medium, for a similar over-recovery is encountered in controls incubated in distilled water and then recentrifuged in which most of the activity remains sedimentable. The activation is not due to mere "aging," for particle suspensions kept at 0°C. or at 35°C., in the absence or presence of 1 mM PP or ATP, showed no change in amylase activity over a 1 hour period. We do not have any explanation for this phenomenon, but irrespective of the mechanism involved, amylase distribution can be used for our purpose

TABLE I

The Effect of Various Treatments upon the Enzymatic Activities of RNP Particles

The guinea pigs were fed after a fast of 24 hours and pancreatectomized 1 hour after feeding. The RNP particles were prepared as described under Experimental.

Particle aliquots equivalent to 0.5 gm. tissue were incubated in 2 ml. 0.44 M sucrose with the additions listed in the table. After incubation, the aliquots were combined two by two in samples equivalent to 1 gm. tissue which were centrifuged for 90 minutes at 105,000 g to separate the particles (pellet) from the incubating medium (supernatant). Enzymatic assays were carried through on both. One gram tissue equivalent of RNP particles (line 1) was not incubated, but kept at ~0°C. until the end of the incubation and centrifugation of the treated particles (~2 hours).

Amylase activity is given in mg. maltose released from a starch digest by 1 gm. tissue equivalent, whereas RNase and TAPase activities are given as micrograms of enzyme per gm. tissue equivalent, calculated from curves obtained with crystalline bovine pancreatic RNase and chymotrypsinogen.

Treatment	Amylase (activity/gm.)			RNase (activity/gm.)			TAPase (activity/gm.)		
	Pellet	Superna-tant	Additive total	Pellet	Superna-tant	Additive total	Pellet	Superna-tant	Additive total
1. No incubation............	—	—	66.8*	—	—	26.8*	—	—	62.7*
2. Incubated in water........	83.0	20.8	103.8	16.2	5.4	21.6	43.3	9.2	52.5
3. Incubated in 0.5 mM PP.....	16.3	83.6	99.9	1.0	21.8	22.8	14.3	41.0	55.3
4. Incubated in 0.5 mM ATP...	13.3	89.0	102.3	0.9	21.8	22.7	13.1	45.0	58.1

* These figures were obtained experimentally as described above.

since the activation is general and proportional and since the enzyme responds to various agents in the same way as do RNase and TAPase. Table II gives the results of two experiments representative of a long series of tests in which we followed the effects of various reagents upon the amylase of the RNP particles, while Table III shows similar experiments in which RNase and TAPase activities were assayed. ATP, GTP, and PP caused extensive release at concentrations of 0.5 mM; AMP and GMP had little effect even at 1 mM; whereas IP released the enzyme only at 10 mM. Like IP, MgCl₂ had no effect at 1 mM, but caused extensive amylase release at a concentration ten times higher. Among the other compounds tested, CaCl₂ at 6 mM had the same effect as MgCl₂ at 10 mM (about 70 per cent release in one experiment). EDTA at 1.0 mM caused almost complete release of RNase and TAPase activities. Its effect on amylase could not be tested since at the concentration mentioned the chelator completely inhibited this enzyme. p-Chloromercuribenzoate (0.1 mM), iodoacetate (1 mM), and iodoacetamide (1 mM) were inactive by themselves and did not affect release by 0.5 mM PP. Negative results were also obtained with 1 mM glutamine and glutamic acid.

Since RNP particles have little ATPase and

GTPase activity under our assay conditions,[4] the most plausible explanation for our findings is that the organic and inorganic pyrophosphates we tested act as Mg⁺⁺ complexing agents. The assumption is supported by the fact that enzyme-releasing and Mg⁺⁺-chelating abilities vary in the same way in the series PP, ATP, AMP, IP (24) and by the finding that the addition of F⁻ (1 mM) to IP (0.5 mM) causes 20 per cent release (presumably by the formation of a Mg⁺⁺ fluorophosphate complex), whereas the same reagents are completely ineffective when tested separately. The explanation implies that the enzymes are bound to the other components of the RNP particles by Mg⁺⁺ which is known to occur in relatively high concentrations in RNP particles isolated from other sources (25). We believe that the experiments shown in Tables IV and V strongly support our view on the role played by Mg⁺⁺ in the reaction studied. These experiments (Table IV) indicate that the ATP and PP effects can be completely counteracted by the addition of Mg⁺⁺ in appropriate concentrations. At the same concentration (1.0 mM) Ca⁺⁺ is about only one-half as effective

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[4] For example, under the experimental conditions described for enzyme release, only 0.05 μmoles of IP and no PP were liberated upon the addition of 2 μmoles of ATP or GTP to the assay system.

TABLE II

Release of Amylase Activity from Pancreatic RNP Particles

The guinea pigs were fed after a fast of 24 hours and pancreatectomized 1 hour after feeding. The RNP particles were prepared as described under Experimental. Particle aliquots equivalent to 0.25 gm. tissue were incubated in 2 ml. of 0.44 M sucrose with the additions indicated in this table. After incubation, the aliquots were centrifuged for 90 minutes at 105,000 g and the ensuing pellets and supernatants assayed for amylase activity. The latter is expressed as in Table I. Percentages are calculated by considering additive activity figures as 100.

Exp.	Additives		Amylase in the pellet		Amylase in the supernatant		Additive activity*
			Activity	Per cent	Activity	Per cent	
1	0		179	91	18	9	197
	ATP	(0.1 mM)	162	80	41	20	203
	ATP	(0.3 mM)	62	26	175	74	237
	ATP	(0.5 mM)	45	18	198	82	243
	GTP	(0.5 mM)	43	18	200	82	243
	PP	(0.1 mM)	167	74	59	26	216
	PP	(0.3 mM)	122	62	75	38	197
	PP	(0.5 mM)	24	10	209	90	233
	MgCl₂	(1.0 mM)	165	83	34	17	199
	MgCl₂	(10.0 mM)	20	10	180	90	200
2	0		234	87	34	13	268
	AMP	(1.0 mM)	213	76	67	24	280
	GMP	(1.0 mM)	199	74	69	26	268
	IP	(1.0 mM)	174	68	81	32	255
	IP	(5.0 mM)	95	38	155	62	250
	IP	(10.0 mM)	45	17	218	83	263

* The activity of the original particle pellets (unincubated and untreated) was 135 in Experiment 1 and 165 in Experiment 2.

as Mg^{++}. Since Mg^{++} blocks the release when introduced at the beginning of the incubation, and has no effect when added at the end, it can be concluded that it can successfully compete for ATP and PP with the particle-bound Mg^{++}, but it cannot replace the latter once it is removed from the particles. Table IV records another interesting observation: the Mg^{++} effect is partially reversed by DNP (0.1 mM). Since DNP has no effect on the release induced by high Mg^{++} concentration (10 mM), the results suggest that ATP, Mg^{++}, and DNP compete in forming bimolecular complexes. DNP by itself is inactive. One may wonder whether

TABLE III

Release of RNase and TAPase Activities from Pancreatic RNP Particles

Indications about methods and activity figures are the same as for Table I.

