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THE ISOLATION OF YEAST RIBOSOMES ASSOCIATED WITH TRIOSE PHOSPHATE DEHYDROGENASE*

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The role of the ribosome in the synthesis of proteins has been clarified with the development of *in vitro* systems which incorporate isotopically labeled amino acids into peptide linkages.[1] Recently, the older concept that the ribosome contained template RNA for the synthesis of specific proteins has been expanded by the demonstration of a metabolically active RNA designated as "messenger" RNA, which exists in bacterial cells both in the soluble fraction and associated with ribosomes.[2, 3] The ability of reticulocytes to synthesize protein in the absence of DNA[4] suggests that there may be cases in which the "messenger" RNA is stable. If it were possible to isolate a family of ribosomes which was involved in the synthesis of a specific enzyme, it might be possible to demonstrate that the RNA of these ribosomes differed in structural and functional properties from that of the total population. The data reported in this paper concern an immunological method of isolation. Antiserum to crystalline yeast triose phosphate dehydrogenase was used to precipitate a small percentage of the total ribosomal population. By this method, enrichment of the triose phosphate dehydrogenase activity per unit of ribosomal RNA was obtained.[5]

Materials and Methods.—Triose phosphate dehydrogenase (TPD) was prepared from baker's yeast (Red Star Yeast and Products Co., Cleveland, Ohio) by the method of Krebs.[6] Different preparations of the enzyme were recrystallized either two or three times. Crystalline aldolase, prepared from rabbit muscle by the method of Taylor *et al.*,[7] was provided by H. Z. Sable. The assay for triose phosphate dehydrogenase activity was as follows. To a cuvette with a 1 cm light path were added 0.6 ml of 0.06 *M* sodium pyrophosphate pH 8.5, 0.04 ml of 0.17 *M* sodium arsenate, 0.01 ml of 0.05 *M* DPN, 0.144 ml of 0.05 *M* cysteine, 0.27 mg of aldolase, an aliquot of the enzyme, and water to a final volume of 1.1 ml. After preincubation for 3 min, 0.1 ml of 0.05 *M* fructose diphosphate was added and the rate of reduction of DPN at 340 mμ between 30 and 90 sec was measured. Preparations with an activity of approximately 25 μm/min/mg of protein, when assayed by this procedure, were obtained.

Alcohol dehydrogenase was prepared from baker's yeast (Red Star), recrystallized three times, and assayed by the methods of Racker.[8] For protein determinations, the Folin-phenol method[9] was employed as well as that of McDuffie and Kabat,[10] employing an extinction coefficient at 277 mμ of 0.01 per μg of protein nitrogen per ml.

Antisera to triose phosphate dehydrogenase and alcohol dehydrogenase were prepared by immunization of rabbits. This was done either by intravenous injections (12 injections of 5 mg each over a 4-week period) or by subcutaneous administration of enzyme in adjuvant. Thirty-two mg of protein in 4 ml of Bacto-Adjuvant Complete, Difco Laboratories, Detroit, Michigan, were injected into 4 loci on the back. One month after injection, 2 mg of enzyme were injected intravenously and 8 days later the rabbits were bled. The amounts of antibody obtained by the two methods were comparable. Sera were heated at 56°C for 30 min. Preparations of antisera

were tested for activity by the precipitin reaction, by inactivation of enzyme activity, and by the method of Ouchterlony.[11] The preparation of antiserum to egg albumin was provided by A. Stavitsky. Recrystallized egg albumin was obtained from Pentex Inc., Kankakee, Illinois.

Ribosomes were isolated from baker's yeast[12] (Red Star) by disrupting the cells with glass beads. A $MgCl_2$-buffer solution containing 0.005 *M* potassium phosphate pH 6.85, 0.0005 *M* $MgCl_2$ was found to produce the least clumping and precipitation of ribosomes on successive low-speed centrifugation cycles. Concentrations of Mg^{++} from 5×10^{-3} to 10^{-4} *M* were tested. A Nossal shaker[13] or a cell homogenisator (Model MSK, Firma Braun Apparateau, Melsungen, Germany) was used for preparation of ribosomes. 37.5 gm of yeast were suspended in 100 ml of the $MgCl_2$-buffer, and aliquots were shaken at 0°C with an equal volume of Ballotini No. 12 glass beads for 12 sec in the Nossal shaker or for 45 sec in the Braun apparatus. After settling of the beads, a supernatant fraction was decanted and centrifuged at $15{,}000 \times g$ for 20 min at 0°C. The resulting supernatant fraction was centrifuged at $105{,}000 \times g$ for 1 hr. The supernatant fraction was decanted and the inside walls of the tubes were rinsed three times with the suspending medium. The precipitate was then suspended by homogenization in the $MgCl_2$-buffer solution and centrifuged for 1 hr at $105{,}000 \times g$. The tubes were again rinsed and the ribosomal pellets were resuspended. This washing procedure was performed as often as five times. Ribosomes prepared in this manner contained 30–35 per cent RNA and 65–70 per cent protein. RNA determinations were done by either an orcinol[14] or an optical density[15] method. Enzyme activity

