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Preparation and Properties of Beef Pancreas Microsomal Fraction*

SHERMAN R. DICKMAN, JAMES T. MADISON, AND ROBERT L. HOLTZER

From the Department of Biological Chemistry, University of Utah College of Medicine, Salt Lake City 12, Utah

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The microsomal fraction from beef pancreas homogenates has been prepared. It contains almost four times as much protein as RNA and about 25% phospholipid. It is most stable in solutions of low ionic strength at *p*H 7.0–7.4. Ribonucleoprotein particles of 80 *S* sedimentation rate can be separated from the membranous component by adjustment to *p*H 8.0. These particles are also detached from the membranous material by the detergents deoxycholate and lubrol. Chelating agents, on the other hand, dissociate the nucleic acid from the protein, with the latter forming aggregates of high molecular weight.

Current interest in the mechanism of protein biosynthesis has directed attention to the properties of the endoplasmic reticulum (microsomal fraction) and the ribonucleoprotein particles (ribosomes) of cells. Electron micrographs suggest that tissues which produce proteins primarily for secretion possess an extensive reticulum with attached particles, whereas tissues which synthesize proteins for utilization inside the cell of manufacture possess many unattached ribosomes (Harris, 1961). The pancreas, as an example of the former type, has been shown to synthesize RNase[1] in the microsomal fraction in the mouse (Morris and Dickman, 1960) and to incorporate labeled amino acid into chymotrypsinogen of guinea pig ribosomes (Siekevitz and Palade, 1960). To determine whether the attached ribonucleoprotein particles are the sole site of synthesis in a particular tissue, however, requires knowledge of their separation from the other components of the microsomal fraction. Results of such a study on beef pancreas microsomal fractions are included in this report. These data serve as a basis for the subfractionation procedures utilized in the accompanying paper (Dickman *et al.*, 1962), in which the incorporation of C^{14} amino acids into the proteins RNase, trypsinogen, and chymotrypsinogen A has been investigated in beef pancreas slices.

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[1] The abbreviations used are EDTA, ethylenediaminetetraacetic acid; PP_i, inorganic pyrophosphate; RNase, ribonuclease; RNA, ribonucleic acid.

EXPERIMENTAL

Homogenization and Fractionation.—Pancreas, preferably from Holstein cattle, was obtained about 10 minutes after slaughter and immediately immersed in ice-cold 0.25 M sucrose. All subsequent operations were carried out at 4°. Gross fat and connective tissue were removed, the glandular tissue forced through a stainless steel screen (1-mm holes), and the resulting pulp mixed with 9 volumes of chilled solution and homogenized with 4 strokes in a Potter-Elvejhem type homogenizer equipped with a Teflon pestle.

Differential centrifugation was accomplished with a Spinco Model L centrifuge. A large granule fraction (cell debris, nuclei, zymogen granules, mitochondria) was separated at 26,000 $\times$ *g* for 10 minutes and the microsomal fraction at 90,000 $\times$ *g* for 40 minutes. Each pellet was washed once by resuspension and recentrifugation.

Treatment of Microsomal Fraction.—When the microsomal fraction was to be further treated with detergents or by *p*H adjustment, the washed pellet was usually resuspended in mM phosphate by gentle homogenization. One-ninth volume of the detergent was added and the chilled suspension adjusted to the desired *p*H with the aid of a Leeds and Northrup *p*H meter equipped with miniature

TABLE I

DISTRIBUTION OF COMPONENTS IN FRACTIONS OF BEEF PANCREAS HOMOGENATES

Twenty per cent homogenates of beef pancreas were prepared in three media and separated into three fractions by centrifugation as follows: large granules, 26,000 × *g* for 10 minutes, microsomal fraction, 105,000 × *g* for 40 minutes. Each pellet was washed once.

Centrifugal Fraction	Concentration of Sucrose	Amount of Component in Fraction					
		Total Nitrogen	Protein Nitrogen	RNA		Ribonuclease	
	(M)	(%)	(mg)	(%)	(μg/mg N)	(%)	(μg/mg Protein N)
Large granules	0	26	7.6	32	85	28	87
	0.25	42	13.8	29	60	67	98
	0.88	41	14.9	34	70	59	85
Small granules (microsomes)	0	23	7.2	51	155	28	92
	0.25	17	5.2	36	167	13	49
	0.88	13	4.6	24	145	18	84
High-speed supernatant	0	51	17.1	17	25	44	60
	0.25	42	13.7	35	67	20	30
	0.88	46	16.5	42	70	23	30

electrodes. Any aggregated material was removed by a slow-speed centrifugation, and the insoluble material was collected at 90,000 × *g* for 60 minutes.

