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GLUTAMINE SYNTHETASE

II. THE INTRACELLULAR LOCALIZATION IN THE RAT LIVER

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SUMMARY

The intracellular distribution of glutamine synthetase (L-glutamate: ammonia ligase (ADP), EC 6.3.1.2) in normal and regenerating rat livers has been studied. The distribution of the enzyme activity in liver in these two situations is similar. The highest enzyme activity occurred in the microsome fraction, which comprised about one half of the activity in liver. Although most of the enzyme activity was identified with the particulate fractions, most of the free glutamine in liver was found in the soluble fraction.

In the microsome fraction, the enzyme was associated with the vesicles, and the ribonucleoprotein particles contained no significant activity. NaCl in concentrations less than that of physiological saline caused solubilization of the enzyme from the vesicles. The evidence indicates that the enzyme was attached to the outer surface of the vesicular membrane in the microsome fraction, and that it was probably attached also to the surface of the nuclear and mitochondrial membranes.

INTRODUCTION

A preliminary report[1] has shown that, although glutamine synthetase activity could be found in all four subcellular fractions of rat-liver homogenate, the microsome fraction had the highest activity. Since the microsome fraction is known to contain membrane-bound structures and dense particles[2], the localization of glutamine synthetase in the organelle of this fraction has been investigated further. This investigation has led to the finding that the microsomal enzyme could be made soluble with sodium chloride. The results of these studies are the subject of this report.

EXPERIMENTAL PROCEDURE

Male Sprague-Dawley rats weighing about 300 g were used without prior fasting. The animals were lightly anesthetized with ether, and the throats were cut to permit as much bleeding as possible. The liver was removed, immediately chilled in ice-cold 0.25 M sucrose, and cut coarsely with a pair of scissors. An all-glass homogenizer of the Potter-Elvehjem type served to prepare the liver homogenate containing 100 mg of

liver/ml. During the homogenization and subsequent centrifugation, the operation was carried out near 5°. The enzyme retained its activity under these conditions. The method of SCHNEIDER AND HOGEBOOM[3] was used with modifications to obtain the subcellular fractions. The nuclear fraction was collected on centrifuging the homogenate at 1000 × *g* for 10 min. It was washed once by resuspending in one half its original volume of 0.25 M sucrose and centrifuged again. The original supernatant fluid and the supernatant fluid from the washing were combined and centrifuged at 15 000 × *g* for 10 min to obtain the mitochondria. The mitochondria were washed once also with the sucrose solution and centrifuged again. A Servall Type SS-3 automatic superspeed centrifuge served to separate these two fractions. The original supernatant fluid and the supernatant fluid from washing the mitochondria were combined and centrifuged at 144 000 × *g* for 60 min in a Spinco Model L preparative ultracentrifuge to obtain the microsome fraction. The supernatant fluid from the last operation formed the soluble fraction. The pellets of the nuclear, mitochondrial, and microsome fractions were finally resuspended in suitable volumes of 0.25 M sucrose for analyses.

When the regenerating rat liver was used, partial hepatectomy was performed according to the technique of HIGGINS AND ANDERSON[4]. The animals were killed 68–72 h after the operation. The liver homogenate and subcellular fractions were prepared exactly as for normal liver.

The activity of glutamine synthetase (L-glutamate: ammonia ligase (ADP), EC 6.3.1.2) in the homogenate and the fractions prepared from it was assayed according to a procedure described in detail in the first paper of this series[5]. One unit of enzyme activity is defined as the amount of enzyme that will cause the formation of 1 μmole of γ-glutamylhydroxamic acid in 1 h at 37°. The tissue activity is expressed in units/g of liver or an equivalent amount of each fraction, and the specific activity in units /mg of protein. Protein was determined by the method of LOWRY *et al.*[6]. Crystalline bovine albumin (Pentex, Inc.) served as the standard. Glutamine was determined by a procedure described previously[5]. RNA was extracted by SCHNEIDER's modification[7] of the method of SCHMIDT AND THANNHAUSER and determined by the MEJBAUM's orcinol reaction[8]. Yeast RNA (Mann Research Laboratories) containing 9.0% P served as the standard without further purification.