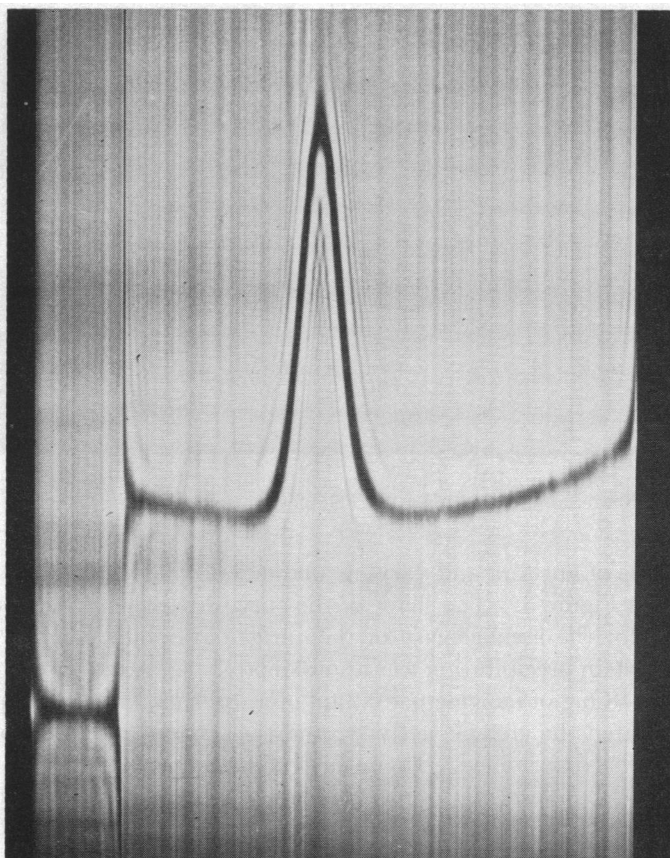

FIG. 1.—Ultracentrifugation of a 1.4 per cent solution of triose phosphate dehydrogenase in potassium phosphate buffer 0.1 μ, pH 7.2. The picture was taken 48 min after a speed of 59,660 rpm was attained.

of ribosomes was determined exactly as with crystalline enzymes. Prior to the determination, all aliquots from a series of centrifugations were adjusted to the same RNA concentration.

For precipitation of a fraction of yeast ribosomes with antiserum specific for triose phosphate dehydrogenase, ribosomes which had been washed 2 or 3 times were diluted in $MgCl_2$-buffer to give an RNA concentration of 3–5 mg/ml. This suspension was centrifuged at 3,000 $\times$ g for 15 min to remove any aggregated particles. Increasing amounts of ribosomes were added to a series of tubes containing 0.2 ml of antiserum or control serum, and the final volume was adjusted as indicated in the tables. After standing at 0°C for the specified time, the tubes were centrifuged at 3,000 $\times$ g for 5 min. The precipitates, unless otherwise noted, were washed three times with 3 ml aliquots of cold $MgCl_2$-buffer solution and then dissolved in 0.2 ml 0.5 N NaOH and made up to 2.0 ml by addition of 0.25 M acetic acid. Aliquots were taken for determination of RNA.

Results.—Triose phosphate dehydrogenase and antiserum: The immunological isolation of ribosomal material depends upon the purity of the antigen used and the antibody produced. The triose phosphate dehydrogenase, prepared from yeast did not show evidence of significant heterogeneity when examined by ultracentrifugation (Fig. 1) or by electrophoresis (Fig. 2). Precipitin curves obtained with