Methods.—Intracellular fractions were extracted with cold trichloracetic acid, organic solvents, and hot trichloracetic acid (Schneider, 1957). The extracts and residues were analyzed for total phosphorus (Gomori, 1942), for ribose by the orcinol method (Schneider, 1957), and for protein by a colorimetric method (Nayyar and Glick, 1954) or with bovine serum albumin as standard (Lowry *et al.*, 1951). The amounts of acid-soluble nucleotides were calculated from ribose determinations of the combined cold trichloracetic acid extracts. The factor 4.67 was used to convert ribose (mg) to RNA (mg) or to nucleotides. Total nitrogen was determined by the micro-Kjeldahl method (Hiller *et al.*, 1948). Trypsin-activated protease was assayed with Hammarsten casein used as substrate (Hirs, 1953). Under these conditions an enzymatically inactive protein is formed from trypsinogen.

RNase was assayed by the method of Dickman *et al.* (1956). Madison (1962) observed that if magnesium acetate is added to the *t*-butanol–glacial acetic acid solution at mM concentration, not only is the precipitation of the undigested RNA accomplished more rapidly, but also the final supernatant solution is less likely to be cloudy. This slight modification has speeded up the assay and resulted in greater consistency without affecting its sensitivity and range.

Ultracentrifugation.—The sedimentation patterns were obtained at 20° with a Spinco model E ultracentrifuge equipped with a Schlieren optical system. The patterns were projected in an enlarger and the relative proportion of components estimated with a planimeter.

Materials.—Deoxycholic acid (Matheson, Coleman and Bell) was neutralized immediately before use. Lubrol WX was a gift from Arnold Hoffman Co. and was purified by treatment with 95% ethanol before use.

RESULTS

Homogenizing Medium.—Since the introduction of sucrose as a homogenizing medium (Hogeboom et al., 1948), knowledge and understanding of intracellular organelles has increased greatly. The assumption is frequently made, however, that tissues other than liver will fractionate in the same manner as the hepatic. In the pancreas especially, isolation of particles is complicated by the presence of the zymogen granules which may release hydrolytic enzymes, and by the occurrence of a variety of basic proteins in considerable quantity. Knowledge and control of these factors are necessary prerequisites for the planning and evaluation of experiments with this tissue.

The effects of variations in sucrose concentration in the homogenization medium on the distribution of components of beef pancreas were examined. Table I lists the amounts of total N, protein N, RNA, and RNase in the large granule and microsomal fractions and in the high-speed supernatant solution. Homogenization in H_2O resulted in the disruption of many granules, since the supernatant solution contained 51% of the total N and 44% of the RNase activity. Homogenization in 0.25 M sucrose altered the distribution markedly. The large granules now became equal to the supernatant in N content and exhibited over three times as much RNase activity.

The microsomal fraction changed relatively little in N content in the three media, but its share of the total RNA decreased progressively from 50% to 24% in going from the hypo- to the

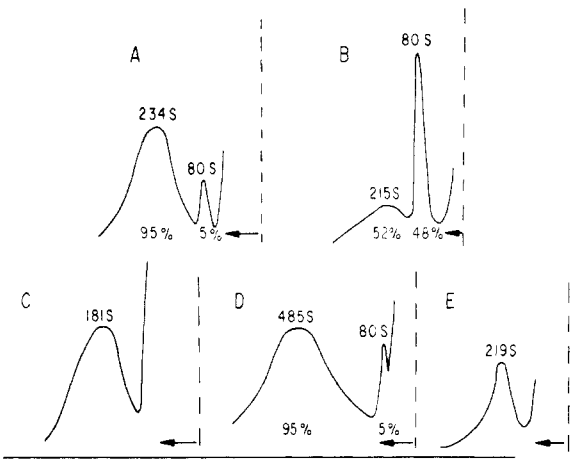

FIG. 1.—Sedimentation patterns of beef pancreas microsomal fractions. The washed microsomal fraction was resuspended in the solution indicated and centrifuged in the Spinco model E ultracentrifuge at 20°. (*A*) mM phosphate, *p*H 7.3. (*B*) mM phosphate, *p*H 8.0. (*C*) 0.1 M bicarbonate, 2 mM phosphate, 0.1 M EDTA, *p*H 8.2. (*D*) 0.1 M bicarbonate, 2 mM phosphate, mM Mg^{++}. (*E*) as in (*D*) plus 10 mM pyrophosphate, *p*H 8.2.