Deoxycholic acid (Calbiochem) was dissolved in NaOH and was back titrated with HCl to a pH of 7.5–7.6 shortly before use. Crystalline RNAase, prepared from bovine pancreas and purchased from the Sigma Chemical Company, contained 45 Kunitz units/mg. EDTA was a product of Eastman Organic Chemicals. γ-Glutamylhydroxamic acid, which was used as the standard in the glutamine synthetase assay, was synthesized according to ROPER AND MCILWAIN[9] and recrystallized to a constant molar extinction coefficient as determined with the HCl–$FeCl_3$ reagent[10].

RESULTS

The results for the distribution of glutamine synthetase and of glutamine in the subcellular fractions of rat liver will be presented first. This presentation will be followed by a study of the localization of this enzyme in the microsome fraction.

Localization in the subcellular fractions

A total of 8 normal rat livers has been used in this study. Although the recovery

of glutamine synthetase activity was satisfactory in each experiment, the percentage of the enzyme activity in a particular fraction varied somewhat from one experiment to another. Most likely this variation was due to the manner in which each fraction was separated, because the demarcation between the supernatant fluid and the pellet was never physically sharp. For this reason, the results from different experiments are not averaged.

Table I shows a typical result for the intracellular distribution of glutamine

TABLE I

GLUTAMINE SYNTHETASE ACTIVITY AND GLUTAMINE CONTENT IN SUBCELLULAR FRACTIONS OF NORMAL RAT LIVER

In this and the following two tables, the results are expressed on the basis of per gram liver or an equivalent amount of each fraction.

Preparation	*Protein content (mg/g)*	*Synthetase activity*			*Glutamine content*	
		Tissue activity		*Specific activity (units/mg protein)*		
		(units/g)	*(%)*		*(μmoles/g)*	*(%)*
Homogenate	214.0	273.0	(100.0)	1.28	5.02	(100.0)
Nuclear	53.9	66.6	24.4	1.24	0.45	9.0
Mitochondria	46.9	51.1	18.7	1.09	0.42	8.4
Microsome	41.6	126.9	46.5	3.05	0.24	4.8
Soluble	67.0	24.0	8.8	0.36	3.77	75.1
Recovery	209.4	268.6	98.4		4.88	97.3

synthetase in rat liver. The recoveries of protein, enzyme activity, and glutamine of the homogenate were 97.9, 98.4 and 97.3%, respectively. Although enzyme activity was found in all fractions, the microsome fraction had the highest activity, with 126.9 units/g liver and the soluble fraction had the lowest, with only 24.0 units/g liver. As far as the specific activity is concerned, only the microsome fraction, with 3.05 units/mg protein, yielded an activity significantly higher than did the homogenate, with 1.28 units/mg protein. The specific activities of the nuclear and mitochondrial fractions were essentially the same as that of the homogenate. The extremely low specific activity of 0.36 unit/mg protein found in the soluble fraction makes it appear probable that the enzyme activity found in this fraction might have its origin in one of the particulate fractions, especially the microsome fraction and was released during cell homogenization. When the pellet of the microsome fraction was resuspended even gently, 5–15% of its enzyme activity appeared in the supernatant fluid after centrifuging.

The intracellular distribution of glutamine in rat liver is shown also in Table I. In contrast with glutamine synthetase, which was present essentially in the particulate fractions, glutamine occurred predominantly in the soluble fraction. Thus, the particulate fractions contained 89.6% of the enzyme activity, the soluble fraction contained 75.1% of the glutamine. The apparent contrast in the distribution between the enzyme and its reaction product will be discussed later.