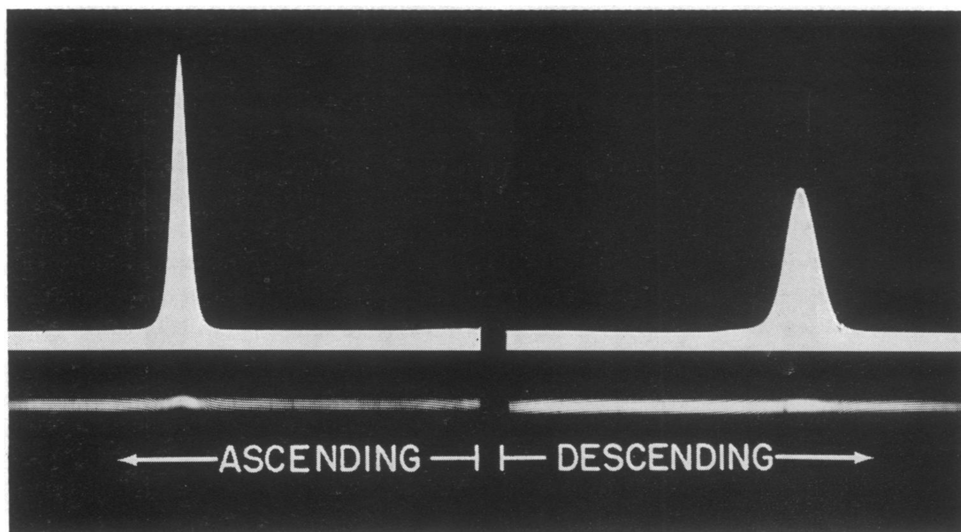

FIG. 2.—Electrophoretic analysis of a 2.8 per cent solution of triose phosphate dehydrogenase in potassium phosphate buffer 0.1 μ, pH 7.2. The picture was taken after 569 min at 80 volts and 10 milliamps.

two preparations of antisera and varying amounts of triose phosphate dehydrogenase are shown in Figure 3. The shape of the curves (particularly curve 2) provides some evidence for the homogeneity of the antigen-antibody system.[16] The point of maximum protein precipitation was approximately the same for each preparation of antiserum, 0.18 mg of enzyme per 0.2 ml of antiserum, or 0.90 mg per ml. The antiserum inhibited the enzyme activity, and the amount required to give 75 per cent inhibition was determined. From this value, the ratio of enzyme to antiserum necessary for 100 per cent inhibition was calculated to be 1.09 mg of enzyme per ml of antiserum. This figure is close to that obtained from the precipitin curve. Results of experiments which utilized the gel diffusion method of Ouchterlony[11] were variable depending on the conditions. When the purified protein interacted with antiserum in agar containing sodium azide (Fig. 4), either a broad diffuse line

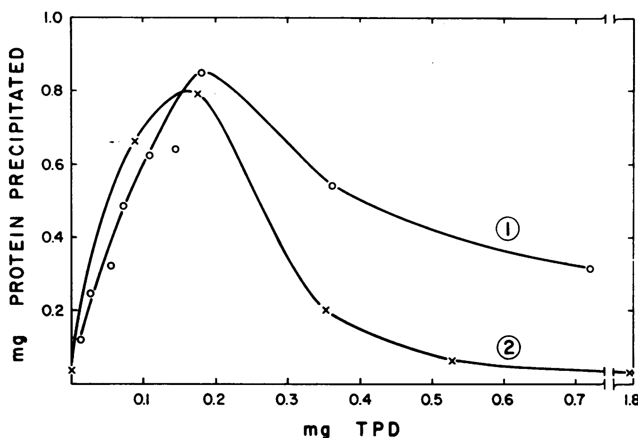

FIG. 3.—Precipitin curves of crystalline triose phosphate dehydrogenase with antiserum. Points were determined with 0.2 ml of antiserum. Antiserum preparation A was used for determination of curve 1, and preparation B for curve 2.

or multiple discrete lines appeared. By gentle aeration of the antigen with O_2, the precipitation band could occasionally be made more broad and diffuse. This result was obtained with different dilutions of the antigen and was also noted in agar preparations without azide. In contrast to this, when the antigen-antibody reaction occurred in agar containing either merthiolate or para-chloromercuribenzoate, only a single sharp line was observed. Since triose phosphate dehydrogenase is known to contain sulfhydryl groups,[17] the most reasonable interpretation is that the enzyme can exist in several forms depending upon the oxidation-reduction state of the SH groups.

Association of triose phosphate dehydrogenase with ribosomes: In order to isolate a family of ribosomes, it was necessary to demonstrate that the enzyme associated with the ribosomes was not loosely adsorbed. Triose phosphate dehydrogenase activity was measured after successive washings of the ribosomes by high-speed centrifugation. The results are presented in Table 1. It is apparent that the activity following the first cycle decreased only gradually, which suggests that whatever enzyme activity was bound to the ribosomes was bound rather tightly. In experiment 6*a* and 6*b*, crystalline triose phosphate dehydrogenase was added

TABLE 1

TRIOSE PHOSPHATE DEHYDROGENASE ACTIVITY IN RIBOSOMES FOLLOWING SUCCESSIVE CENTRIFUGATION CYCLES

		μm DPNH/min/mg RNA Centrifugation cycle				
Experiment	Additions	1	2	3	4	5
1	None	6.7	3.4	2.9	2.0	1.4
2	None	5.0	3.4	2.0	1.8	
3	None	6.0	4.0	3.3	2.8	
4	None	6.3	5.7	3.5		
5	None	4.3	3.1	1.9		
6	None	8.2	6.8	4.1		
6*a*	0.4 mg TPD*/mg RNA	17.3	2.3	1.6		
6*b*	0.8 mg	20.4	1.7	0.8		

Experiments were done as described in the *Methods* section.
* Triose phosphate dehydrogenase.