hypertonic solution.[2] This variation in RNA distribution is accounted for by the presence of increasing amounts in the high-speed supernatant solution. On the other hand, the specific amount (μg/mg N) of RNA in the microsomal fraction remained relatively constant in the three media.

The RNase activity of the microsomal fraction decreased considerably in 0.25 M sucrose, both in percentage as well as in specific activity compared to the water homogenate. There was a striking increase in RNase specific activity of the microsomal fraction prepared in hypertonic sucrose. The microsomal fraction prepared in 0.25 M sucrose possessed the highest specific amount of RNA and the least RNase of the three types of homogenates. Consequently this solution was utilized as the homogenizing medium throughout this work.

Properties of Microsomal Fraction.—The sedimentation characteristics of beef pancreas microsomal fraction are markedly affected by *p*H, ionic strength, and presence of chelating agents in the medium. As the sedimentation pattern of Figure 1a demonstrates, 95% of the material sedimented with an S_{20} of 234 in mM phosphate buffer, *p*H 7.3. A 77 *S* component accounted for the remainder. Since the S_{20} of free ribonucleoprotein particles is strongly dependent on their concentration, the material in this latter peak will be referred to as the 80 *S* component. When the microsomal fraction was adjusted in mM phosphate to *p*H 8.0 (Fig. 1b), a large number of 80 *S* particles were detached from the membranes. This pattern suggests that the 80 *S* particles are not artifacts of the fixation technique, as has been suggested by Sjostrand and Baker (1957) and Hanzon *et al.* (1959), but may be separated from other microsomal components by relatively mild procedures.

At 0.11 ionic strength, however (Fig. 1d), the proportion of 80 *S* particles decreased to 5%, and the remainder of the microsomal fraction aggregated to form 485 *S* material. The omission of Mg^{++} from the bicarbonate medium did not affect the sedimentation pattern, but the presence of 0.1 M EDTA or 0.01 M pyrophosphate decreased the sedimentation constants to 181 *S* and 219 *S* respectively (Fig. 1c,e). No 80 *S* particles were observed under these conditions.

An analysis of the sedimentable components due to these treatments was made, and the results are included in Table II. An increase in ionic strength at *p*H 8.2 lowered recovery of RNA to 89% of the original value even though the percentage in the pellet remained relatively constant. Inclusion of either EDTA or PP_i in the solution lowered RNA recovery to approximately half that of the control. These losses in RNA are largely accounted for by an increase in acid soluble nucleotides. The chelating agents have apparently stimulated RNA hydrolysis. Protein recovery, however, was not affected by the chelating agents, although the proportion of sedimentable protein decreased progressively with increase in their concentration.

These data demonstrate that the composition of the material comprising the major peaks illustrated in Fig. 1 varies widely. As calculated in the last column of Table II, the presence of the chelating agents approximately tripled the protein–RNA ratio of the pellet. Thus the S_{20} of the major peak was decreased owing to the presence of EDTA or PP_i, and the sedimentable material contained much less nucleic acid than the control. Sachs (1958) and Palade and Siekevitz (1956a) have reported effects similar to these with rat liver microsomes.

Figure 2 shows the effect of several detergents on the sedimentation pattern of components of beef pancreas microsomal fraction. A solution containing 0.5% deoxycholate (Fig. 2b) completely disrupted the 234 *S* peak material, and only 80 *S* particles (ribosomes) were observed; 0.125% deoxycholate (Fig. 2a) appears to have only partially solubilized the membranous component of the microsomes, but did reduce the sedimentation coefficient from 234 *S* to 146 *S*. A solution containing 0.5% lubrol (Fig. 2c) and 0.5% lubrol plus 0.125% deoxycholate (Fig. 2d) completely destroyed the faster sedimenting peak of the microsomes yet did not completely solubilize the microsomal phospholipid. It appears likely that the turbidity visible to the left in Figures 2c and 2d represents a large portion of nondispersed membranous components of the microsomes.