TABLE II

GLUTAMINE SYNTHETASE ACTIVITY IN RECOMBINED SUBCELLULAR FRACTIONS

*Fractions recombined**	*Tissue activity*		
	Determined (units/g)	*Calculated (units/g)*	*Recovered (%)*
Nu + Mt + Mc	237.0	244.6	96.9
Nu + Mc	213.0	193.5	110.1
Nu + So	90.6	90.6	100.0
Nu + Mt + Mc + So	246.0	268.6	91.6
Mt + Mc	175.1	178.0	98.4
Mt + So	72.0	75.1	95.9
Mc + So	153.8	150.9	101.9

* Nu, nuclear; Mt, mitochondria; Mc, microsome; So, soluble fraction.

The four subcellular fractions were recombined in various combinations as indicated in Table II; in each case the fractions were recombined in the same proportion as they occurred in the homogenate. The table compares glutamine synthetase activity found for each of these combinations with the summed activity of the constituent fractions, as shown in the preceding table. The good agreement between the determined and the calculated values further indicates that the synthetase activity in a given fraction was neither stimulated nor inhibited by any other fraction.

The activity of glutamine synthetase in the regenerating rat liver has been found to be significantly lower than that in the lobes removed during partial hepatectomy. A general account of these experiments will be presented in a subsequent communication. The following experiment was made, nevertheless, to see if the decrease in the enzyme activity in the regenerating liver cell was due to a decrease in the activity in a certain subcellular fraction. The results on the distribution of the tissue activity in the regenerating liver, as shown in Table III, agree well with those in Table I for the normal liver and show that the enzyme activity in the regenerating liver, although diminished, was distributed through the cell in the usual way.

The microsome fraction is morphologically heterogeneous[2]. It contains two types

TABLE III

GLUTAMINE SYNTHETASE ACTIVITY IN SUBCELLULAR FRACTIONS OF REGENERATING RAT LIVER

Preparation	*Protein content*		*Synthetase activity*		
			Tissue activity		*Specific activity (units/mg protein)*
	(mg/g)	*(%)*	*(units/g)*	*(%)*	
Homogenate	205.0	(100.0)	191.4	(100.0)	0.94
Nuclear	74.8	36.5	62.8	32.8	0.86
Mitochondria	29.8	14.5	26.4	13.8	0.90
Microsome	30.4	14.8	92.6	48.4	3.06
Soluble	64.5	31.5	11.0	5.7	0.17
Recovery	199.5	97.3	192.8	100.7	

of organelles, the vesicles* and the ribonucleoprotein particles. Since a major portion of the enzyme activity in the rat liver occurred in this fraction, an effort was made to find out whether the enzyme in this fraction was associated with the vesicles or the ribonucleoprotein particles or both. The principle involved in the experiments to be described next consists of selectively disrupting one type of the organelles, centrifuging at 144 000 × *g* for 60 min to sediment the undisrupted structure, and determining the redistribution of the protein, RNA, and glutamine synthetase activity in the supernatant fluid as well as in the pellet. For these purposes the microsome fraction was used only.

Treatment with deoxycholate

Deoxycholate has been shown to cause an almost immediate and complete disruption of the vesicles but little damage to the ribonucleoprotein particles[2]. Deoxycholate was added to the microsome fraction to give the final concentrations indicated in Fig. 1, and the mixtures were centrifuged at once without incubation. Deoxycholate

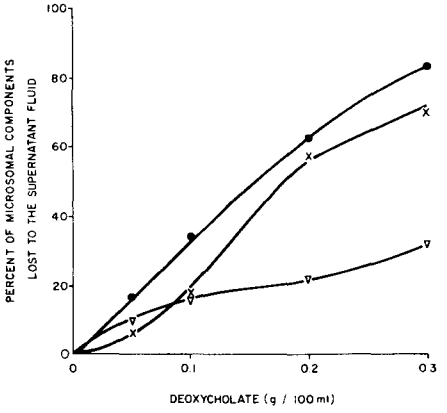

Fig. 1. Effect of deoxycholate on the solubilization of glutamine synthetase (×—×), RNA (▽—▽) and protein (●—●) of the microsome fraction of rat liver.

