Bovine Pancreatic Ribosomes.* I. Preparation and Some Properties

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Bovine pancreatic ribosomes of both the microsomal and postmicrosomal classes include several molecular species with different sedimentation rates. One sedimenting species predominates in fresh preparations of either class, however. The present manuscript describes some attempts to define the optimum conditions for stabilization of this species during isolation. The influence of the ionic and osmolar environment during detachment of microsomal ribosomes from the membrane was studied. A procedure is described for the isolation of microsomal ribosomes in yields of approximately 4 mg/g of tissue, wherein the major sedimenting species constitutes more than 90% of the total sedimenting material. A provisional $s_{20,w}$ of 75 *S* was calculated for this species. In the absence of magnesium, two species with $s_{20,w}$ values of 57 *S* and 33 *S*, respectively, arise at the expense of the 75 *S* ribosomes. The enzymic complement released from microsomal ribosomes by acid extraction or incubation at neutral *p*H was analyzed and compared with the total spectrum of enzymic proteins secreted by the gland. All the expected basic enzymic proteins (trypsinogen, chymotrypsinogen A, and ribonuclease) were present, whereas none of the acidic members (chymotrypsinogen B, procarboxypeptidase A and B, deoxyribonuclease) was present in equivalent amounts. Postmicrosomal ribosomes were isolated in yields of about 5 mg/g tissue and exhibited a population of sedimenting species similar to that seen with microsomal ribosomes. The two types of pancreatic ribosomes are similar in their gross chemical composition and their tendency toward association and aggregation. Postmicrosomal ribosomes also bind basic enzymic protein but appear to release the bound protein exponentially during successive washes.

An impressive body of information has accumulated during the past several decades concerning the structural and functional features of the digestive proteins secreted by the bovine pancreas (Green and Neurath, 1954; Desnuelle and Rovery, 1961). More recently information has been acquired on the biosynthesis and transport of these proteins within the pancreatic acinar cells (Siekevitz and Palade, 1960a; Morris and Dickman, 1960; Dickman *et al.*, 1962a). The bovine pancreas thus affords a rich material for the study of the biochemical relationships between a family of well-characterized, specific enzymic proteins and the ribosomes,[1] which appear to be the intracellular sites of biosynthesis of the respective proteins. Such a study, however, requires a stable preparation of pancreatic ribosomes.

The existence of two classes of ribosomes in pancreatic acinar cells was reported by Palade and Siekevitz (1956), who demonstrated in electron micrographs of guinea-pig pancreas the presence of two kinds of particles: those which appeared to be bound to the membrane of the endoplasmic reticulum, and those which appeared to be free in the cytoplasmic matrix. Homogenates of the same tissue were also shown to contain two types of ribosomes; namely, those which were sedimented with the microsomal fraction, and those which were sedimented as postmicrosomal material. On the basis of cytochemical studies and *in vivo* labeling experiments, it was concluded that the microsomal ribosomes correspond to attached particles and postmicrosomal ribosomes to free particles, and that the two classes are metabolically discrete (Siekevitz and Palade, 1958a, 1959).

Homogenates of bovine pancreas also contain two classes of ribosomes which are distinguishable during preparative ultracentrifugation, and both classes include several molecular species with different sedimentation properties. One molecular species, however, predominates in sedimentation patterns of freshly prepared ribosomes of either class. The present manuscript reports the results of some experiments aimed at isolation of this molecular species in a stable form from each class of ribosomes. Some chemical and enzymologic properties of bovine pancreatic ribosomes of both classes are described.

EXPERIMENTAL PROCEDURES AND RESULTS

General Procedures

Sedimentation analyses were carried out at 5° or below with a Spinco Model E Ultracentrifuge; both ultraviolet absorption and schlieren optical systems were used. Viscosity measurements were made with a viscometer of the type described by Frensdorff *et al.* (1953) in a visibility bath with the temperature controlled to within 0.005° by means of a mercury-toluene thermoregulator. A parallel stem pycnometer and Mettler Gramomatic Balance were employed for density measurements.

Preparative ultracentrifugation was accomplished with a Spinco Model L Ultracentrifuge. Procedures for the measurement of protein, RNA, and enzymic activity were the same as described previously (Keller and Cohen, 1961; Keller *et al.*, 1958a), except that, in the present work, ribonuclease activity was measured in the Radiometer *p*H-Stat, with cytidine-2',3'-phosphate as substrate (Gundlach *et al.*, 1959).

Preparative Procedures

Figure 1 presents a diagram of the principal steps in the isolation of bovine pancreatic ribosomes. The first step entails preparation of a homogenate of fresh bovine pancreas in 0.88 M sucrose. The second step

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[1] The terms ribosomes and ribonucleoprotein are used interchangeably in the manuscript, and the following abbreviations are employed: RNP = ribonucleoprotein particles or ribosomes; EDTA = ethylenediaminetetraacetic acid (Na salt).