Additives	RNase activity /gm.			TAPase activity/gm.		
	Pellet	Supernatant	Additive	Pellet	Supernatant	Additive
None	28.4	5.2	33.6	65.6	5.6	71.2
PP (0.5 mM)	4.6	29.2	33.8	3.8	60.0	63.8
Mg^{++} (1.0 mM)	29.2	2.7	31.9	60.0	1.9	61.9
PP (0.5 mM) plus Mg^{++} (1.0 mM)	29.5	3.5	33.0	61.9	6.4	68.3
IP (1.0 mM)	29.2	4.9	34.1	61.9	11.2	73.1
AMP (1.0 mM)	30.3	5.2*	35.5	71.2	0*	71.2
EDTA (1.0 mM)	2.7	31.4	34.1	3.8	63.8	67.6

* Assays were corrected for absorption at 280 and 260 mμ due to the amount of AMP in the medium.

TABLE IV

Release of Amylase Activity from Pancreatic RNP Particles

Indications about methods and activity figures are the same as for Table II.

Additions to the incubating medium	Per cent activity released		
	Exp. 1	Exp. 2	Exp. 3
None	9	25	10
ATP (0.5 mM)	81	94	92*
Mg^{++} (1.0 mM)	17	31	6
ATP (0.5 mM) plus Mg^{++} (1.0 mM)	20	36	10
ATP (0.5 mM) plus Mg^{++} (1.0 mM) plus DNP (0.2 mM)	51	—	66
Ca^{++} (1.0 mM)	—	36	—
ATP (0.5 mM) plus Ca^{++} (1.0 mM)	—	63	—
PP (0.5 mM)	—	—	81
PP (0.5 mM) plus Mg^{++} (1.0 mM)	—	—	30

* When the particles were incubated at 0°C. only 26 per cent of the amylase activity was released.

this finding has any bearing upon the well known inhibitory effect of DNP on mitochondrial oxidative phosphorylation.

Release of Mg^{++} from RNP Particles.—Table V gives the results of Mg^{++} determinations on particles either untreated or treated with PP, ATP, IP, or AMP. All the Mg^{++} in the particles was extracted by cold 10 per cent TCA; none was found

TABLE V

Occurrence of Mg^{++} in, and its Release from,
RNP Particles

For control, RNP particles were prepared as described under Experimental and their Mg^{++} content determined in a pelleted sample equivalent to 1 gm. tissue.

For studying Mg^{++} release, particle aliquots equivalent to 0.5 gm. tissue were incubated in 2 ml. 0.44 M sucrose with the additions specified in this table. After incubation, the aliquots were combined to give samples equivalent to 1 gm. tissue which were centrifuged for 90 minutes at 105,000 g to separate the particles from the incubating medium. The pelleted particles were taken up in 10 per cent TCA to extract the cold acid-soluble Mg^{++}. The residue was further extracted at 90°C. for 20 minutes with 5 per cent TCA to remove the RNA and the hot acid-soluble Mg^{++}, and finally digested in H_2SO_4 to solubilize the protein N and the protein bound Mg^{++}.

Mg^{++} was determined as described under Experimental in the acid extracts and final digest. In the supernatant Mg^{++} was too diluted for measuring. No Mg^{++} was found in the hot acid extract or in the H_2SO_4 digest.

Exp.	μmoles Mg^{++}/10 μmoles RNA-P	μmoles Mg^{++}/10 μmoles protein N
1	1.2	0.25
2	1.2	0.3
3	—	0.2
4	0.8	0.2
5	2.6	0.4
6	1.5	0.3
7	1.4	0.3

Exp.	Additives		RNA in pellet	Protein in pellet	Mg^{++} in cold acid-soluble extract
			$\mu g.$	$\mu g.$	$\mu moles$
1	No incubation		312	525	0.24
	None		260	525	0.21
	PP	(0.5 mM)	85	450	0.00
	ATP	(0.5 mM)	90	455	0.00
	IP	(1.0 mM)	297	525	0.20
	AMP	(1.0 mM)	312	518	0.17
2	No incubation		630	669	0.25
	None		637	625	0.25
	PP	(0.5 mM)	195	515	0.03
	IP	(1.0 mM)	579	613	0.23

in the hot 5 per cent TCA extract, nor was there any in the H_2SO_4 protein digest. In this respect, our results agree with those reported by Ts'o *et al.* (25) for pea seedling particles, but in their case

TABLE VI

Release of Protein and RNA from Pancreatic
Ribonucleoprotein Particles

For the condition of the animals, the preparation of the RNP particles, the incubation procedure, and the separation of particles from the incubating medium see protocol given in Table I. RNA and protein were determined in particles pelleted after incubation. The samples were equivalent to 1 gm. tissue and the amounts of RNA and protein are given in μg. per gram original tissue. To calculate percentage losses, the protein and RNA content of the original pellets were equated to 100.

Exp.	Additions	Protein in pellet	RNA in pellet
		$\mu g.$	$\mu g.$
1	H_2O	755*	384‡
	ATP (2 mM)	667	74
	PP (2 mM)	442	36
2	H_2O	917*	342‡
	ATP (1 mM)	757	110
	ATP (1 mM); plus $MgCl_2$ (2 mM)	800	298

Additions	Per cent protein lost (average and range)	Per cent RNA lost (average and range)
ATP (0.5–2.0 mM) (7 exps.)	22 (12–46)	77 (65–84)
PP (0.5–2.0 mM) (8 exps.)	30 (14–50)	81 (67–93)

* The amount of protein in the untreated particles was 843 μg. in Experiment 1 and 947 μg. in Experiment 2.

‡ The amount of RNA in the untreated particles was 394 μg. in Experiment 1 and 352 μg. in Experiment 2.

the ratio Mg^{++}/RNA was 3 times greater than in ours. If the extreme values of Experiments 4 and 5 in Table V are disregarded, it can be calculated that the pancreatic RNP particles contain from 1 to 2 atoms of Mg^{++} for every 10 nucleotides in the RNA and for every 50 amino acids in the protein. In several experiments, practically all the Mg^{++} was released from particles upon treatment with 0.5 mM PP or ATP, with a concomitant release of 68 to 71 per cent of their RNA. IP and AMP, both at 1.0 mM, removed from 0 to 10 per cent RNA and from 0 to 20 per cent of the Mg^{++}.