inhibited the activity of glutamine synthetase, when its concentration in the reaction mixture[5] exceeded 0.28 mg/ml. In the assay of the enzyme activity in the deoxycholate treated microsome fractions, however, only a small aliquot was used, and the deoxycholate concentration in the reaction mixture was sufficiently low so that no inhibition was observed. All three components, RNA, total protein, and the enzyme were determined in the treated preparation before centrifuging, henceforth called the "unspun", as well as in the supernatant fluid and the pellet obtained from it. Within the experimental error, what was lost from the pellet could be quantitatively recovered in the supernatant fluid.

From the redistribution curves shown in Fig. 1, we can see that, as the concentra-

* This term is used here to include all membrane-bound structures of different profiles observed in the microsome fraction, *e.g.* vesicles, tubules, and cisternae.

tion of deoxycholate increased, the increase in the protein content in the supernatant fluid was large, but the increase in the RNA content was slight. These results agree well with those of PALADE AND SIEKEVITZ[2]. Obviously, most of the protein made soluble by deoxycholate came from the vesicles. Furthermore, the increase in the enzyme activity in the supernatant fluid with increasing concentrations of deoxycholate bears no resemblance to that of the RNA, but is closely related to that of the protein. When a final concentration of 0.5 g of deoxycholate/100 ml was used (not shown in the figure), 95.9% of the enzyme activity and 97.1% of the protein appeared in the supernatant fluid. Apparently, less than 5% of the enzyme activity escaped solubilization even with such a high concentration of deoxycholate. These results indicate that glutamine synthetase activity in the microsome fraction is primarily, if not exclusively, associated with the vesicles.

In addition to the microsome fraction, the enzyme in the nuclear fraction was made soluble readily by deoxycholate also (Fig. 2).

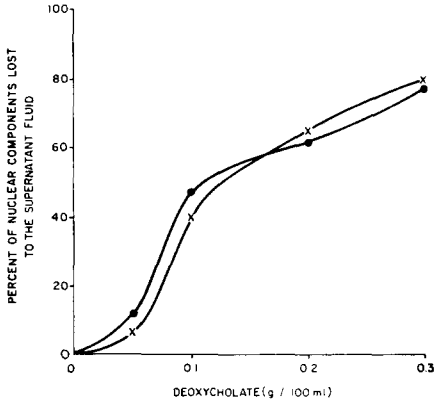

Fig. 2. Effect of deoxycholate on the solubilization of glutamine synthetase (×—×) and protein (●—●) of the nuclear fraction of rat liver.

Treatment with RNAase

In contrast with deoxycholate, RNAase ([polyribonucleotide 2-oligonucleotidotransferase (cyclizing)], EC 2.7.7.16) will disrupt the ribonucleoprotein particles and cause the vesicles to agglutinate[2]. The RNAase was dissolved in a glycylglycine buffer (pH 7.2), prepared in 0.25 M sucrose. The final concentration of the buffer in the incubation mixtures was uniformly 0.016 M. The incubation of the microsome fraction with RNAase was carried out at 15° to minizime the agglutination of the vesicles, but the reaction was allowed to proceed for 4 h. RNAase did not affect glutamine synthetase activity. When the microsome fraction was treated with RNAase, however, the total RNA determinable with the method of SCHNEIDER[7] was always less than that in the untreated microsome fraction. The higher the concentration of RNAase, the greater was the decrease in total RNA. This result means that, under the conditions used, RNAase not only disrupts the ribonucleoprotein particles, but it also degrades RNA molecules to such small units that they are no longer precipitated by cold trichloroacetic acid and hence cannot be determined by the method. Almost all

the RNA left after the RNAase treatment remained in the pellet after centrifuging. Evidently, the RNA made soluble is not precipitable with cold trichloroacetic acid. Nevertheless, the protein and glutamine synthetase activity in the unspun preparation were readily recovered in the supernatant fluid and in the pellet.