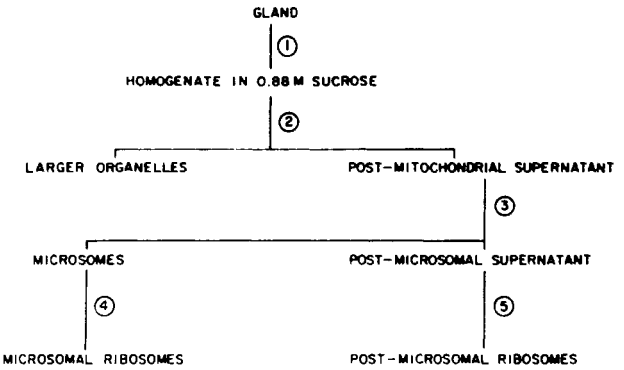

FIG. 1.—Diagram of the principal stages in the preparation of microsomal and postmicrosomal ribosomes from bovine pancreas.

leads to a postmitochondrial supernatant solution freed of the larger cellular organelles. The third step consists in the collection of microsomes and a postmicrosomal supernatant solution. These three stages were accomplished by procedures which were detailed in an earlier communication (Keller and Cohen, 1961), with the following modification in step 2. In the present work, cellular debris and the larger cellular organelles—*viz.*, nuclei, zymogen granules, and mitochondria—were not subfractionated but were eliminated in a common pellet by centrifugation of the freshly prepared homogenate in the No. 30 rotor for 45 minutes at 26,000 × *g*.

The present report will be concerned primarily with the isolation of ribosomes from washed microsomes (step 4) and the isolation of postmicrosomal ribosomes (step 5).

Isolation of Microsomal Ribosomes (Step 4).—Siekevitz and Palade (1958b) showed that treatment of microsomes with sodium deoxycholate effected release of the attached particles. The release is depicted in the ultracentrifugal patterns of Figure 2. Washed microsomes from 1.25 g of tissue were suspended in 1 ml of the solvents indicated in the legend. In the absence of sodium deoxycholate, the suspension of microsomes was turbid, and the early pictures were partially masked. The turbidity settled during centrifugation and was followed by a broad peak of polydisperse material and, in turn, by a small peak of ribonucleoprotein. When deoxycholate was included in the solvent, the turbidity and polydisperse microsomal matter disappeared. Early photographs indicate the presence of some material of lower density than the solvent which is probably membranous matter. The most pronounced effect of the addition of deoxycholate, however, is the increased peak of ribonucleoprotein.

In the experiment shown in Figure 2, most of the ribonucleoprotein material sedimented as a single peak. In some other experiments, however, variable amounts of ribonucleoprotein with faster and slower sedimentation rates were seen in addition to the major component. For operational purposes, and in accordance with the precedent set by Petermann in her studies of ribosomes from rat liver and Jensen sarcoma (Petermann and Hamilton, 1961), the major component was designated component B. The heavier material was designated component A, and those sedimenting more slowly than B were designated components C and D.

The deoxycholate reaction was carried out under varying ionic and osmolar conditions and the reaction followed by analytical ultracentrifugation in an attempt to establish those conditions favoring the isolation of component B in optimum yield and in a stable form. In all cases, the microsomes from 1.25 g of tissue were suspended by means of a Potter-Elvejhem type homogenizer in 1.0 ml of solvent.

The effect of deoxycholate concentration was studied under conditions identical to those stated in the legend of Figure 2. A concentration of 0.01 M deoxycholate was found to be optimum; lesser concentrations (3 mM and 6 mM) failed to release the ribosomes in equivalent yields.

The effect of magnesium concentration was studied in solvents composed of 0.88 M sucrose–10 mM deoxycholate–8 μM crystalline soybean trypsin inhibitor at *p*H 7.5. Magnesium at low concentrations (0.5 mM and 1 mM) favored the presence of the B component at the expense of components C and D. At higher concentrations (2.5 mM) magnesium caused aggregation and gross clumping of the B component. At a magnesium concentration of 1 mM, component B constituted 90% of the total ribosomal material, 2% being heavier and 8% lighter than the major component. However, the high density and viscosity of the solvent used in these experiments necessitated prolonged periods of centrifugation (*ca.* 8 hours) for collection of the ribosomes in the preparative ultracentrifuge. Invariably, material so prepared was aggregated and could not be redissolved in any of the solvents tested. Accordingly, efforts were made to expedite the isolation procedure by working in media of lesser densities and viscosities.