Release of RNA and Protein from the RNP Particles.—Tables V and VI show that, under the
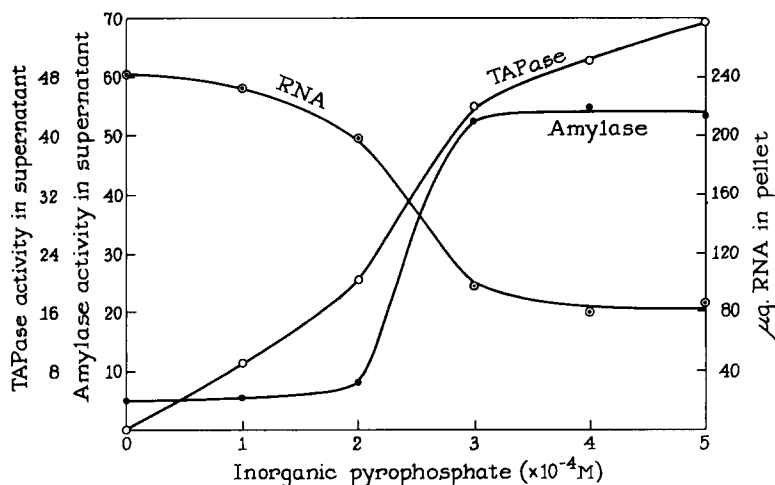
Text-Fig. 2. The effect of increasing inorganic pyrophosphate concentrations upon the release of RNA, amylase, and TAPase activities from isolated RNP particles.

The condition of the animals, the preparation of the RNP particles, the incubation procedure, and the separation of particles from the incubation medium were the same as in the protocol for Table I, except that the animals were starved 48 hours before feeding.

Amylase and TAPase activities are expressed as in Table I. RNA amounts are given in μg. per 1 gm. tissue equivalent.

influence of ATP and PP, the RNP particles lose not only their enzymatic activity, but also most of their RNA and part of their total protein. A comparison of Tables IV to VI indicates that the enzymes, RNA, and Mg^{++} react in exactly the same way to various incubation conditions. As in the case of the enzymes, RNA release by ATP or PP is completely or almost completely blocked by Mg^{++}. The parallelism in behavior is extended by the finding that Mg^{++} can counteract ATP or PP effects only when introduced from the beginning into the incubating medium. It has no effect when added at the end of the incubation.

The connection between enzyme and RNA release is further illustrated by the experiments in Text-figs. 2 and 3 which show that amylase, TAPase, and RNA are released from the particles at the same concentration of PP (Text-fig. 2), and that all these components (plus RNase) start to be released more or less simultaneously, 5 to 10 minutes after the beginning of the incubation (Text-fig. 3). In any case, it is clear that RNA release is not delayed until completion of enzyme, particularly RNase, discharge.

Part of the RNA released in the medium can still be precipitated by TCA, but most of it is TCA-soluble. In 3 out of 4 experiments complete recovery of RNA, as measured by the orcinol

eaction, was obtained between the TCA-insoluble and TCA-soluble fractions of the incubation medium. A preliminary survey has shown that the latter fraction contains a mixture of dialyzable and non-dialyzable orcinol-reacting components. Ion-exchange chromatography showed that less than 10 per cent of the material in this fraction is in the form of simple mono- and dinucleotides. It seems therefore that the release is accompanied by various degrees of degradation, the spectrum of breakdown products beginning with ribonucleoprotein (TCA-precipitable) and ending with small dialyzable polynucleotides.

As far as the total proteins of the RNP particles are concerned, ATP or PP caused a loss of from 20 to 30 per cent, and in this case recovery between the resedimented particles and the incubation medium was almost complete.

It is noteworthy that 1 mM EDTA was less effective than either 0.5 mM ATP or 0.5 mM PP: it released only $\sim$50 per cent of the RNA and $\sim$19 per cent of the total proteins from RNP particles.

Release of Labeled Proteins from RNP Particles.— It might be assumed that the proteins lost by the particles upon ATP or PP treatment are mostly newly synthesized enzymes. Indeed, enzymes are preferentially lost in this process and at least one
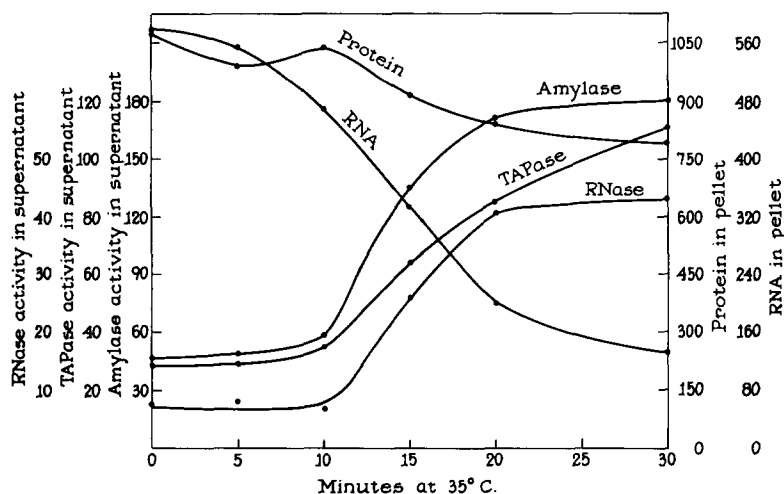
Text-Fig. 3. The Effect of time of incubation at 35°C. upon the release of RNA, protein, amylase, RNase and TAPase activities from RNP particles treated with 0.5 mM inorganic pyrophosphate.

The condition of the animals, the preparation of the particles, and the incubation procedure were the same as in the protocol of Table I, except that the guinea pigs were starved for 48 hours. After incubation for the time interval indicated, cold distilled water was added to each sample (1 gm. tissue equivalent) and the latter placed in an ice bath until the end of the experiment. All treated samples were centrifuged at the same time to separate the particles from the incubating medium. RNA and protein amounts were determined in the pellets whereas amylase, TAPase, and RNase activities were assayed in the corresponding supernatants. RNA and protein are given in μg. per gm. tissue equivalent. Amylase, TAPase, and RNase activities are expressed as in Table I.

of those involved, namely α-chymotrypsinogen, appears to be rapidly synthesized *in vivo* by the RNP particles (12).

To test this assumption, DL-leucine-1-C¹⁴ was injected into guinea pigs, the general circulation stopped 1 to 3 minutes later, and the pancreas removed and homogenized immediately after. At the time chosen, the specific radioactivity of the chymotrypsinogen is known to be markedly high in the RNP particles (12). In each experiment, attached particles pooled from 2 animals were divided into two aliquots: one was incubated as control in distilled water, while the other was incubated in 1 mM ATP in the first experiment, and in 1 mM PP in the second. At the end of the incubation period, the particles were resedimented and the pellets obtained treated with TCA, washed, and counted. The ATP- and PP-treated particles lost 30 and 55 per cent more counts than the controls. In one experiment, after adding crystalline bovine chymotrypsinogen and RNase as carriers to the decanted supernatant of the resedimented particles, the proteins of the preparation were precipitated by TCA, washed, and counted. They were found to contain nearly all the counts released from the particles. Since the

specific radioactivity of the sedimentable protein in PP-treated particles is 40 per cent lower than in controls, and since PP releases ~20 per cent of the total protein of the particles, it can be calculated that the specific radioactivity of the protein released in the incubation medium is 4 times higher than the corresponding figure for the protein retained in the particles. The result is reminiscent of Sachs's findings (26, 27) that PP releases protein of high specific radioactivity from whole liver microsomes and is in agreement with our observations (12) that α-chymotrypsinogen isolated from RNP particles has a high specific radioactivity.