[2] The centrifugal force utilized was insufficient to sediment all of the microsomal fraction in 0.88 M sucrose. The main conclusions of this experiment, however, are based on analyses of the microsomal pellets and are relatively uninfluenced by yield.

TABLE II

SOLUBILIZATION OF MICROSOMAL COMPONENTS

Equal amounts of washed beef pancreas microsomal fraction were suspended in the indicated solutions. The suspensions were centrifuged for 75 minutes at 90,000 × *g* and the supernatant solution and pellets analyzed. For each component, the sum of the amount in the pellet and in the supernatant solution from each treatment is termed "total."

Treatment	Total Acid-Soluble Nucleotides	Ribonucleic Acid		Protein		Protein / RNA of Pellet
		Total	Pellet / Total	Total	Pellet / Total	
	(mg)	(mg)	(%)	(mg)	(%)	
1. mM P_i, *p*H 8.2	2.6	9.99	95	49.9	93	4.9
2. 2mM P_i, 0.1 M $KHCO_3$, *p*H 8.2	4.5	8.88	97	52.0	90	5.4
3. as 2 + mM EDTA	9.8	4.09	74	50.8	86	14.5
4. as 2 + 10 mM EDTA	11.2	4.81	73	66.8	85	16.2
5. as 2 + 0.1 M EDTA	10.3	5.58	59	51.6	75	13.0
6. as 2 + mM PP_i	9.4	4.62	81	57.3	85	13.0
7. as 2 + 10 mM PP_i	10.2	4.21	62	49.8	78	14.7

The 112 *S* and 104 *S* peaks in these patterns probably represent dimers of the 80 *S* particles.

Chemical Analyses of Microsomal Fraction and Ribonucleoprotein Particles.—Ribonucleoprotein particles (ribosomes) occur as unattached organelles in many cells. In others, especially those which produce proteins for secretion, an extensive endoplasmic reticulum with attached granules can be observed with the electron microscope in osmium-fixed preparations (Palade, 1958). In preparations from liver both the microsomal fraction and the ribosomes have been demonstrated to incorporate amino acids into protein, with the latter exhibiting a higher stability in this important capability (Kirsch et al., 1960). The ribosome is presently conceived as a ribonucleoprotein particle which contains most of the RNA of the microsomal fraction, but with little or no phospholipid (Palade, 1958; Cohn and Butler, 1957, 1958).

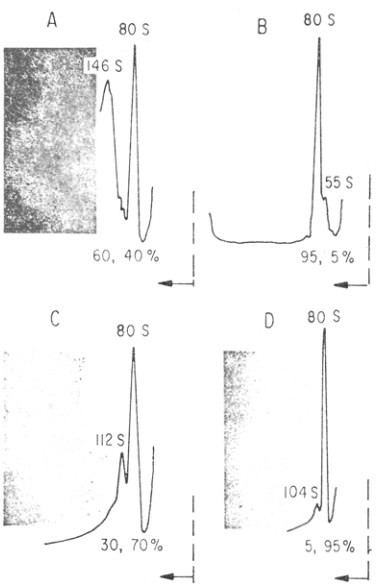

FIG. 2.—Effect of detergents on sedimentation patterns of beef pancreas microsomal fractions. Washed microsomal fraction was resuspended in 0.25 M sucrose and brought to the concentration of detergents indicated. The suspensions were centrifuged at 90,000 × *g* for 75 minutes. The pellets were resuspended in mM phosphate, *p*H 7.3, and centrifuged in the Spinco model E ultracentrifuge at 20°. (A) 0.125% deoxycholate. (B) 0.5% deoxycholate. (C) 0.5% lubrol. (D) 0.5% lubrol-0.125% deoxycholate. The stippled area to the left in (A), (C), and (D) represents partially insoluble membranous material.

Various agents, mostly detergents, have been employed in the preparation of ribosomes. Since the sedimentation patterns of the microsomal fractions demonstrated a marked *p*H effect, variations in this factor as well as in concentration of detergent on the composition of the resulting ribonucleoprotein particles were determined. Figure 3 shows the effect of two substances, 0.125% deoxycholate and 0.5% lubrol WX on the solubilization of five components: total N, RNA, phospholipid, RNase, and trypsin-activated protease.