TABLE I
MICROSOMAL RIBOSOMES RELEASED IN SEVERAL SOLVENTS

Method	Solvent[a]	Mg^{++}	Component B	
			% Area[b]	$s_{20,w}$
1	0.88 M sucrose, *p*H 7.5	1×10^{-3} M	92	59
2	0.25 M sucrose, *p*H 7.5	1×10^{-3} M	89	56
3	0.001 M potassium phosphate, *p*H 7.5	2×10^{-3} M	92	53

[a] In each case the solvent contained sodium deoxycholate (1×10^{-2} M) and crystalline soybean trypsin inhibitor (8×10^{-6} M). [b] See footnote 3.

The deoxycholate treatment of washed microsomes was carried out in less concentrated sucrose solutions (0.25 M), and in buffer containing no sucrose, at several magnesium concentrations. The results are summarized in Table I. Again low concentrations of magnesium favored component B over components C and D, whereas concentrations greater than 2.5 mM effected prompt aggregation of the ribosomes. The concentrations of magnesium found to be optimum for maintenance of component B in each solvent are presented in Table I along with the relative proportions and sedimentation coefficients of the B species. The relative proportions of sedimenting material represented by component B in the three media were estimated from area measurements on enlarged tracings of the sedimentation patterns. Observed sedimentation rates were corrected for the densities and relative viscosities of the added solvents.[2]

[2] In the deoxycholate reaction mixture and thus during the initial collection of detached ribosomes, the ribosomes are sedimenting through a medium made up not only of added solvent but also of those proteins and that membranous matter made free by deoxycholate. No attempt was made in these experiments to correct for these constituents.

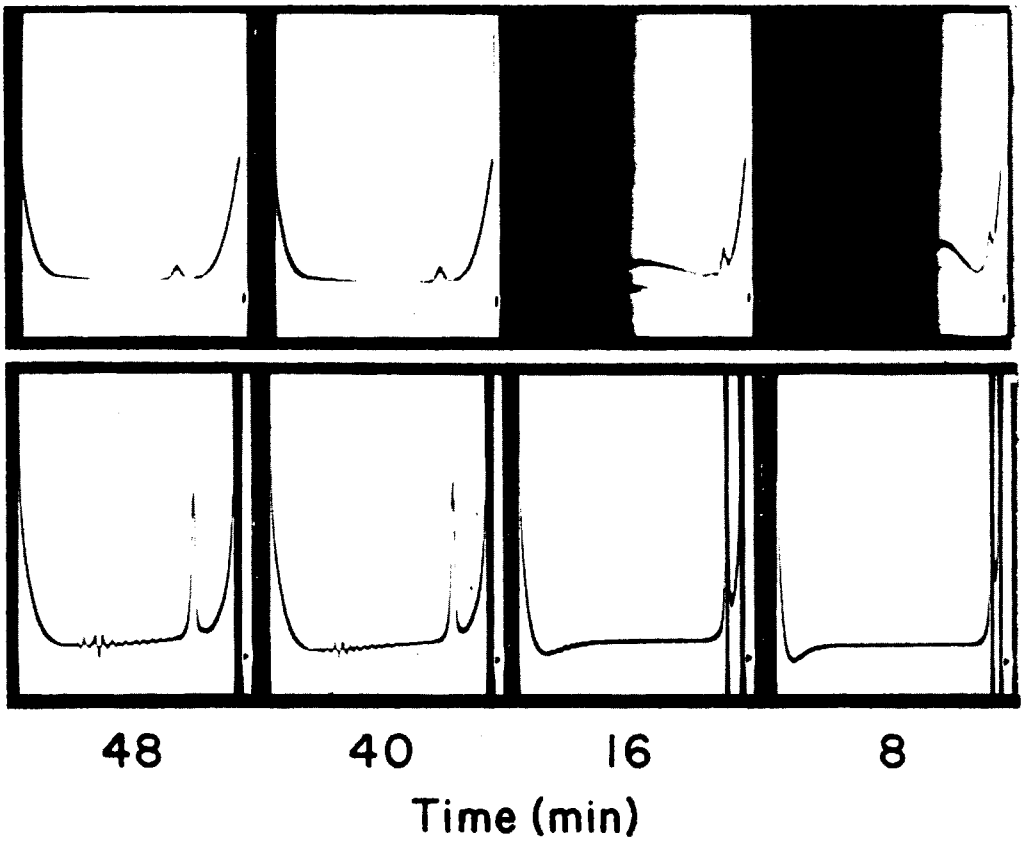

FIG. 2.—Sedimentation diagrams of bovine pancreatic microsomes in a solvent of 0.88 M sucrose, 1 mM potassium phosphate, and 0.5 mM Mg at *p*H 7.5 (upper frame), and in the same solvent plus 0.01 M sodium deoxycholate (lower frame). The photographs were taken at the times indicated, after the rotor had reached a speed of 37,020 rpm. The temperature was 7°.