Morphological Changes.—When resedimented after incubation in ATP or PP, the RNP particles show noticeable changes in their behavior: they stick easily to the lateral wall of the centrifuge tube and as a result form a thin pellet with a long lateral tail. The pellet is thinner at the center than at the periphery and is frequently less translucent than the parental preparation. In the electron microscope, the pellet as well as its tail appear to consist of altered particles: by comparison with fresh untreated RNP particles (d = ~150 A) (Figs. 1 and 2), or with the controls (Fig. 3) (d = ~180 A), the ATP- (Fig. 4), PP- (Fig. 7),
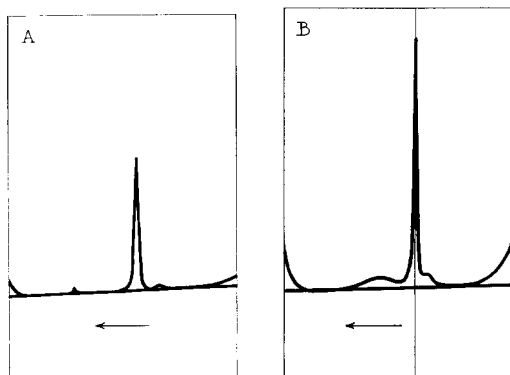
Text-Fig. 4. Ultracentrifugal patterns.

(A) Fresh, untreated RNP particles; (B) Particles stored overnight in the cold room. Patterns after 11 minutes at 37,000 r.p.m. at room temperature. The concentration in (A) was calculated to be about 3 mg./ml. while in (B) it was about 7 mg./ml.

and EDTA- (Figs. 5 and 6) treated particles appeared of greater (250 to 320 A) and more varied diameter. Many of these swollen particles have a central zone of lesser density (Fig. 4). Finally, although they appear in rows like their untreated counterparts, their individuality seems lost: frequently, no intervening space is left between successive granules, the rows being transformed into branching cylindrical bodies of variable diameter (Figs. 5 and 6). It follows that ATP, PP, and EDTA treatments result in swelling and extensive aggregation of the particles. This aggregation, however, is not entirely fortuitous, leading to random-cluster formation, but seems to be directed, in the sense that many particles line up end-to-end.

Ultracentrifugal Analysis.[2]—Text-fig. 4 shows the ultracentrifugal patterns after 11 minutes at 37,020 r.p.m., of: (A) the fresh, untreated RNP particles, and (B) the particles stored overnight in the cold as a pellet overlaid with the suspending medium. The concentrations of the components present were estimated from the areas in the Schlieren diagrams. The major component, comprising 80 to 85 per cent of the total in (A), has an $S_{20_w}^0$ of 85 ± 3 S (Svedberg = 10^{-13} secs.). The other components in (A) are: a fast moving polydisperse one, comprising about 15 per cent of the total (as estimated early in the run) and having an s_{20_w} of 105 to 125 S; a slower moving one, comprising about 2 per cent of the total and having an s_{20_w} of 60 to 65 S; and finally a very small amount of material at the meniscus (s_{20_w} of 2 to 4 S). The

major component is probably similar to those having sedimentation coefficients of 70 to 83 S, previously described in bacteria (28, 29), yeast (30), pea seedlings (25, 31), and liver (20). The pancreatic RNP particles seem to be more labile than those isolated from liver (cf. 20), for storage overnight (Text-fig. 4 B) reduced the major component to about 50 to 60 per cent, increased the faster-sedimenting component to about 20 per cent, and the slower sedimenting one to about 10 per cent, and in addition brought up the material at the meniscus (s_{20_w} of 2 to 4 S) to about 20 per cent of the total. When the particles were incubated in water as described, centrifuged, and then resuspended for analysis, there was no clumping visible to the naked eye, but all the material moved very quickly in the ultracentrifuge, indicating extensive aggregation. As mentioned earlier, this aggregation became visible and affected the gross appearance of the pellets when ATP or PP was added during the incubation.

Release of Enzyme from Free RNP Particles.—As shown by our previous work, the cytoplasmic matrix of the exocrine pancreatic cells contains free RNP particles which can be isolated as postmicrosomal fractions (11). They also exhibit enzymatic activities and contain chymotrypsinogen which, after short labeling *in vivo*, has a relatively high specific radioactivity (12), but lower than that of the proenzyme isolated from the attached RNP particles.

The free RNP particles react to PP and ATP treatment much like the attached particles. Their amylase appears to be more loosely bound, since incubation in water or in 1 mM AMP or IP releases 35 to 38 per cent of the enzymatic activity. The release is increased, however, to 65 to 70 per cent in 1 mM ATP, 1 mM PP, or 10 mM $MgCl_2$. In 1 mM PP the free particles also lose ~55 per cent of their RNA and ~30 per cent of their total proteins. These results increase the number of features that free and attached particles have in common, but leave still unsettled the problem of their relationship.

Release of RNA and Protein from Liver RNP Particles.—ATP and PP have been previously used in experiments on whole liver microsomes by Sachs (26, 27). He found that 1 mM PP inhibits (about 75 per cent) the incorporation of labeled amino acids into the proteins of a liver system comprised of microsomes and final supernatant. And he showed that prelabeled microsomes release most of their radioactive proteins together with almost 60 per cent of their RNA when incubated in the presence of 1 mM PP. In his case the released protein was 2 to 7 times more radioactive than the protein left in the particles. Sachs obtained similar results with EDTA and citrate, but recorded

variable effects with 10 mM ATP. His general conclusion was that PP, possibly by complexing microsomal Mg^{++}, detaches the RNP particles from the microsomal membrane.

In our experiments RNP particles, detached by DOC treatment from liver microsomes, reacted like pancreatic RNP particles when incubated in the presence of ATP or PP but required higher concentrations for comparable effects. For instance, 5 mM ATP caused the release of 34 per cent of the protein and 57 per cent of the RNA whereas 2 mM PP solubilized 17 per cent of the protein and only 39 per cent of the RNA. When microsomes, rather than detached RNP particles, were used, 5 mM ATP had no detectable effect, whereas 20 mM caused the release of 35 per cent of their RNA with no concomitant loss in protein. It appears, therefore, that hepatic particles react in the same general way as, but are sturdier than, pancreatic RNP particles. And it also follows that, in Sachs' experiments, PP was not only detaching, but also breaking up, RNP particles.