The results shown in Fig. 3, which were corrected for the changes in a control containing no RNAase, indicate that more than 90% of the enzyme activity remained in the pellet after the treatment, and, except for an initial drop, the distribution of the enzyme activity was not affected by increasing concentrations of RNAase. The initial drop of less than 10% of the enzyme activity can be taken to mean that a very small portion of it is associated with the ribonucleoprotein particles, and this portion is made non-sedimentable by RNAase. At this point we may recall that, as shown earlier,

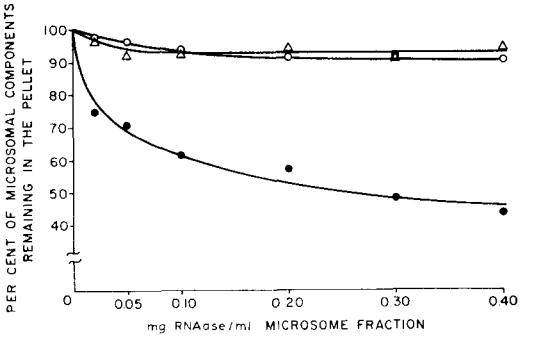

Fig. 3. Solubilization by RNAase of glutamine synthetase (△—△), RNA (●—●) and protein (○—○) of the microsome fraction of rat liver).

a few percent of the enzyme activity could not be made soluble by deoxycholate. Like the enzyme activity, most of the microsomal protein remained in the pellet. For example, at the highest concentration of RNAase employed, with the loss of 54% of RNA from the pellet, only 10% of the protein was lost. The results in the following tables will show that on a weight basis, the ratio of protein to RNA in a given microsome fraction was often greater than 10. In the ribonucleoprotein particles, the same ratio was about 1 (see ref. 11). Hence, even if all the protein in the ribonucleoprotein particles were lost to the supernatant fluid, the microsomal protein content could not have been reduced by more than 10%. ROTH[12] has made a similar observation on the distribution of the microsomal protein and RNA after RNAase treatment. The evidence presented here is consistent with that obtained following the deoxycholate treatment and again shows that glutamine synthetase is associated with the vesicles but not the ribonucleoprotein particles.

The enzyme molecules found so far to be associated with the vesicle may (a) form a part of the contents of the vesicle, (b) form an integral part of the membrane, (c) be attached to the inner surface of the membrane, or (d) be attached to the outer surface of the membrane. If the enzyme molecules are attached to the outer surface of the vesicular membrane, it should be possible to detach them without disrupting the vesicles. As a result of this detachment, the enzyme would be made soluble and would

appear in the supernatant fluid after centrifuging. At the same time, a large portion of the protein should remain in the pellet, since most of the vesicles are not disrupted. The results of the following experiments demonstrate that this is the case.

Treatment with EDTA

According to the electron microscopic observation of PALADE AND SIEKEVITZ[2], EDTA causes detachment of the ribonucleoprotein particles from the vesicles with no appreciable damage to the latter. This reagent was prepared in 0.25 M sucrose and adjusted to pH 7.3. When added to the microsome fraction to a final concentration of 0.054 M, EDTA caused an almost instantaneous release of the enzyme activity from the vesicles into the supernatant fluid (Table IV). Thus, 88.8% of the enzyme activity was instantly released into the supernatant fluid, whereas 95.2 and 98.3%,

TABLE IV

SOLUBILIZATION BY EDTA OF GLUTAMINE SYNTHETASE IN PARTICULATE FRACTIONS

In this and the following tables, the results are expressed on the basis of per ml of the fraction used, which, in the case of the microsome fraction, usually contained the equivalent of about 300 mg liver/ml. EDTA, when used, was 0.054 M (pH 7.3). The incubation was done at 5°.