The effectiveness of 0.125% deoxycholate and 0.5% lubrol increased with increase in *p*H. At *p*H 7.6, RNA recovery in the pellet was high with both detergents, but solubilization of phospholipid phosphorus, total N, RNase, and trypsin-activated protease activity was considerably greater in the lubrol-treated suspensions, so that lubrol was employed in many of the studies described in the accompanying paper (Dickman *et al.*, 1962).

Data on the composition of microsomal fraction subjected to a wide range of *p*H treatments are set out in Table III. The effect of 0.5% deoxycholate at three *p*H values is included for comparison. An increase in *p*H by itself resulted in a decrease in RNA recovery as well as its percentage in the pellet. Most of this "lost" RNA was recovered as acid-soluble nucleotides. There was a tendency for slightly higher hydrolysis of RNA in the range *p*H 9–10. The presence of deoxycholate, however, resulted in the formation of relatively large amounts of acid-soluble nucleotides even at neutral *p*H. Since deoxycholate possesses chelating properties in addition to its

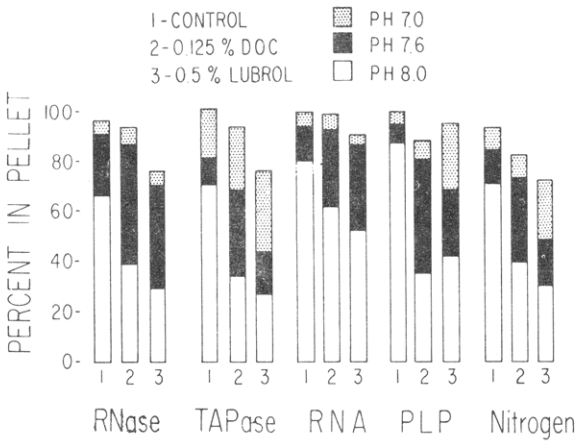

FIG. 3.—Solubilization of microsomal components by detergents. Washed beef pancreas microsomes were resuspended in mM phosphate, *p*H 7.0, and brought to 0.125% deoxycholate or 0.5% lubrol. The suspensions were then adjusted to *p*H 7.0, 7.6, or 8.0 and centrifuged at 90,000 × *g* for 75 minutes. The pellets and supernatant solutions were each analyzed for total nitrogen, phospholipid phosphorus (PLP), RNA and RNase, and trypsin-activated protease (TAPase) activities.

detergent action, this effect may be compared to those obtained with EDTA and PP_i reported in Table II.

The amount of ribose-containing material which became alcohol soluble was also determined. The disruption of the microsomal fraction at high *p*H did not result in the formation of appreciable alcohol-soluble ribose after the trichloracetic acid treatments. Protein and phospholipid recovery remained relatively constant, but the amounts in the pellets were lower at the higher *p*H values. The protein–RNA ratio of the pellet was practically constant up to *p*H 8.0, but an increase was found at higher *p*H values. Deoxycholate addition resulted in almost complete solubilization of the phospholipid and much of the protein. The ribonucleoprotein particles obtained in this manner contained about twice as much protein as RNA.

DISCUSSION

This work was undertaken to help elucidate the structure of beef pancreas microsomal fraction and to compare certain of its properties to those of other species. To this end Table IV has been assembled; this table contains data from four separate beef pancreas samples of known breed, and the data of Palade and Siekevitz (1956b) on guinea pig pancreas and of Hokin (1955) on dog pancreas. Analyses of rat and mouse liver microsomal fraction have been included for comparative purposes.

Of the three types of pancreas the guinea pig pancreas is highest in protein and lowest in phospholipid. The high phospholipid content of beef pancreas is noteworthy. The protein–RNA ratios are remarkably similar, considering the diversity of the tissue samples. As might be expected, the liver preparations contain less RNA than the pancreas, with the protein–RNA ratio accordingly somewhat higher. The lower protein–RNA ratio of beef compared to other species suggests that RNA hydrolysis by RNase is absent or minimal under these conditions of preparation despite the presence of RNase in this pellet (Table I).