DISCUSSION

The significance of our findings depends entirely upon the character of the association between the RNP particles and the digestive enzymes studied. Accordingly, the first question to be discussed concerns the genuine or spurious character of this association.

Past work on cell fractions has already detected spurious localizations for RNase and deoxyribonuclease (32), arginase (33), cytochrome c (34), hemoglobin (35), and other miscellaneous proteins (36). In these cases, either a soluble protein, or a protein released from another cell compartment, was shown to be adsorbed on microsomes during and after tissue homogenization. In our case, relocation by adsorption must be considered because part of each enzyme studied is found in the supernatant fraction, and because the RNP particles are microsomal derivatives which represent a large, probably charged surface. We believe, however, that the association described reflects the situation *in situ* because the enzymes studied are 2 to 3 times more concentrated in the RNP particles than in the parental microsomes; the enzymes can not be washed from the particles; their release is accompanied by the loss of other particle constituents, *i.e.*, RNA and Mg^{++}; and leucine turnover in one of the enzymes studied, *i.e.*, α-chymotrypsinogen, is higher in the RNP particles than in any other cell fraction.[5] We

[5] It might be argued that such cationic proteins as α-chymotrypsinogen, trypsinogen, and pancreatic

realize, though, that the absolute and relative amounts of enzyme recovered in the particles are very small, and for this reason we are inclined to consider as tentative, rather than final, the conclusion that the localization of digestive enzymes in the RNP particles is physiologically significant. On comparable grounds past work has postulated a similar physiological association between microsomes or "microsomal particles" and amylase (37, 38), aldolase (39), RNase (40–42), hemoglobin or "pre-hemoglobin" (43), albumin (44, 45), and antibodies (46).

RNase activity associated with RNP particles isolated from *E. coli* was found to be in a still latent condition (42), but in our case, the enzymes studied seem to be finished products of relatively stable activity. Only amylase shows a noticeable degree of activation for which we do not have an explanation, but which may have some bearing on the increase in amylolytic activity repeatedly reported in the past in pancreatic cell fractions (47, 48). An inactive amylase has been noted before in pancreatic microsomes (38) and its "activation" ascribed to an increase in availability of enzyme to substrate.

Our experiments indicate that RNase, chymotrypsinogen, trypsinogen, and amylase can be released from the RNP particles by various reagents which have in common the complexing of bivalent cations, such as Mg^{++}. Together with these enzymatic activities, the treated particles lose 20 to 30 per cent of their total proteins, 70 to 80 per cent of their RNA, and all of their Mg^{++}. Our estimates suggest that most, if not all of the proteins lost are digestive enzymes. The fact that the specific radioactivity of the released proteins is much higher than that of the proteins left behind suggests, in addition, that newly synthesized enzymes predominate among the former, whereas structural proteins prevail among the latter.

The results of these release experiments can be explained in part by available information. It is known, for instance, that Mg^{++} ions are required for the stability of RNP particles (sedimentation coefficient ~80 S) isolated from a variety of sources, such as mammalian liver (20, 49), pea seedlings (25, 31), yeast (30, 50), and bacteria

RNase, whose isoelectric points are 9.3, 11.0, and 7.8 respectively, could be electrostatically bound to acidic groups of the RNA in the particles, but this argument would not hold for amylase, whose isoelectric point is 5.3.

(28, 29, 51), which in the absence of Mg^{++} break down into smaller particles of unequal size (30 S, 46 to 50 S, and 70 S) but similar RNA/protein ratios (25, 28, 20). It is also known that mononucleotides like ATP, or even inorganic PP can complex Mg^{++} by forming probably monometallic (52) and possibly also bimetallic complexes (53). But the details of the mechanism by which enzymes and RNA are released from particles remain obscure.

Adjacent RNA chains seem to be linked together either by hydrogen bonds, expected to be sensitive to urea (cf. 54) and salt concentration (cf. 55), or by Mg^{++} complexes that would respond to PP or ATP treatment. In fact, even yeast RNA, fractionated a number of times by acetic acid precipitation, can still be appreciably disaggregated by PP treatment.[6] In pea seedling particles, Mg^{++} is assumed to be involved only in the coupling of the RNA macromolecules (subunits) to each other (25), and in E. coli particles the protein and RNA moieties are apparently linked by hydrogen bonds (56). Our results would suggest that in the pancreatic RNP particles, Mg^{++} is involved not only in binding the RNA subunits to one another but also in binding these subunits to the structural proteins, on the one hand, and possibly to the newly synthesized proteins, on the other. A possible alternative explanation is that disaggregation of the RNA chains through Mg^{++} removal leads to a weakening of some of the hydrogen bonds between the protein and RNA moieties and eventually to the release of most of the RNA and enzymes. As already shown (Table V), complete Mg^{++} removal is not accompanied by total RNA release, as if only $\sim\frac{2}{3}$ of the RNA were Mg^{++} complexed with the remaining $\frac{1}{3}$ being hydrogen-bounded. The fact that a large part of the released RNA is found in the form of acid-soluble and even dialyzable material can be due either to Mg^{++} removal from between RNA subunits or to RNase activity in the incubation medium.[7]

[6] Disaggregation was tested by following the precipitability of the treated RNA by 0.25 per cent uranyl acetate in 0.75 M perchloric acid. As increasing amounts of PP were added to a solution of purified RNA in tris (hydroxymethyl) aminomethane buffer (pH 7.8), more and more RNA remained soluble after the addition of the precipitating reagent. The reaction followed a straight line at least up to 5 mM PP, at which concentration 10 per cent of the RNA was rendered soluble.

[7] Roth (57) has postulated that in liver RNP parti-

It will be noted that in our experiments the removal of Mg^{++} by chelating agents produces results qualitatively different from those previously obtained in Mg^{++}-free media (cf. above). Upon PP or ATP treatment most of the RNA becomes soluble and the residual material, which has a low RNA/protein ratio, aggregates severely. It can be assumed that the rapid and extensive removal of Mg^{++} by the chelating agents leads to the disruption of everything in the particle except the structural protein. More gradual and probably incomplete Mg^{++} withdrawal, as effected by incubating the particles in an Mg^{++}-free medium, splits the particles into subunits of unequal size but similar RNA/protein ratios without releasing any material in solution. The discrepancy between these results might be due to a difference in the stability of Mg^{++} complexes between RNP and RNP subunits on one hand, and RNA and protein on the other.