The densities and viscosities of the sucrose solvents used in these experiments were measured at three temperatures. The measured values are presented in Table II. From these data factors were obtained for the viscosities of the respective solvents relative to water at the temperature at which the ultracentrifugal analyses were performed. These factors were applied in conjunction with a factor for the temperature dependence of the viscosity of water.

The $s_{20,w}$ values given in Table I apply for the ribonucleoprotein concentration existing in that experiment, a value approximately equal to 0.2% in each case.[3] The data presented in Figure 2 and Table I demonstrate that, under optimum ionic conditions, one ribosomal species predominates in reaction mixtures examined immediately upon release of the ribosomes from pancreatic microsomes, and that the same species predominates in media of different osmolarity. In all subsequent experiments, therefore, the deoxycholate reaction was carried out by Method 3, in 1 mM potassium phosphate buffer–2 mM magnesium, *p*H 7.5. The sedimentation pattern of a typical reaction mixture of this composition is shown in Figure 3. The detached ribosomes were collected subsequently by ultracentrifugation for 90 minutes with use of the SW-39 rotor.

TABLE II

DENSITIES AND VISCOSITIES OF SUCROSE-SOLVENTS[a]

Temp. (°C)	ρ	η	ρ	η
	I-a		I-b	
2	1.0339	1.2982	1.0351	1.3278
12	1.0329	1.2805	1.0341	1.3071
20	1.0314	1.2663	1.0324	1.2928
	II-a		II-b	
2	1.1184	3.0752	1.1192	3.1627
12	1.1162	2.8681	1.1167	2.9406
20	1.1136	2.7247	1.1144	2.7912

[a] I-a = 0.25 M sucrose. I-b = Same plus 0.02% soybean trypsin inhibitor + 0.01 M sodium deoxycholate. II-a = 0.88 M sucrose. II-b = Same plus 0.02% soybean trypsin inhibitor + 0.01 M sodium deoxycholate. ρ = Densities at the temperatures indicated. η = Viscosities relative to water at the temperatures indicated.

Isolation of Postmicrosomal Ribosomes (Step 5).—As shown in Figure 1, postmicrosomal ribosomes can be isolated directly from the postmicrosomal supernatant by centrifugation. In the experiments reported herein the solvent composition of the postmicrosomal supernatant was the same as that of the original homogenate (0.88 M sucrose). Hence, 10 hours at a centrifugal force of 105,000 × *g* were required for collection of postmicrosomal ribosomes.

Figure 4 presents the sedimentation pattern of postmicrosomal ribosomes prepared in this way and resuspended in 1 mM potassium phosphate, *p*H 7.5. As with microsomal ribosomes, the population of postmicrosomal ribosomes includes at least four species in addition to some material which did not separate from the meniscus under the conditions of centrifugation. With the respective sedimenting boundaries again

[3] The RNP concentration cited must be considered to be a low estimate because of the Johnston-Ogston effect (Johnston and Ogston, 1946).

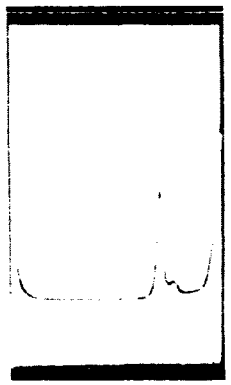

FIG. 3.—Sedimentation pattern of microsomal ribosomes in a solvent composed of 1 mM potassium phosphate, 2 mM Mg^{++}, and 0.01 M deoxycholate at *p*H 7.5. The picture shown was made after 32 minutes of centrifugation at 29,500 rpm and 0.7°.

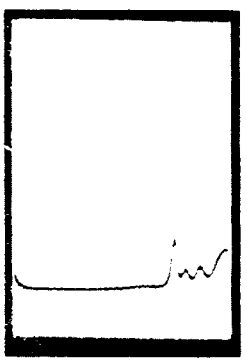

FIG. 4. Sedimentation of postmicrosomal ribosomes in 1 mM potassium phosphate buffer, *p*H 7.5. The pattern was made after 16 minutes of centrifugation at 1.8° and 29,500 rpm.