Addition	*Incubation (min)*	*Fraction**	*Synthetase*		*Protein*		*RNA*	
			(units/ml)	*(%)*	*(mg/ml)*	*(%)*	*(μg/ml)*	*(%)*
			A. Microsome fraction					
None	120	U	9.60		5.10		406	
		S	1.84	19.2	0.62	12.2	28	6.9
		P	7.84	81.7	4.37	85.7		
None	1200	U	8.16		5.00		406	
		S	1.44	17.6	0.74	14.8	34	8.4
		P	7.28	89.2				
EDTA	0	U	15.50					
		S	13.76	88.8				
		P	0.82	5.3				
EDTA	20	U	15.76		4.50		437	
		S	15.00	95.2	1.21	26.9	119	27.3
		P	0.78	4.9	3.26	72.4	298	68.1
EDTA	120	U	15.50		4.40		430	
		S	15.24	98.3	1.36	30.9	221	51.4
		P	0.76	4.9	3.17	72.0	232	54.0
			B. Nuclear-mitochondrial fraction					
None	120	U	37.72		25.60			
		S	0.64	1.7	2.60	10.5		
		P	37.52	99.5	22.30	87.2		
EDTA	120	U	54.36		25.60			
		S	52.20	96.0	4.50	17.6		
		P	5.24	9.6	19.40	75.8		

* U, the unspun preparation, was subsequently centrifuged at 144 000 × *g* for 60 min to obtain S, the supernatant fluid, and P, the pellet.

respectively, appeared following incubation at 5° for 20 and 120 min. On the other hand, only about 30% of the total protein was concurrently solubilized. Had most of the vesicles been disrupted by EDTA, the percentage of the protein appearing in the supernatant fluid should have been much higher. This interpretation is consistent with the electron microscopic observation[2] mentioned above. Consequently, the results for the solubilization by EDTA of the enzyme activity and the total protein indicate that the enzyme is merely attached to the surface of the vesicle. The results also suggest a difference between the enzyme and the ribonucleoprotein particles in the way of attachment to the vesicles, because under the same conditions, the percentage of RNA released into the supernatant fluid was much less than that of the enzyme activity. The solubilizing effect of EDTA shown here and that of some others to be described in the next section may be due to a common denominator, the sodium ion.

As it was not always possible to complete a series of experiments on the same day the microsome fraction was prepared, microsome preparations frozen at −30° for different lengths of time were used also. The loss of the enzyme activity from storage[5], coupled with the use of microsome fractions having originally varying levels of the

TABLE V

SOLUBILIZATION OF GLUTAMINE SYNTHETASE UNDER DIFFERENT CONDITIONS

Treatment	*Conditions*		*Fraction**	*Synthetase*		*Protein*		*RNA*	
	Temperature	*Time (min)*		*(units/ml)*	*(%)*	*(mg/ml)*	*(%)*	*(μg/ml)*	*(%)*
None added	5	0	U	14.20		4.10			
			S	1.44	10.1	0.80	19.5		
			P	13.28	93.5	3.32	81.1		
NaCl, 0.06 M	5	0	U	18.88					
			S	10.08	53.4				
NaCl, 0.10 M	5	0	U	18.88		5.80		369	
			S	14.72	78.0	1.34	23.1	55	13.5
			P	5.76	30 5	4.40	75.9	307	83.2
Ultrasonic oscillation	8	5	U	4.88		3.20		358	
			S	1.36	27.9			79	22.0
			P	3.12	64.0	1.90	59.4	271	75.7
Ultrasonic oscillation	8	10	U	4.80		3.20		356	
			S	1.64	34.2			114	32.0
			P	2.76	57.5	1.60	50.0	209	58.7

* See Footnote under Table IV.

enzyme activity, has resulted in preparations with different values of specific activity as can be noted in Tables IV and V. This difference in specific activity, however, should not affect the interpretation of the results.