Our experiments are to a certain extent comparable to those carried out by Sachs (26, 27) who reported that PP treatment resulted in the release of RNA and a highly labeled protein from liver microsomes. Moreover, it has been shown (58) that the release of labeled protein from reticulocyte ribonucleoprotein is stimulated by ATP, that an ATP-requiring process seems to be associated with the in vitro transfer of labeled protein from liver microsomes to "cell sap" (59), and that ATP, along with GTP and coenzyme A, stimulate the transfer of radioactive protein from an RNA-containing oviduct fraction to the soluble phase (60). After prolonged labeling in vitro of slices, however, Peters (45) succeeded in releasing radioactive albumin from microsomes only by DOC treatment, PP and EDTA being inactive. EDTA also showed little activity in releasing protein counts from RNP particles of pea seedlings (61), after prolonged labeling. The discrepancy between Peters' results (45) and ours could be due to differences in timing. It is known that protein counts are transferred from RNP particles to microsomal (endoplasmic reticulum) cavities or other cell compartments both in liver (44, 62, 63) and in the pancreas (10, 12). If the radioactive proteins are already within microsomal vesicles by the end of the experiment, DOC should be more efficient than PP as a releasing agent (cf. 9).

Whether the release of enzymes and labeled

cles the RNA is in the form of small chains individually linked to protein.

protein by ATP or PP is of physiological significance is not known at present. Protein synthesis and protein release from the "template" may be parts of the same chemical process (64) but it is also possible, and our experiments give some support to this view (see also 12), that the newly synthesized proteins have a finite period of residence on the particles and are released from this site by a distinct process. If this is so, the GTP requirement for amino acid turnover (23) might be related to the release of newly synthesized protein. Inorganic PP, made available through one or possibly more reactions leading to protein synthesis (cf. 65), might also be used in the same process. But the concomitant release of RNA upon PP or GTP treatment, when previous experiments indicate that *in vivo* the RNA of the particles is relatively stable (11), and the extensive aggregation of particles that follows, indicate that either the conditions of our experiments are still very far from those operating *in vivo* or that Mg^{++} complexing by pyrophosphates is of no physiological significance in enzyme release.

Interpreted at the structural level, our results suggest that the pancreatic RNP particles have a solid framework of structural proteins to which are bound in succession, probably by Mg^{++} complexes, the RNA and the synthesized enzymes. The fact that, after PP or ATP treatment, the particles, though swollen and frequently cavitated, are still recognizable morphologically, suggests that the structural framework is a protein shell in which the RNA and the enzymes are centrally but accessibly located. This tentative representation of the structure of the RNP particle is reminiscent of that postulated for small spherical viruses (66) and for the Rous sarcoma virus (67). In the latter case Epstein has succeeded in removing the density of the central "nucleoid" by RNase treatment (67). An RNA-containing core has already been postulated for liver RNP particles (68).

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EXPLANATION OF PLATES

FIGS. 1 to 7. Pellets of ribonucleoprotein (RNP) particles isolated from pancreatic microsomes (Figs. 1 and 2) by DOC treatment, then incubated for 30 minutes at 35°C. (Figs. 3 to 7) in the media indicated under each figure.

Osmium tetroxide fixation. Methacrylate embedding. Sections stained with lead hydroxide and covered with a protective film of carbon.
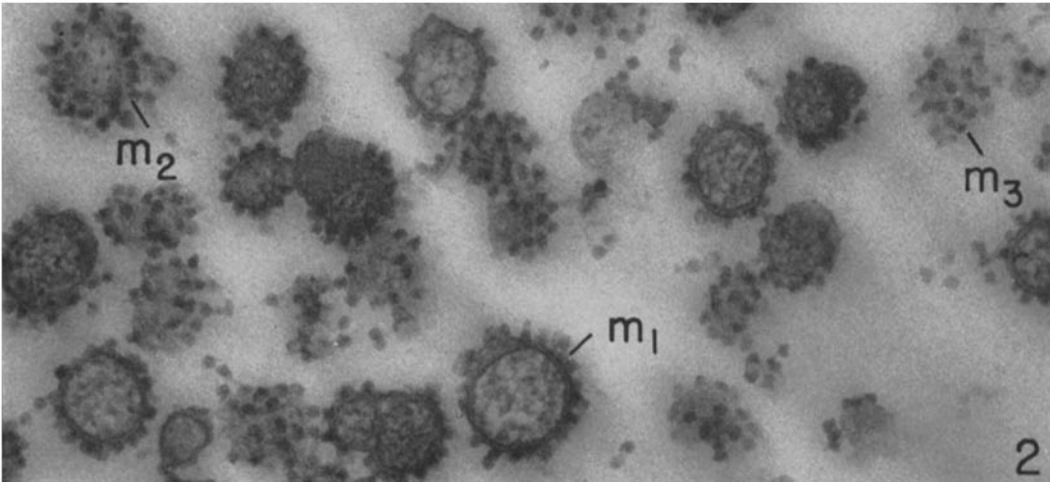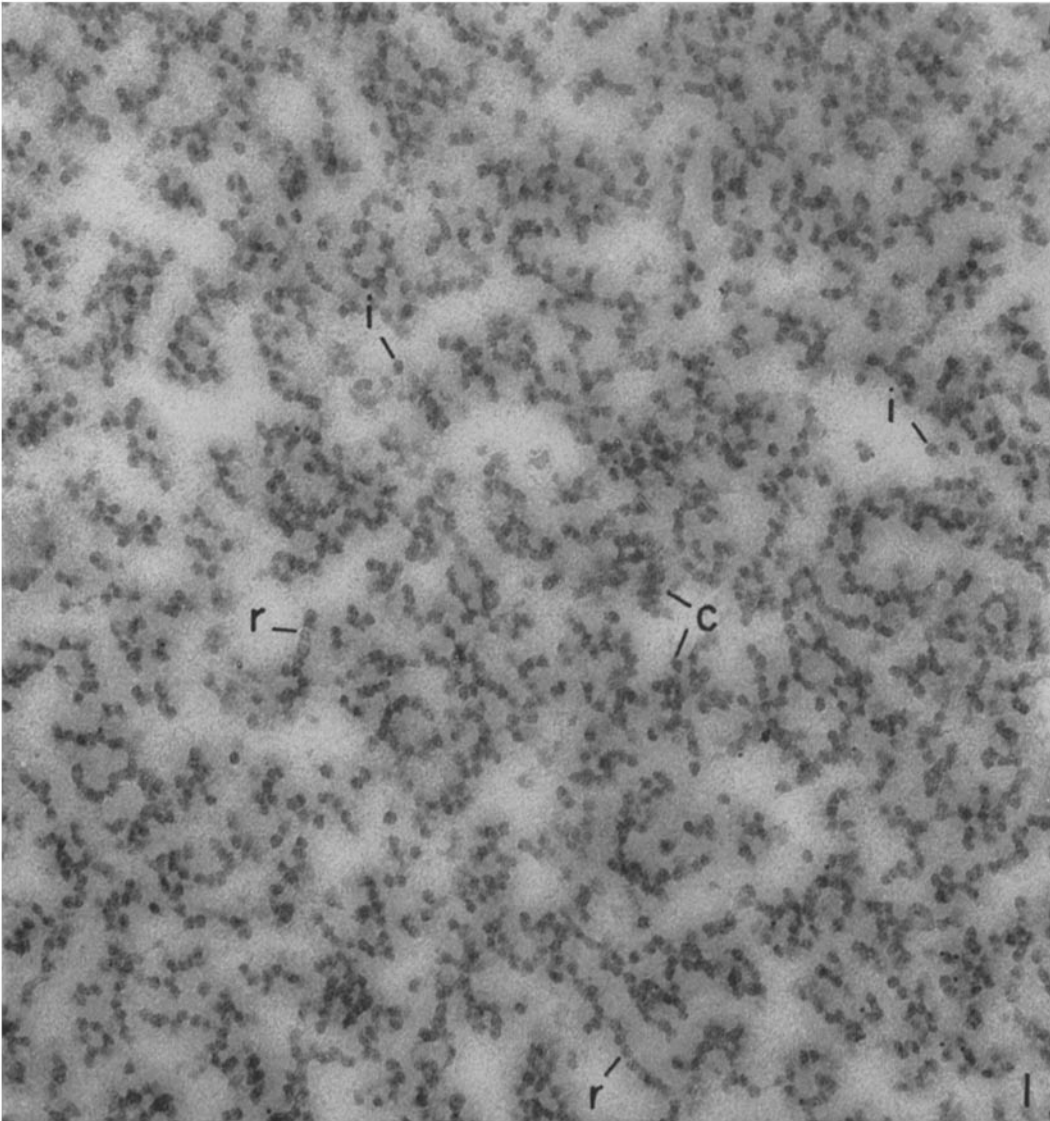
PLATE 319