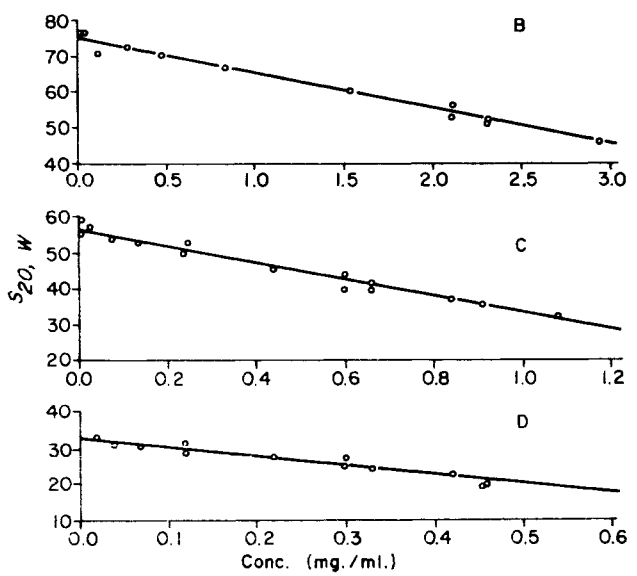

FIG. 5. The concentration dependence of sedimentation rates of microsomal ribosomes in a solvent composed of 0.25 M sucrose 0.02% soybean trypsin inhibitor–0.01 M deoxycholate. The abscissa presents the RNP concentration of the species for which sedimentation rates are plotted, uncorrected for the Johnston-Ogston effect (Johnston and Ogston, 1946).

designated A, B, C, and D in order of decreasing sedimentation rates, component B predominates in fresh preparations.

In the experiment shown in Figure 4, the total ribonucleoprotein concentration was approximately 0.4%. Under these conditions, the $s_{20,w}$ values of components B, C, and D, respectively, were 58 *S*, 46 *S*, and 32 *S*.

Properties

Sedimentation Constants of Microsomal Ribosomes.—During the course of study of the deoxycholate-mediated release of microsomal ribosomes in 0.25 M sucrose solutions, the effect of ribonucleoprotein concentration on the sedimentation rates was examined. In order to study also the behavior of the dissociation products these experiments were conducted in the absence of added magnesium. The ribosomal population was thus made up of 70% component B, 20% component C, and 10% component D. The concentration of each species was evaluated from knowledge of the total ribonucleoprotein concentration and the relative proportion of each species. The data are presented in Figure 5. Upon extrapolation to zero ribonucleoprotein concentration, the $s_{20,w}$ values of 75 *S*, 57 *S*, and 33 *S* were obtained from components B, C, and D, respectively.

The data, although limited by the considerations cited concerning corrections applied,[2] provide provisional sedimentation constants for the major species of microsomal ribosomes (component B) and its dissociation products. The values indicate that the population of these pancreatic ribosomes is similar in sedimentation properties to those found in mammalian and other systems (Petermann and Hamilton, 1961). Experiments designed to define more precisely the sedimentation coefficients of both microsomal and postmicrosomal ribosomes, as well as their respective concentration dependencies, are in progress.

Stability.—Fresh preparations of microsomal or postmicrosomal ribosomes, isolated by the procedure described, consist predominantly of the respective species designated "B." However, this species of ribosome, from either class, exhibits marked tendencies toward association and aggregation during washing or dialysis. In 1 mM potassium phosphate buffer at *p*H 7.5, association often occurred even in the absence of added magnesium, but these events were always accelerated by low concentrations of magnesium (0.1 mM) and potassium in excess of 5 mM.

It has become increasingly apparent that the behavior of crude pancreatic ribosomes cannot be regulated by ionic factors alone but relates, in part at least, to their high complement of basic enzymic protein, in particular ribonuclease. Experiments detailing the content of enzymic protein are reported below.

Yields and Composition.—Table III presents the respective yields and gross composition of pancreatic ribosomes isolated by the procedures described. The quantity of ribosomes harvested from the postmicrosomal supernatant exceeded slightly that from the microsomal fraction. In other respects, however, the two classes of ribosomes are grossly similar. In both

TABLE III

YIELD AND COMPOSITION OF RIBOSOMES

The analyses were carried out by methods indicated in the text. Postmicrosomal ribosomes had been washed once with 1 mM potassium phosphate–0.5 mM $MgCl_2$. Microsomal ribosomes were unwashed.

	Microsomal	Postmicrosomal
RNP	3.9 mg/g of tissue	4.7 mg/g of tissue
RNA	40.0% of RNP	38.0% of RNP
Ribonuclease	3.0% of total ribosomal protein	3.8% of total ribosomal protein

cases the ribosomes were composed of approximately 40% RNA and 60% protein. In each instance, 3–4% of the total ribosomal protein was accounted for by the enzyme, ribonuclease. Washing with cold buffer reduced the relative proportion of enzymic protein bound. Some typical properties of once-washed microsomal ribosomes are presented in Table IV.