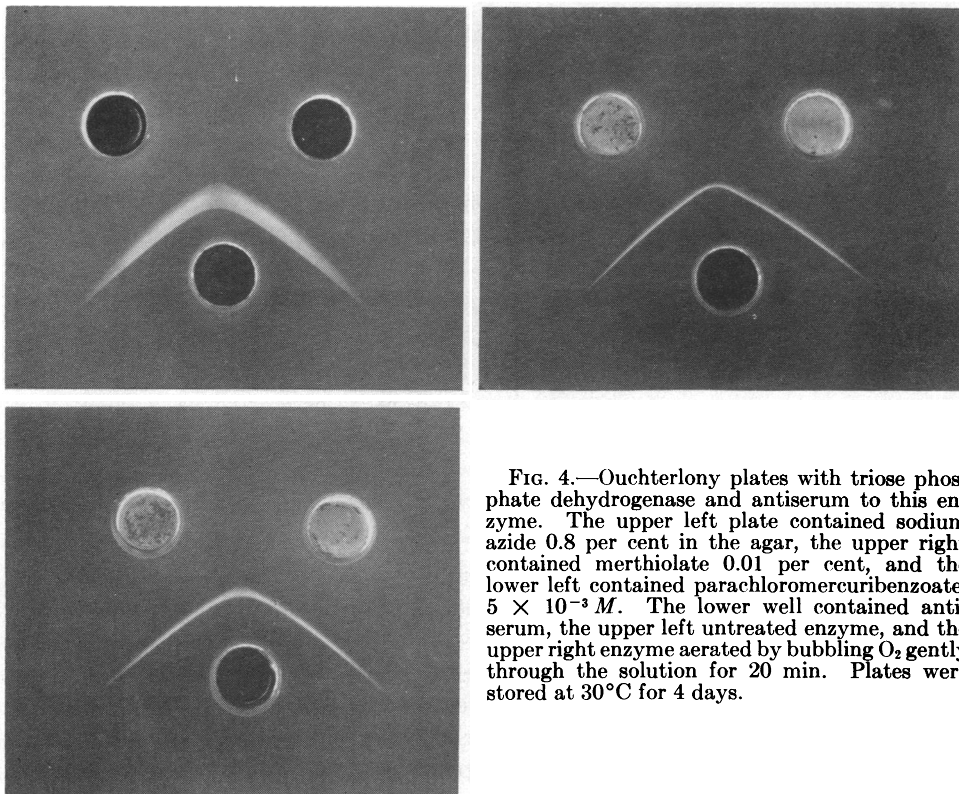

FIG. 4.—Ouchterlony plates with triose phosphate dehydrogenase and antiserum to this enzyme. The upper left plate contained sodium azide 0.8 per cent in the agar, the upper right contained merthiolate 0.01 per cent, and the lower left contained parachloromercuribenzoate, $5 \times 10^{-3} M$. The lower well contained antiserum, the upper left untreated enzyme, and the upper right enzyme aerated by bubbling O_2 gently through the solution for 20 min. Plates were stored at 30°C for 4 days.

in two different concentrations to the ribosomal suspension prior to the first centrifugation cycle. This addition did not increase the amount of activity found on the ribosomes after the first cycle but actually decreased it. The reason for this is not known; it was not due to an increase in ionic strength, since the enzyme was dialyzed against $MgCl_2$-buffer prior to addition. When mercaptoethanol in concentrations up to 0.1 M was added to ribosomes and they were then recentrifuged and analyzed for triose phosphate dehydrogenase, there was no change in enzymatic activity compared to controls. These data support the assumption that adsorption of the soluble enzyme on the ribosomes or linkage of the enzyme to the ribosomes through disulfide bonds does not account for the ribosomal enzymatic activity. The amount of enzymatic activity measured with an aliquot of ribosomes could be increased by disrupting the ribosomes. The ribosomes were suspended in 1 N NaCl at 37°C for 5 min, and then eleven volumes of 0.005 M potassium phosphate buffer pH 6.85, 0.0005 M $MgCl_2$ were added. After centrifugation at 105,000 $\times$ g for 1 hr, 170 per cent of the original enzyme activity as measured with intact ribosomes was found in the supernatant fraction.

Repeated washing and centrifugation did not alter the size of the major component of the ribosomes. In Figure 5, the left-hand picture shows the ultracentrifugation pattern of the ribosomes after the first centrifugation cycle. The major component has an S_{20w} value of 71, uncorrected for concentration of ribosomes. The right-hand picture shows the same preparation after the fourth centrifugation