FIG. 1. Freshly isolated RNP particles fixed without previous incubation. The dense, sharply outlined particles measure ~150 A in diameter and occur either individually (i), or in rows (r), or in small clusters (c).

A comparison with Fig. 2 shows that these particles are similar in size and density to those attached to the membrane of the microsomes in the parent preparation. ×100,000.

FIG. 2. Small field in a microsomal pellet. Because of its thinness the section comprises median (m_1), medial (m_2), and lateral (m_3) slices through microsomal vesicles. The first (m_1) show the microsomal membrane cut normally with the dense particles attached to its outer surface. The last (m_3) allow a full face view of the same particle-studded membrane.

Note the apparent stratification of the microsomal membrane which measures 100 to 120 A in thickness. × 100,000.

PLATE 320

FIG. 3. RNP particles incubated in 0.44 M sucrose.

Note that like in the unincubated control (Fig. 1), they occur individually (i), in short rows (r), or small clusters (c). They are, however, less sharply outlined and appear slightly increased in size ($d = \sim180$ A).

These particles have retained their RNA and their enzymatic activities. ×100,000.

FIG. 4. RNP particles incubated in 0.44 M sucrose plus 0.5 mM ATP.

Note the increase in the diameter of the particles ($d = 250$ to 300 A), the fact that they form long, extensively branched rows (r), and the fact that most of the spaces separating the rowed-up particles have disappeared. A large cluster can be seen at c. Many particles have acquired a light core (the arrows point to a few examples).

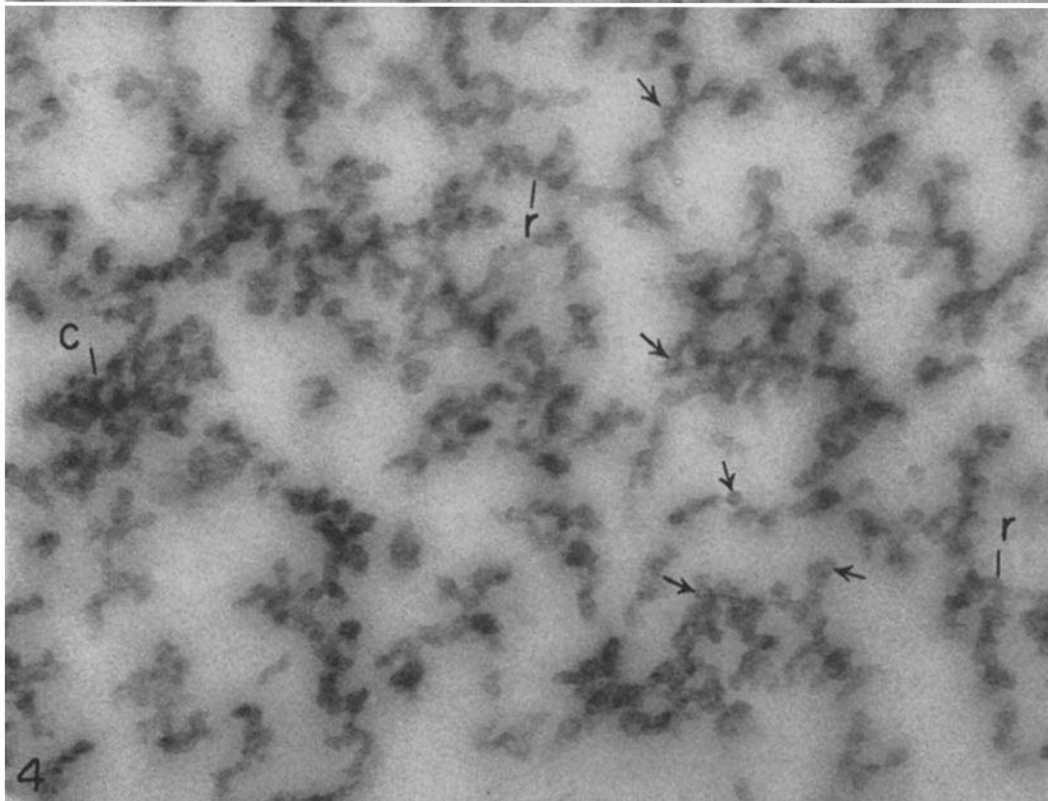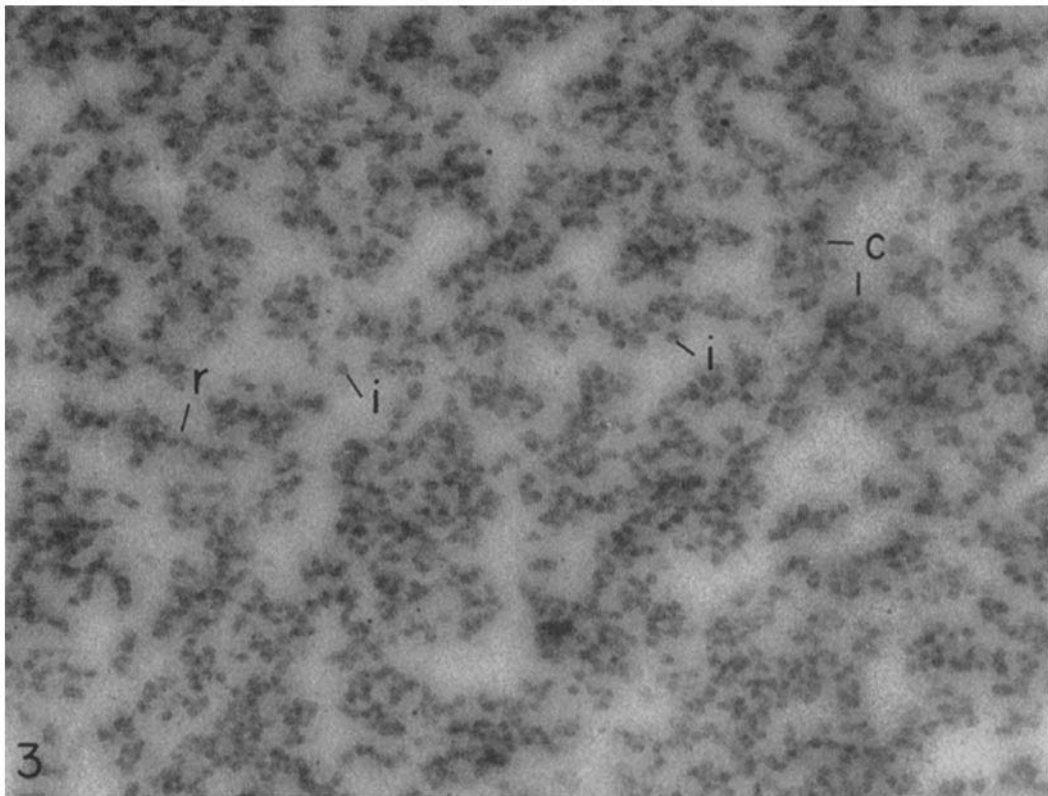These particles have lost ~90 per cent of their enzymatic activities and ~80 per cent of their RNA. ×100,000.