The enzyme activity in the nuclear-mitochondrial fraction, like that in the microsome fraction, was readily made soluble by EDTA. The pellet obtained on centrifuging the liver homogenate at 15 000 × *g* for 10 min was resuspended in 0.25 M sucrose. After addition of EDTA and incubation at 5° for 2 h, an aliquot of the mixture was centrifuged at 144 000 × *g* for 60 min to obtain the supernatant fluid and the pellet. The results, shown in Part B of Table IV, indicate that the extent of solubiliza-

tion by EDTA of the enzyme activity and protein was much like that observed with the microsome fraction. Since most of the protein remained in the pellet, these subcellular structures appeared to be intact following the EDTA treatment. Perhaps glutamine synthetase found in this combined fraction is attached also to the surface of the nuclear and mitochondrial membranes.

Under the standard conditions of assaying glutamine synthetase activity[5], EDTA began to reduce the activity when added to the reaction mixture to a final concentration of 6.0 mM. Apparently, EDTA inhibits the enzyme by forming a chelate with Mg^{2+} in the reaction mixture. The inhibition could be prevented by either moderately increasing the Mg^{2+} concentration or dialyzing the EDTA-containing preparations against distilled water. Dialysis at 5° for 6 h did not affect the enzyme activity. When the inhibition by EDTA had been prevented, the synthetase activity in the nuclear-mitochondrial fraction was significantly increased following the EDTA treatment, as can be seen in Part B of Table IV. The treatment resulted in a similar but smaller increase, about 10%, in the enzyme activity in the microsome fraction.

Effect of NaCl

NaCl caused the release of glutamine synthetase activity from the microsome pellet into the supernatant fluid. The microsome fraction was treated with varying concentrations of NaCl dissolved in 0.25 M sucrose; the mixtures were immediately centrifuged at 144 000 × *g* for 60 min to separate the pellet from the supernatant fluid. At a final concentration of 0.04 M or below, NaCl did not cause any significant solubilization of the enzyme activity. But when the final concentration of NaCl reached 0.06 M, an abrupt increase in the enzyme activity in the supernatant fluid took place. Thus, 53.4 and 78.0% solubilization occurred, respectively, with 0.06 and 0.10 M of NaCl (Table V). Complete solubilization of the enzyme activity occurred with 0.20 M NaCl. On the other hand, the distribution of the total protein and the RNA appeared to be unaffected by the NaCl treatment. On a molar basis, KCl caused a similar solubilization of the enzyme activity.

The microsomal enzyme after solubilizing with 0.20 M NaCl was dialyzed against 160 times its volume of 0.25 M sucrose at 2° for 4 h. The dialyzed preparation was centrifuged at 144 000 × *g* for 60 min. No enzyme activity remained in the supernatant fluid; all was sedimented. The result suggests that the enzyme was re-attached to the membrane upon dilution of NaCl.

The concentrations of NaCl found effective in solubilizing the enzyme activity were lower than that in physiological saline. The treatment with NaCl was brief and at 5°. Under these conditions, the disruption of the vesicles was virtually non-existent. Hence, the results obtained from the NaCl treatment strengthen the view that glutamine synthetase is attached to the surface of the vesicle and suggest that the attachment probably takes place through adsorption. Indeed, the solubilizing effect observed with EDTA can, at least in part, result from Na^+ in the reagent. Moreover, both EDTA and NaCl showed a similar effect on the solubilization of the enzyme activity and the protein.

Other treatments

When acetic acid, oxalic acid, Tris, and *tert.*-butylamine, prepared in 0.25 M

sucrose and adjusted to a pH of 7.2 with NaOH or HCl, were added to the microsome fraction to a final concentration of 0.05 M, they caused the solubilization of glutamine synthetase, similar to that observed with NaCl. But the effectiveness of these reagents in making the enzyme soluble, independent of Na^+ or Cl^-, has not been established. Under similar conditions, however, glycine, serine, alanine, β-alanine, γ-aminobutyric acid, ε-aminocaproic acid, ω-aminocaprylic acid, glycylglycylglycine, and 2-mercaptoethanol caused no release of the enzyme activity into the supernatant fluid.