The differences in composition between beef and guinea pig microsomal fractions may be due to species differences or to the conditions of preparation. The data of Table I show that the RNase concentration of the microsomal fraction from 0.88 M sucrose homogenates of beef pancreas was almost double that obtained from 0.25 M

TABLE III

EFFECT OF *p*H AND DEOXYCHOLATE TREATMENTS ON COMPOSITION OF BEEF PANCREAS MICROSOMAL FRACTIONS

Washed microsomal fractions were suspended in mM phosphate, *p*H 7.0, and adjusted to the indicated *p*H value. Three suspensions were brought to 0.5% deoxycholate before the *p*H adjustment. All suspensions were centrifuged at 90,000 × *g*. for 60 minutes, and the pellets and supernatant solutions were frozen until analyzed. For each component, the sum of the amount in the pellet and in the supernatant solution from each treatment is termed "total."

Treatment	Acid-Soluble Nucleotides			Phospholipid		—Ribonucleic Acid—			—Protein—		Protein / RNA of Pellet
	Supernatant	Pellet	Total	Total	Pellet / Total	Total	Pellet / Total	Recovery	Total	Pellet / Total	
	(mg)	(mg)	(mg)	(mg)	(%)	(mg)	(%)	(%)	(mg)	(%)	
*p*H 7.0	—	1.4	—	21.0	100	13.3	100	100	54.2	98	4.0
7.2	4.4	1.4	5.8	20.5	100	13.2	98	100	52.2	96	3.8
7.5	4.9	1.4	6.3	20.0	100	12.6	98	95	51.0	94	3.8
8.0	5.0	1.5	6.5	21.6	97	12.0	97	90	51.4	91	4.0
9.0	5.9	1.6	7.5	20.1	95	9.8	96	74	50.2	87	4.8
10.0	7.8	1.9	9.7	23.4	90	7.9	85	60	45.8	72	4.9
Deoxycholate											
*p*H 7.0	8.5	1.6	10.1	20.0	2	5.3	83	40	62.8	15	2.1
7.2	9.1	1.6	10.7	20.3	4	4.9	84	36	60.3	15	2.3
8.0	12.5	1.5	14.0	21.9	2	5.1	76	38	67.7	11	2.0

TABLE IV
COMPOSITION OF MICROSOMAL FRACTIONS FROM VARIOUS SOURCES

Sample	Protein (%)	RNA (%)	Phospholipid (%)	Protein / RNA
Pancreas				
Beef				
Holstein 1	59.5	14.0	26.5	4.3
Holstein 2	58.5	14.8	26.8	3.9
Hereford 1	60.5	16.0	23.8	3.8
Hereford 2	57.0	16.7	26.4	3.4
Average	58.8	15.4	25.9	3.85
Guinea pig 1	74.5	16.4	8.9	4.5
Guinea pig 2 (Palade and Siekevitz, 1956b)	76.3	17.1	7.0	4.5
Dog (Hokin, 1955, Table II)	61.8	17.6	20.8	3.5
Liver				
Rat (Palade and Siekevitz, 1956a)	55.3	9.8	34.8	5.6
Mouse (Barnum and Huseby, 1948)	56.1	9.1	22.0	6.2

sucrose homogenates. Keller and Cohen (1961) demonstrated the adsorption of the basic protein, chymotrypsinogen A, during the isolation of beef pancreas ribosomes. It is possible that this adsorption first occurs on the microsomal fraction and is greater from 0.88 M sucrose than from 0.25 M sucrose solutions. This suggestion is consistent with the high content of protein in the microsomal fraction from guinea pig pancreas reported by Palade and Siekevitz (1956b).

The relative stability of beef pancreatic microsomal fraction is of interest in connection with its inability to incorporate amino acids into protein.[3] Palade and Siekevitz observed that the microsomal fraction from guinea pig pancreas was less stable than that from liver. While an extensive comparison of the relative stabilities of guinea pig and bovine pancreatic microsomes was not the objective of this work, the data at hand suggest that the bovine is somewhat less labile than the guinea pig. In the latter, for example, approximately 30% of the protein and RNA became nonsedimentable after incubation for 30 minutes at 0° in 0.88 M sucrose. The bovine sample, however, quantitatively retained these components in mM P_i, *p*H 7.2 (Table II). This difference may also be due to differing conditions rather than to species. A difference in procedure that deserves mention in this connection concerns the washing of the fraction. The bovine sample was washed once and the wash solution discarded before further treatment and analysis, whereas no description of such a step is included by Palade and Siekevitz (1956b). If the amount of material which was removed from the sedimented pellet be considered as degradation products, then the beef fraction would appear considerably more labile than it does.