FIGS. 5 and 6. These RNP particles, which were incubated in 0.44 M sucrose plus 1.0 mM EDTA, have an increased diameter (d = 250 to 300 A) and form long, slightly branched rows (r). Within the latter, individual particles can still be made out, although most of the intervening spaces have disappeared. Note the presence of a few "vacuolated" elements (arrows).

These particles have lost ∼90 per cent of their enzymatic activities. ×120,000.

FIG. 7. RNP particles incubated in 0.44 M sucrose plus 0.5 mM pyrophosphate. As after incubation in ATP, the diameter of the particles is increased (260 to 320 A) and the rows (r) are longer and more extensively branched. A few relatively tight clusters (c) are also present.

These particles also had lost ∼70 per cent of their RNA and ∼90 per cent of their enzymatic activities. ×100,000.
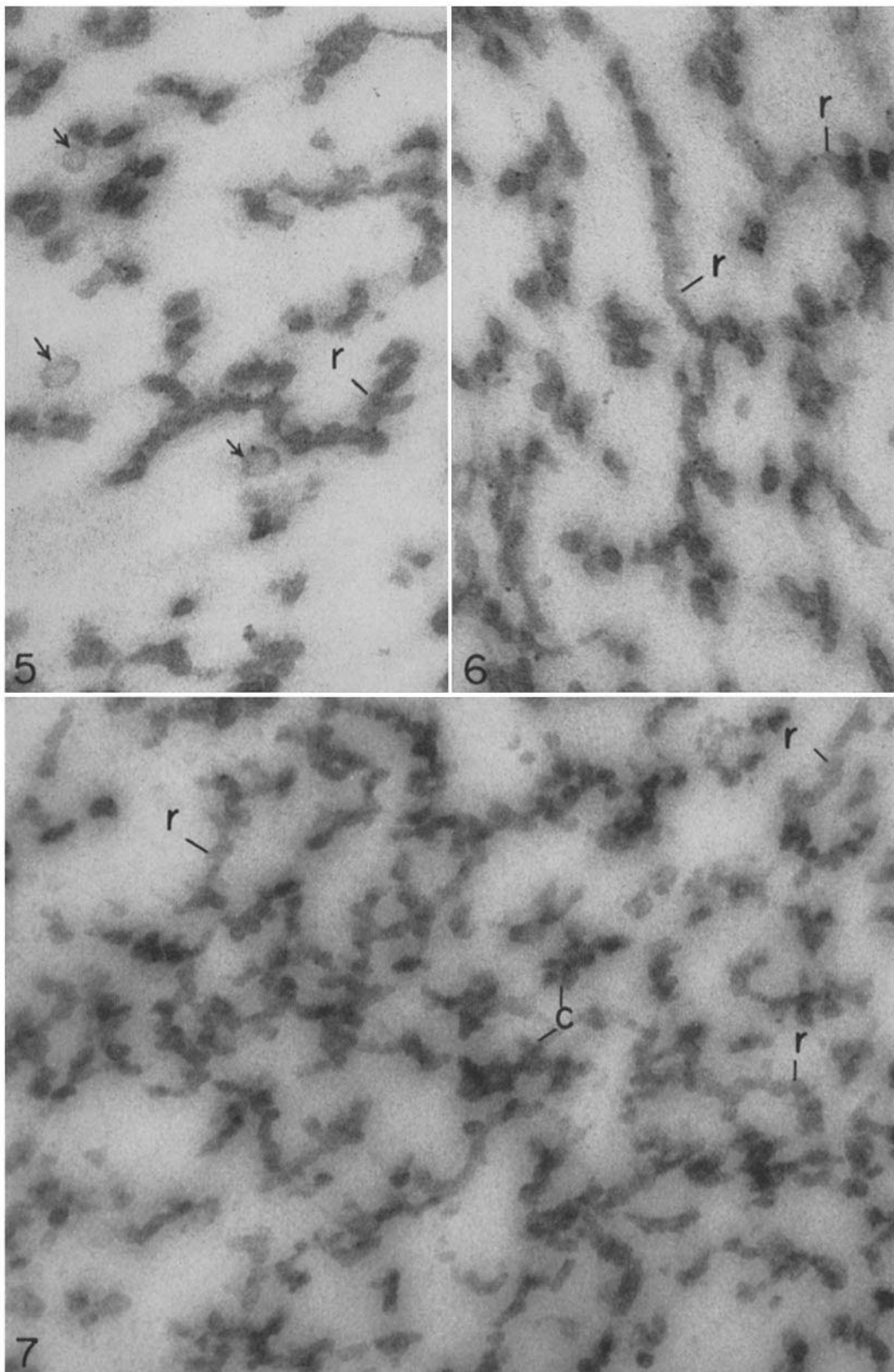
(Siekevitz and Palade: Cytochemistry of pancreas. VI)