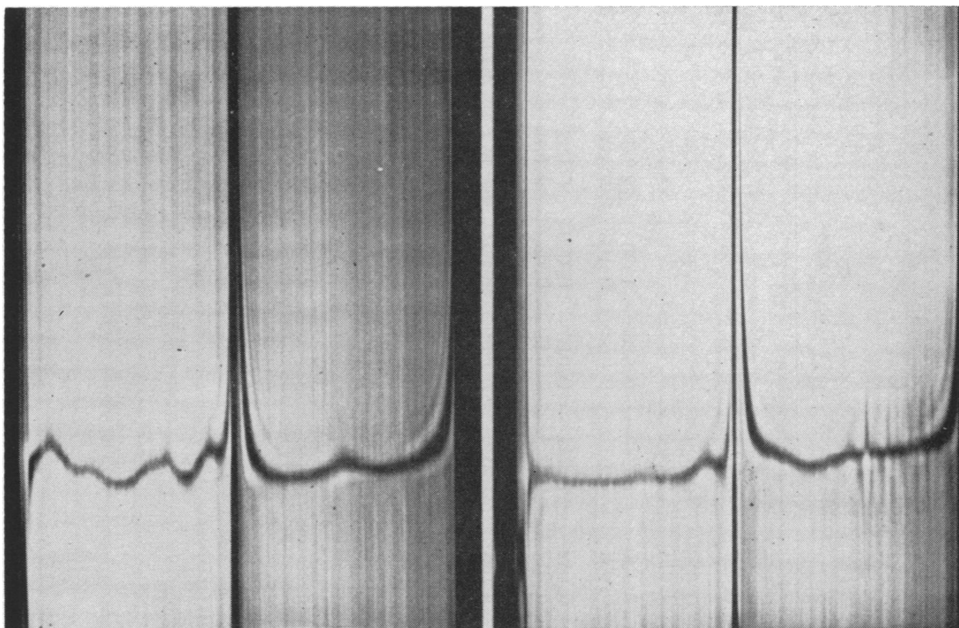

FIG. 5.—Ultracentrifugation pattern of yeast ribosomes. Left, ribosomes after first centrifugation cycle; right, after fourth centrifugation cycle. Pictures taken 12 min after reaching speed of 42,040 rpm.

cycle. There was a loss of lower-molecular-weight material, but the major peak still retained the same mobility. It is therefore apparent that the triose phosphate dehydrogenase was not loosely adsorbed or bound by disulfide bonds to the ribosomes and that repeated washing did not alter the major sedimenting component.

Precipitation of ribosomes with antiserum to triose phosphate dehydrogenase: With the antisera to crystalline yeast triose phosphate dehydrogenase, it was possible to precipitate selectively a small percentage of the total ribosomal population.

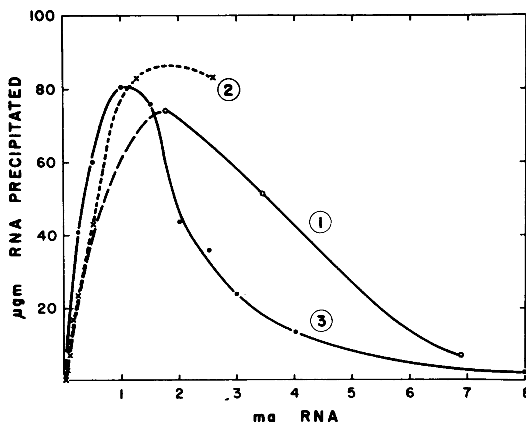

FIG. 6.—Precipitin curves of ribosomes with triose phosphate dehydrogenase antiserum. Points of curves were determined with 0.2 ml of antiserum plus ribosomes equivalent to the amount of RNA indicated. Antiserum preparation A was used for determination of curves 1 and 2 and preparation C for curve 3.

TABLE 2

PRECIPITIN REACTION BETWEEN TRIOSE PHOSPHATE DEHYDROGENASE ANTISERUM AND YEAST RIBOSOMES

Experiment	Conditions	Tube No. 1	2	3	4	5	6	7	8	9
1	RNA added (mg)	1.73	3.46	6.92	10.38	13.84				
	RNA precipitated with antiserum (mg)	0.084	0.065	0.023	0.027	0.027				
	RNA precipitated with normal serum (mg)	0.010	0.013	0.016	0.016	0.023				
	RNA, antiserum minus control (mg)	0.074	0.052	0.007	0.011	0.004				
2	RNA added (mg)	0.026	0.052	0.104	0.130	0.260	0.520	1.30	2.60	
	RNA precipitated with antiserum (mg)	0.009	0.009	0.019	0.031	0.037	0.060	0.103	0.106	
	RNA precipitated with normal serum (mg)	−0.013	0.007	0.012	0.014	0.014	0.017	0.020	0.022	
	RNA, antiserum minus control (mg)	−0.004	0.002	0.007	0.017	0.023	0.043	0.083	0.084	
3	RNA added (mg)	0.25	0.5	1.0	1.5	2.0	2.5	3.0	4.0	8.0
	RNA precipitated with antiserum (mg)	0.045	0.065	0.086	0.078	0.043	0.037	0.025	0.015	0.005
	RNA precipitated with normal serum (mg)	0.004	0.005	0.005	0.002	0.000	0.001	0.001	0.002	0.003
	RNA precipitated with antiserum minus control (mg)	0.041	0.060	0.081	0.076	0.043	0.036	0.024	0.013	0.002
	TPD precipitated with antiserum (μm DPNH/min)	0.13	0.48	0.49	0.50	0.14	0.15	0.12	0.06	0
	TPD precipitated with antiserum (μm/min/mg RNA)	2.9	7.4	5.7	6.4	3.2	4.2	4.8	3.7	0
	$\frac{\text{TPD precipitated } \mu\text{m DPNH/min/mg RNA}}{\text{TPD added, } \mu\text{m DPNH/min/mg RNA}}$	1.2	3.1	2.4	2.7	1.4	1.8	2.0	1.6	0