TABLE IV

COMPOSITION OF WASHED MICROSOMAL RIBOSOMES

Analyses were carried out by procedures cited in text. Enzymic protein was extracted from the ribosomes with *p*H 2 HCl.

	mg/g Tissue[a]	%[a]
RNA	0.91	45
Protein	1.13	55
Trypsinogen	0.05	4.4
Chymotrypsinogen A	0.031	2.7
Ribonuclease	0.019	1.7

[a] The percentages of RNA and protein are expressed in terms of total ribonucleoprotein; the percentages of enzymic protein are expressed in terms of ribosomal protein.

Enzymic Protein of Microsomal Ribosomes.—In bovine pancreatic juice and zymogen granules the basic enzymic proteins (trypsinogen, chymotrypsinogen A, and ribonuclease) account for approximately one third of the total protein, whereas the acidic enzymes and zymogens (chymotrypsinogen B, deoxyribonuclease, and the procarboxypeptidases A and B) make up the remaining two thirds (Keller and Cohen, 1961). Table IV shows that the full complement of the basic proteins is bound to the once-washed ribosomes of pancreatic microsomes and that together these make up approximately 9% of the total ribosomal protein. In the experiments summarized in Table IV, enzymic protein was extracted from ribosomes with *p*H 2 HCl, and the acid extract was examined chromatographically for the anionic as well as the cationic proteins. None of the family of anionic proteins was detected, despite the fact that at least two members (chymotrypsinogen B and deoxyribonuclease) would be expected to tolerate the extraction procedure.

Several prominent members of the anionic family of secreted proteins, notably the procarboxypeptidases, are unstable in dilute acid. Hence, to test for their presence, attempts were made to effect release of the enzymic protein at neutral *p*H. Siekevitz and Palade (1960b) had noted that incubation of microsmal ribosomes from guinea pig pancreas in the presence of magnesium chelating agents effected release of the enzymic protein. Accordingly, experiments were conducted to test the effects of similar treatment of the bovine pancreatic ribosomes. Experimental details of these experiments are presented in the legend of Figure 6. Figure 6 shows that enzymic protein was released into the supernatant in each medium, including the control mixture to which no chelating agent had been added. The rates of release were only slightly affected by the presence of pyrophosphate but were accelerated by the presence of EDTA. The amounts of enzymic protein released were similar in all cases and equal to the level found in *p*H 2 extracts of the same preparation of ribosomes.

Accordingly, microsomal ribosomes were incubated for 90 minutes at 30° in 0.5 mM potassium phosphate buffer at *p*H 7.5, and the enzymic composition of the supernatant solution was examined by a combination of chromatographic and enzymatic techniques (Keller and Cohen, 1961). Again only cationic enzymes and zymogens were found in significant amounts. The levels found are reported in Table V.

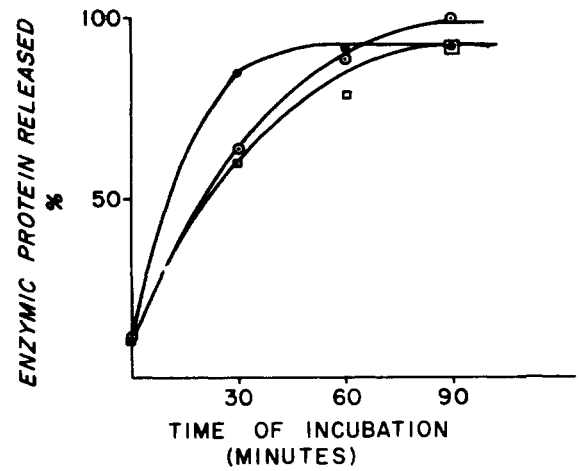

FIG. 6.—The release of enzymic protein from microsomal ribosomes during incubation at 30°. Microsomal ribosomes which had been washed once and subsequently dialyzed for 18 hours at 4° against 0.5 mM potassium phosphate buffer at *p*H 7.5 were incubated in the same buffer fortified with: 1 mM EDTA (●), 0.5 mM sodium pyrophosphate (□), no additional reagent (○). At intervals aliquots were removed from the incubation mixture and centrifuged at 127,000 × *g* for 90 minutes. The amount of enzymic protein released to the supernatant was estimated from enzymatic activity against acetyl-L-tyrosine ethyl ester after activation by trypsin and is expressed relative to the activity found in acid extracts of the same ribosomes.

It is noteworthy that enzymatic hydrolysis of the synthetic substrate acetyl-L-tyrosine ethyl ester after tryptic activation is a property common to three known pancreatic zymogens, namely, chymotrypsinogen A, chymotrypsinogen B, and the endopeptidase associated with procarboxypeptidase A (Keller *et al.*, 1958b). In pancreatic juice and zymogen granules approximately half of the potential activity *versus* acetyl-L-tyrosine ethyl ester resides in chymotrypsinogen B and procarboxypeptidase A. However, 92% of the total activity which was released from microsomal ribosomes was recovered as chymotrypsinogen A.