[3] S. R. Dickman, unpublished.

The increase in acid-soluble nucleotides on treatment of beef pancreas microsomes with chelating agents is more readily attributable to an alteration in the status of RNA than to an activation of RNase, as was found in mouse pancreas fractions (Dickman and Morrill, 1959). The loss of 80 *S* particles and the large increase in protein-RNA ratio of sedimentable material suggests that RNA of ribosomes is bonded to protein by divalent cations. On the addition of chelating agents the RNA becomes susceptible to attack by RNase and the proteins aggregate.

The data presented in this work support the dual nature of the pancreatic endoplasmic reticulum: (1) a membranous component, and (2) attached ribonucleoprotein particles, as suggested by Palade and Siekevitz (1956b) from their studies of guinea pig pancreas. In addition, the sedimentation patterns demonstrate a similarity of the beef pancreas ribosomes to those of other tissues (Roberts, 1958). In one of the few sedimentation studies of the microsomal fraction which have been reported, Petermann *et al.* (1953) resuspended rat liver microsomes in buffer (*p*H 8.6, $\mu = 0.1$) and observed sedimentation constants from 130 *S* down to 27 *S*. Although these conditions have not been reproduced exactly in the present work, the large increase in the amount of 80 *S* particles at *p*H 8.0 compared to *p*H 7.3 at low ionic strength suggests that the forces which are responsible for the maintenance of microsomal structure are extremely weak. Furthermore, one might postulate that the release of 80 *S* particles is dependent on two factors: (1) solubilization of a certain proportion of the phospholipid, and (2) decrease in ionic attraction between the ribonucleoprotein particles and membranous components at higher *p*H values.

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Protein Synthesis by Beef Pancreas Slices*

S. R. DICKMAN, R. L. HOLTZER, AND G. GAZZINELLI

From the Department of Biochemistry, University of Utah College of Medicine, Salt Lake City 12, Utah

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Beef pancreas slices constitute a very active amino acid incorporating system. Rate of uptake of valine-C^{14} and tryptophan-C^{14} into protein indicates that ribosomes are the site of synthesis. Valine-C^{14} has also been shown to be incorporated into the chromatographed proteins trypsinogen, chymotrypsinogen A, and ribonuclease during short (2 and 15 minute) incubations. The specific radioactivities of the latter two purified proteins in the microsomal solubilized portion were much greater than that of total protein in this fraction after a 2-minute incubation. This large difference in specific radioactivity was not found in the ribosomes themselves. The data suggest either that "export" proteins are synthesized many times more rapidly than total protein or that the sites of synthesis of the two types of protein are different.

Recent research has resulted in a marked increase in our understanding of some of the reactions of protein biosynthesis (Harris, 1961). The manner in which individual proteins are synthesized, however, remains unknown. In pursuit of this objective it is essential that the biosynthesis of specific proteins or polypeptides of known amino acid sequence be studied. The pancreas generally is recognized as an organ which is capable of a high rate of protein biosynthesis. In addition, the bovine gland is the source of a number of highly purified proteins (Northrup *et al.*, 1948). The amino acid sequences of several of these proteins (insulin [Sanger and Thompson, 1951, 1953; Ryle *et al.*, 1955], glucagon [Bromer *et al.*, 1957], and RNase [Hirs *et al.*, 1958]) have been determined, and those of the proteolytic zymogens presently are being investigated (Desnuelle, 1960).

In the present work the time course of incorporation of L-valine-C^{14} and DL-tryptophan-C^{14} into the proteins of intracellular fractions of beef pancreas was measured. Some of the factors which affect the rate of incorporation also have been studied. Finally, the uptake of L-valine-C^{14} into the specific proteins trypsinogen, chymotrypsinogen-A, and ribonuclease of two microsomal subfractions has been determined at two time periods.

Experimental Procedures

Bovine pancrease was obtained as soon as possible after the death of the animal and was trimmed and cut into 2-cm cubes and placed in ice-cold Krebs III solution (Krebs, 1950). Holstein tissues were preferred, since they generally are less fatty than those from other breeds, and slicing and homogenization are thereby facilitated. A

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