Varying amounts of yeast ribosomes, indicated by the content of RNA, were added to the tubes. In all experiments, 0.2 ml antiserum was used. Antiserum A was used in experiments 1 and 2, antiserum C in experiment 3. The ribosomes used in experiments 1, 2, and 3 were centrifuged 3, 2, and 2 times, respectively. A solution containing $MgCl_2$, 5×10^{-4} *M*, and potassium phosphate buffer, 5×10^{-3} *M* pH 6.85, was used both for suspension of ribosomes and to bring the final volume of the mixture to 3.2 ml. Tubes were maintained at 0° for 18 hr prior to centrifugation at 3,000 × *g* for 5 min and washed three times with 3 ml aliquots of $MgCl_2$-buffer. The precipitates in experiment 3 were suspended in 0.4 ml of 1 *N* NaCl at 0° for 30 min prior to analysis for TPD activity and RNA.[15] For TPD activity, the NaCl extract was added to the cuvette containing all additions including fructose diphosphate. In experiment 3, when ribosomes containing 2.0 mg of RNA were suspended in 3.2 ml of $MgCl_2$-phosphate buffer and treated as a control, no precipitate containing RNA was isolated. The triose phosphate dehydrogenase activity of the original yeast ribosomes used in experiment 3 was 2.36 μm DPNH/min/mg RNA.

The results of three experiments are presented in Figure 6, and the conditions of the experiments are presented in Table 2. At the point of maximal precipitation of ribosomal material by the specific antisera, the percentage of the added RNA precipitated was 4.3, 6.3, and 8.1 in curves 1, 2, and 3, respectively, shown in Figure 6. As is evident in the figure, less RNA was precipitated on both the antigen-excess and antibody-excess portions of the precipitin curves.

To demonstrate that this precipitation was associated with the antigen-antibody reaction, it was necessary to show that the antibody reacted with the antigen on the ribosomes. Evidence for this was provided by the inactivation of the enzyme associated with the ribosomes on addition of antiserum. Also, triose phosphate dehydrogenase activity could be recovered in the ribosomal-antibody precipitate. This activity was demonstrated by dissociating the antigen-antibody complex with 1 *N* NaCl and then assaying for enzyme activity. The results are indicated in experiment 3, Table 2. The maximum enrichment of the TPD activity per mg of RNA was 2.7 times. At the point of maximum RNA precipitation, 21 per cent of the original enzyme activity associated with the ribosomes was recovered in 8.6 per cent of the original RNA. The recovery might be higher if the antibody did not inactivate the enzyme activity.

To determine whether the precipitation of ribosomal material was due to the specific antibody-antigen reaction, experiments were performed with egg albumin and rabbit antiserum to egg albumin. The results of one experiment are shown in Table 3. In experiment 1, the data for the precipitin reaction with egg albumin are presented. In experiment 2, a constant amount of ribosomal material was added to the tubes with additions as in experiment 1. The amount of RNA precipitate in each of the tubes was determined. It is apparent that there was some nonspecific precipitation of ribosomes associated with the egg albumin antigen-antibody complex. The amount of RNA precipitated in the control tube without egg albumin added was 0.022 mg and the maximum amount precipitated with the egg albumin was 0.053 mg. This compares with a range of 0.084 to 0.106 mg precipitated with the specific triose phosphate dehydrogenase antiserum. The triose phosphate dehydrogenase activity per mg of RNA of the precipitates was compared with that of the original ribosomes. The ratios of activities are shown in Table 3. The highest value, 0.21, was obtained without addition of egg albumin, and all of the values were below 1.0, indicating that there was no specific precipitation of ribosomes associated with triose phosphate dehydrogenase. Neither egg albumin nor egg albumin antiserum inhibited the activity of the enzyme. This control experiment demonstrates that there is some precipitation of ribosomal material which occurs with an unrelated antigen-antibody reaction, but this precipitation is less than that with the triose phosphate dehydrogenase antiserum, and the precipitate shows no enrichment for triose phosphate dehydrogenase per milligram of ribosomal RNA.