Although the enzyme could be made soluble by EDTA and NaCl, it could not be solubilized readily by the ultrasonic oscillation (10 kC, Raytheon), as shown in Table V. The enzyme activity, however, was neither increased nor decreased under the conditions employed. The percentages of the microsomal protein, the enzyme activity, and the RNA remaining in the pellet after the treatment were similar, being about 60%. After the sonic treatment, the microsome preparation became translucent, like that after the deoxycholate treatment; the vesicles appeared to have been disrupted. Perhaps most of the enzyme remained attached to the broken membranes, which were sedimented on centrifuging.

DISCUSSION

Since glutamine synthetase found in the particulate fractions of rat liver appears to be attached to the surface of these particles, the synthesis of glutamine may be visualized as taking place outside the subcellular organelles and in the cytoplasm. Hence, the glutamine synthesized would be found in the cytoplasm until it is transported into such organelles for metabolic needs. Glutamic acid, a substrate of the enzyme, is well known for its sluggishness to pass through the cell membrane, presumably equally so with the subcellular membranes. The localization of the enzyme on the surface of the particles seems to have a topographic advantage; it does not require transport of glutamic acid into the subcellular structures. These views are consistent with the finding that free glutamine was present mostly in the soluble fraction of rat liver. The finding is probably not surprising, because glutamine is thought to be readily diffusible. Nevertheless, a number of metabolites with low molecular weights have been found to be richer in one of the particulate fractions than in the soluble fraction[13].

The solubilization by EDTA of a particle-bound D-allohydroxyproline oxidase in *Pseudomonas striata* has been reported in a brief communication[14]. More recently, SELLINGER AND DE BALBIAN VERSTER[15] reported the solubilization by EDTA and by Tris maleate–buffer of glutamine synthetase in the post-mitochondrial fraction of rat-cerebral cortex and suggested a loose attachment of the enzyme to the surface of the membrane of the endoplasmic reticulum. They also found no significant glutamine synthetase activity in the ribonucleoprotein particles of the cortex.

The observation that NaCl in concentrations below that of physiological saline dissociated glutamine synthetase from the subcellular particles raises the possibility that the enzyme might be soluble *in vivo*. The attachment of the enzyme to the membranous structures would take place during homogenization, when the intracellular fluid is much diluted. This view, supported by the demonstration that the solubilized enzyme became re-sedimentable on dilution of NaCl in the medium, however, is difficult to prove experimentally.

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REFERENCES

1 C. WU, *Federation Proc.*, 20 (1961) 218.
2 G. E. PALADE AND P. SIEKEVITZ, *J. Biophys. Biochem. Cytol.*, 2 (1956) 171.
3 W. C. SCHNEIDER AND G. H. HOGEBOOM, *J. Biol. Chem.*, 183 (1950) 123.
4 G. M. HIGGINS AND R. M. ANDERSON, *Arch. Pathol.*, 12 (1931) 186.
5 C. WU, *Comp. Biochem. Physiol.*, in the press.
6 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
7 W. C. SCHNEIDER, *J. Biol. Chem.*, 164 (1946) 747.
8 W. MEJBAUM, *Z. Physiol. Chem.*, 258 (1939) 117.
9 J. A. ROPER AND H. MCILWAIN, *Biochem. J.*, 42 (1948) 485.
10 F. LIPMANN AND L. C. TUTTLE, *J. Biol. Chem.*, 159 (1945) 21.
11 J. W. LITTLEFIELD, E. B. KELLER, J. GROSS AND P. C. ZAMECNIK, *J. Biol. Chem.*, 217 (1955) 111.
12 J. S. ROTH, *J. Biophys. Biochem. Cytol.*, 7 (1960) 443.
13 C. LONG, *Biochemists' Handbook*, Van Nostrand Co., Princeton, 1961, p. 816.
14 E. ADAMS AND S. L. NEWBERRY, *Biochem. Biophys. Res. Commun.*, 6 (1961) 1.
15 O. Z. SELLINGER AND F. DE BALBIAN VERSTER, *J. Biol. Chem.*, 237 (1962) 2836.