Enzymic Content of Postmicrosomal Ribosomes. Crude postmicrosomal ribosomes are also abundantly charged with the basic enzymic proteins of the cell. Some typical values for the enzymic protein released from crude ribosomes from 1 g of tissue are: 0.28 mg chymotrypsinogen(s) and 0.09 mg ribonuclease. Washing with cold 1 mM potassium phosphate buffer at *p*H 7.5 reduced these levels markedly. As shown in Figure 7, the reduction is exponential through two

TABLE V

ENZYMIC COMPLEMENT OF MICROSOMAL RIBOSOMES

Enzymic protein was released from washed microsomal ribosomes under conditions described in the text and were analyzed by chromatographic and enzymatic procedures described by Keller *et al.* (1958) and Keller and Cohen (1961).

	mg/g Tissue
Cationic proteins	
Trypsinogen	0.05
Chymotrypsinogen A	0.04
Ribonuclease	0.017
Anionic proteins	
Chymotrypsinogen B	0.0002
Procarboxypeptidase A	Negligible

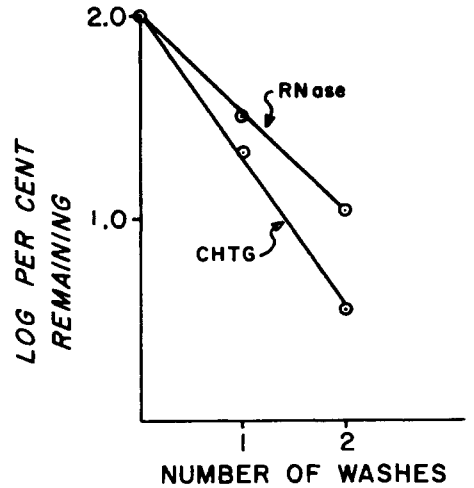

FIG. 7.—Effect of successive washes with cold 1 mM potassium phosphate buffer, *p*H 7.5, on the enzymic content of postmicrosomal ribosomes.

washes, with relatively more chymotrypsinogen than ribonuclease removed in each wash.

DISCUSSION

Pancreatic microsomes and the ribosomes derived from them by treatment with deoxycholate have been cited as the primary sites of biosynthesis of chymotrypsinogen A in guinea-pig (Siekevitz and Palade, 1960a), ribonuclease in mouse (Morris and Dickman, 1960), and trypsinogen, chymotrypsinogen A, and ribonuclease in beef (Dickman *et al.*, 1962a). In general, however, these conclusions were based on data derived from crude ribosomes, and more attention has been paid to the basic enzymes and zymogens than to the full spectrum of secreted proteins.

The objective of the present study was the preparation of both classes of bovine pancreatic ribosomes in forms stable enough to permit characterization and comparisons of both, as well as further study of the relationship of each class to the total spectrum of proteins secreted by the acinar cell. To date the stability requirement has been only partially achieved, but information gained from the present studies has contributed to an understanding of the probable cause of the instability.

The *in situ* state of pancreatic ribosomes as well as the question of relocation of either class during isolation procedures remain moot points (T'so, 1962). Hence, the distinction in the present study between bovine microsomal and postmicrosomal ribosomes is intended only in an operational sense. By the procedures described, the two types were isolated in similar yield (4–5 mg per g tissue), and their gross chemical compositions are similar. The sedimentation behavior of crude ribosomes of either class is also similar. Of the several species seen in fresh preparations, one species predominates in each class, and this species seems to correspond to the ubiquitous "80 *S*" ribosome previously reported in pancreas and other mammalian tissues (Siekevitz and Palade, 1960b; Dickman *et al.*, 1962b; Petermann and Hamilton, 1961). At all stages examined, low concentrations of magnesium have favored this species over those species with lower sedimentation rates. The precise optimum concentration of magnesium, however, as well as the threshold concentration beyond which association and aggregation occurred, have proved difficult to define. These aspects of the magnesium dependency have been seen to vary with the stage in purification of the particles, and, in general, purification has been accompanied by enhanced sensitivity toward magnesium. At all stages, pancreatic ribosomes are aggregated by those concentrations of magnesium which are tolerated—even required—by ribosomes from other mammalian tissues.

The same ultracentrifugal species was released from microsomal membranes by deoxycholate dissolved in solvents of different osmolarity. It is not presently known, however, whether the respective species are functionally identical.