Ribosomes associated with yeast alcohol dehydrogenase: It seemed desirable to prepare antiserum to another yeast enzyme to determine whether a different fraction of ribosomes might be removed from the total population. Alcohol dehydrogenase was present on ribosomes after several centrifugation cycles as indicated in Table 4. When crystalline enzyme was added to the ribosomes prior to the centrifugation cycles, as in experiment 6, no excess activity was noted on subsequent cy-

TABLE 3

PRECIPITIN REACTION WITH EGG ALBUMIN AND ANTISERUM AND PRECIPITIN REACTION WITH EGG ALBUMIN, ANTISERUM, AND RIBOSOMES

Experiment	Conditions	Tube No. 1	2	3	4	5	6	7	8
1	Egg albumin (μg added)	0	2.5	12.5	25	50	100	200	100
	Egg albumin antiserum (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0
	Protein precipitated (μg)	0	29	—	324	456	190	7	0
2	Egg albumin (μg added)	0	2.5	12.5	25	50	100	200	100
	Egg albumin antiserum (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0
	Ribosomes (mg RNA added)	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
	RNA precipitated (mg)	0.022	0.028	0.054	0.048	0.053	0.020	0.026	0.007
	Per cent of added RNA precipitated	1.1	1.5	2.8	2.5	2.8	1.1	1.4	0.4
	TPD μm/min/mg RNA in precipitate	0.59	0	0.12	0.30	0.24	0	0	0
	TPD in precipitate (μm/min/mg RNA) / TPD in original ribosome (μm/min/mg RNA)	0.21	—	0.043	0.11	0.086	—	—	—

For experiment 1, crystalline egg albumin dissolved in $MgCl_2$-buffer was added to 0.2 ml antiserum plus $MgCl_2$-buffer in a final volume of 2 ml. For experiment 2, ribosomes containing 1.9 mg of RNA were added. After 18 hr at 0°, TPD activity and RNA[14] determinations were done as indicated in Table 2, experiment 3.

cles. Antiserum to the crystalline enzyme was prepared and a precipitin curve showed a point of maximum precipitation with 2.5 mg of enzyme per ml of antiserum. When ribosomes were incubated with the antiserum (Table 5), no specific precipitate was observed. From the specific activity of the enzyme, it was calculated that the maximum amount of alcohol dehydrogenase present on the ribosomes after two centrifugation cycles was 2 μg of protein per mg of RNA and, because of this low level, it was technically impossible to precipitate a fraction of ribosomes associated with alcohol dehydrogenase.

Discussion.—Many workers have described the presence of enzyme activity associated with ribosomes.[5, 18–32] The immunological isolation of ribosomes involved in the synthesis of a specific enzyme is based upon the assumption that this enzyme activity associated with the ribosomes represents newly synthesized enzyme rather than enzyme adsorbed from the supernatant fraction. Several lines of evidence support this assumption. Repeated suspension of the particles and centrifugation results in an enzyme activity, associated with the particles which approaches a base-line value,[31, 32] while addition of the purified enzyme to the ribosomes does not increase the activity associated with the ribosomes after several centrifugation cycles.[5, 21, 30–32] A wealth of data indicates that isotopically labeled amino acids are incorporated first into the proteins associated with ribosomes prior to their appearance in protein in the supernatant fraction.[1] This type of isotopic evidence has been extended to two specific proteins, chymotrypsinogen[29] and β-galactosidase,[28] which in kinetic experiments acquire radioactivity first in the ribosome-bound material and then in the soluble enzyme fraction. Finally, p-fluorophenylalanine, which when incorporated into β-glucosidase results in an inactive enzyme, has been demonstrated in pulse experiments to inactivate the ribosomal-bound

TABLE 4

ALCOHOL DEHYDROGENASE ACTIVITY IN RIBOSOMES FOLLOWING SUCCESSIVE CENTRIFUGATION CYCLES

		μm DPNH/min/mg RNA — Centrifugation cycle				
Experiment	Additions	1	2	3	4	5
1	None	0.51	0.093	0.093	0.064	0.029
2	None	0.22	0.051	0.065		
3	None	0.38	0.093	0.056		
4	None	0.18	0.019	0.012		
5	None	0.19	0.047	0.032		
6	None	0.061	0.016	0.000		
	0.18 mg alcohol dehydrogenase/mg RNA	0.45	0.011	0.004		
	0.36 mg alcohol dehydrogenase/mg RNA	0.95	0.018	0.000		
	0.72 mg alcohol dehydrogenase/mg RNA	0.64	0.023	0.000		

TABLE 5

PRECIPITIN REACTION WITH ANTISERUM TO YEAST ALCOHOL DEHYDROGENASE AND YEAST RIBOSOMES

	Tube No.							
	1	2	3	4	5	6	7	8
RNA added (mg)	0	0.082	0.21	0.41	0.82	1.64	2.46	3.28
RNA precipitated with antiserum	0.003	0.007	0.009	0.019	0.013	0.014	0.013	0.015
RNA precipitated with control serum	0.007	0.013	0.014	0.017	0.014	0.011	0.012	0.012

Each reaction mixture, with a final volume of 4.0 ml, contained 0.2 ml of antiserum to alcohol dehydrogenase or 0.2 ml of normal serum, the ribosomes, and $MgCl_2$-phosphate buffer. Tubes maintained at 0° for 18 hr prior to centrifugation, washing, and analysis of RNA.[15]

β-glucosidase without any alteration in the activity of the soluble enzyme.[31] These different experimental approaches support the concept that ribosomal-bound enzyme represents newly synthesized rather than adsorbed material. It should be emphasized, however, that this concept is an assumption which is fundamental to the isolation of any specific ribosomal material by this technique. Relevant to this is the report of Peterman[33] which describes conditions for the binding of hemoglobin to ribosomes.

The isolation by immunological means of a specific protein associated with ribosomes has been accomplished by several groups. Antiserum has been used to isolate C^{14}-labeled β-galactosidase associated with ribosomes.[28] Also, approximately 1 per cent of the *E. coli* ribosomal RNA was precipitated by an antibody to β-galactosidase plus an antibody to this antibody.[32] The usual amount of an induced or a repressible enzyme which is synthesized by *E. coli* is approximately 6 per cent of the total soluble protein.[34] In contrast, triose phosphate dehydrogenase constitutes from 14 to 20 per cent of the soluble protein of baker's yeast as judged by enzyme assay and by immunological techniques, respectively.[35] Between 4 and 8 per cent of the ribosomal population was precipitated with antiserum to this enzyme. If the release of the preformed protein from the ribosome is not the rate-limiting factor and some of the ribosomes have only partially synthesized triose phosphate dehydrogenase which is nonantigenic, then an even larger percentage of the ribosomal population might be involved in the synthesis of this enzyme. However, two factors are responsible for values which are falsely high. The first is the precipitation of ribosomal material by control serum. In experiments 1, 2, and 3 of Table 2, the amount of this nonspecific ribosomal precipitate was 0.6, 1.9, and 0.6

per cent of the total ribosomal material added. The percentages were calculated at the point of maximum ribosomal precipitation with antiserum. This percentage varied with different preparations of ribosomes and with different sera. The second factor is the nonspecific precipitation or occlusion of ribosomal material by an unrelated antibody-antigen system. Both of these factors would lead to the precipitation of ribosomes not involved in the synthesis of the specific enzyme. It should be emphasized that when triose phosphate dehydrogenase antiserum precipitated a fraction of ribosomal material, this enzyme was recovered enriched in relation to the RNA compared to the original ribosomal preparation, while the nonspecific precipitates showed no enrichment. This proves that some enzyme-specific precipitation has occurred. This type of isolation procedure may facilitate the study of structural and functional characteristics of enzyme-specific "messenger" RNA.

Summary.—The enzyme triose phosphate dehydrogenase was closely associated with yeast ribosomes and it was only partially removed by repeated homogenization in buffer and centrifugation. Antiserum to the crystalline yeast enzyme resulted in precipitation of a maximum of 8 per cent of the total ribosomal material with enrichment of enzyme activity per milligram of RNA. Nonspecific precipitation resulted in an additional 0.6 to 1.9 per cent. A nonspecific immunological reaction with egg albumin and its antiserum precipitated 1.7 per cent of the ribosomal population with no enrichment for triose phosphate dehydrogenase. This immunological isolation procedure therefore provides a method for concentrating ribosomal-RNA, associated with a specific enzyme. Although alcohol dehydrogenase activity was demonstrated on yeast ribosomes, the amount of enzyme was so small that antiserum to the enzyme did not precipitate ribosomal material in excess of the controls.

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*2-BENZYLHISTIDINE AND DERIVATIVES AS PILOTS FOR THE SYNTHESIS OF PEPTIDES DESIGNED TO HAVE SPECIFIC ENZYMIC ACTIVITY**

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So much has been written about the participation of serine and histidine residues in the enzymically active sites of hydrolytic enzymes that one of the distinguishing features of enzymes has been neglected by those who would understand the biochemical mechanism of their action. This is despite the fact that most enzymes exhibit a high degree of specificity toward their substrates. The work of recent years, beginning with the pioneering discoveries of Balls *et al.* and Hartley *et al.*,[1-4] has indicated that the active sites of chymotrypsin, trypsin, elastase, cholinesterase, and liver esterase seem to contain a sequence of amino acids, viz. glycylaspartylserylglycine which is identical, or nearly so, for each of these esterases. This similarity of structure of a part of the molecule concerned with the catalytic action in these enzymes of differing specificity has given rise to numerous postulates about the mechanism of hydrolytic enzyme action (e.g. see refs. 5–7). The fact that there is this resemblance in chemical structure of a portion of the active centers of these several enzymes has pushed into the background the fact that each of them carries out a specific reaction. They differ sharply in enzymic activity even though the recognized portion of the active site of each is identical or very similar. What is it then which gives the specificity to each of them?

It is possible that the mechanism of enzyme action will not be understood until the