In the crude, unwashed state, both classes of ribosomes contain significant quantities of enzymic protein. Washing with cold buffer reduced the levels in each instance. Certain differences were noted, however, in the response of the ribosomes to successive washes. In the case of microsomal ribosomes, approximately one half of the enzymic protein was removed in the initial wash, and a second wash removed only an additional 10%. In contrast, elimination of enzymic protein from postmicrosomal ribosomes appeared to proceed exponentially through two washes. Washed microsomal ribosomes contained the full complement of the basic proteins secreted by the cell but failed to exhibit the corresponding acidic proteins in equivalent proportions. This observation is in contrast to the report of Siekevitz and Palade (1960b) that the enzyme complement of guinea pig ribosomes included the acidic enzyme, amylase, as well as the basic proteins, chymotrypsinogen A and ribonuclease. It was not apparent, however, whether amylase was present in amounts commensurate with the basic proteins.

At least part of the enzymic protein bound to the microsomal ribosomes appears to be the nascent protein of the cell (Siekevitz and Palade, 1960a; Morris and Dickman, 1960; Dickman *et al.*, 1962a). However, it has been demonstrated (Keller and Cohen, 1961) that enzymic protein, and in particular the basic proteins, can arrive on the pancreatic ribosomes by relocation. The present data concerning the enzymic complement of pancreatic ribosomes must be interpreted in terms of these considerations as well as in terms of the interdependency of the enzymology and the structural integrity of the ribosomal unit. Tal and Elson (1961) have shown that dissociation of the 70 *S* ribosomes from *E. coli* into subunits was accompanied by release of enzymic protein, namely, deoxyribonuclease. Moreover, Petermann (1962) has shown that dissociation and association affect the binding of extraneous protein by sarcoma ribosomes. Thus in working with unstable particles the possibilities of artifact exist in at least two directions: premature release of nascent protein as well as enhanced binding of relocated protein. A valid description of the enzymic complement of pancreatic ribosomes will require more stable preparations in which the integrity of the molecular structure can be maintained through successive washes and analyses.

The present indications are that stabilization will require control of ribonuclease. Cation adsorbents, and particularly the clay, bentonite, have been used successfully to this end by Petermann and Pavlovec (1962) with tumor ribosomes, and by Fraenkel-Conrat *et al.* (1961) for TMV-RNA. Preliminary experiments in which cation adsorbents have been used to remove ribonuclease from preparations of pancreatic ribosomes have yielded encouraging results. Further experimentation is in progress.

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Beef Pancreas Ribosomes: Isolation and Properties*

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Ribonucleoprotein particles (ribosomes) have been isolated after treatment of beef pancreatic microsomal preparations with deoxycholate. Suspensions of these ribosomes in 1 mM phosphate, *p*H 7.4, consist almost exclusively of 80 *S* particles. In contrast to rat liver ribosomes, however, the beef pancreas particles were aggregated by low concentrations of Mg^{++}, by ionic strength of 0.1, or by EDTA. Spermine also precipitated these ribosomes, with the release of large amounts of protein into solution. Free electrophoresis demonstrated that the pancreatic ribosomes were less acidic than their liver homologs. This could be accounted for by the presence of considerable quantities of the basic proteins ribonuclease and chymotrypsinogen A in association with beef particles. The addition of ribonuclease or lysozyme to rat liver ribosomes resulted in a suspension which closely resembled beef pancreas ribosomes in a number of characteristics. The data obtained in this comparison of beef pancreas and rat liver ribosomes suggest that the extreme instability of beef pancreas ribosomes is most likely due to the presence of ribonuclease, which was adsorbed in the course of isolation.

Ribonucleoprotein particles (ribosomes) have been isolated from a variety of sources and intensively examined since they were first shown to be the site of amino acid incorporation into protein (Littlefield *et al.*, 1955; Roberts, 1958). Siekevitz and Palade (1958) reported that ribosomes of pancreas became labeled more rapidly than other intracellular fractions when guinea pigs were injected with leucine-C^{14}. Dickman *et al.* (1962a) measured the rate of incorporation of valine-C^{14} and tryptophan-C^{14} into the proteins of various intracellular fractions by beef pancreas slices and reached similar conclusions. Despite these evidences of considerable protein-synthesizing ability, isolated microsomal fractions or ribonucleoprotein particles from the pancreas of most species are inactive in the incorporation of amino acids into protein. There must be some factor or condition which is responsible for this inactivity. In this and the succeeding paper we describe the isolation of beef pancreas ribosomes and compare a number of their properties and characteristics with those from rat liver.

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Materials and Methods

(a) *Preparation of Ribosomes*

Pancreas was removed from cattle as soon as possible after slaughter and placed in ice-cold 0.25 M sucrose for transport to the laboratory. The tissue was trimmed of fat and connective tissue and forced through a prechilled French tissue press at a pressure between 2,000 and 10,000 p.s.i. All subsequent operations were performed at a temperature of 0–4°. The pulp was dispersed in sufficient ice-cold 0.25 M sucrose to