The Lactose Synthetase Particles of Lactating Bovine Mammary Gland

PREPARATION OF PARTICLES WITH INTACT LACTOSE SYNTHETASE

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1. The particulate form of lactating bovine mammary lactose synthetase activity is shown to be more highly organized than previously reported. 2. A novel method of shattering frozen mammary tissue with effective cell disruption is described. 3. The apparent subcellular distribution of lactose synthetase was shown to reflect the method of homogenization. 4. After mild homogenization particles associated with a high content of intact lactose synthetase activity sedimented in the lysosome size range between 5×10^4 and 3×10^5 g.-min. 5. Lactose synthetase was dissociated and solubilized by VirTis homogenization and ultrasonic treatment. The activities and behaviour of UDP-galactose hydrolase, succinate dehydrogenase, β -glucuronidase and phosphodiesterase I were compared. 6. Inhibition of UDP-galactose hydrolase by UTP and α -lactalbumin was observed.

Lactose synthetase (UDP-galactose-D-glucose 1-galactosyltransferase, EC 2.4.1.22), which catalyses the final reaction in the synthesis of lactose (Scheme 1), occurs as a soluble enzyme in milk (Babad & Hassid, 1966), but is associated with cell particles in the lactating mammary gland (Watkins & Hassid, 1962). Karimoto & Reithel (1965) solubilized the particulate enzyme by extracting freeze-dried particles with cold butanol followed by acetone, and extracting the acetone-dried powder with tris buffer. Many other methods, including enzymic digestion, proved unsuccessful in solubilizing intact lactose synthetase activity (W. Z. Hassid, personal communication).

Brodbeck & Ebner (1966a,b) and Brodbeck, Denton, Tanahashi & Ebner (1967) reported that the enzyme activity requires two unlike proteins, termed 'A' and 'B'. They found the smaller protein, 'B', to be equivalent to α -lactalbumin. Protein 'B' was stated to be distributed between the microsomes and soluble phase after VirTis homogenization, whereas the larger 'A' protein was

stated to be associated with microsomes. They reported very low activities of lactose synthetase in the particulate fraction unless purified α -lactalbumin were added.

Experiments in our Laboratory indicated that most of the particulate lactose synthetase activity sedimented at forces less than those usually employed for the microsomal elements. No additions were required to demonstrate activity, and the activity was comparable with that reported by Brodbeck & Ebner (1966a,b) for microsomes stimulated by α -lactalbumin. This discrepancy was thought to be due to different methods of homogenization, and led to the investigations reported below. It is shown that mild homogenization provides particles sedimenting in the lysosome-size range, with high lactose synthetase activity.

MATERIALS AND METHODS

Materials. Lactating bovine mammary gland was kindly provided by Falkenstein's Meat Co. (Eugene, Oreg., U.S.A.). UDP-galactose, *p*-nitrophenyl thymidine 5'-phosphate and

Scheme 1. (1) UTP- α -D-glucose 1-phosphate uridylyltransferase (EC 2.7.7.9); (2a) UDP-glucose 4-epimerase (EC 5.1.3.2); (2b) UTP- α -D-galactose 1-phosphate uridylyltransferase (EC 2.7.7.10); (3) UDP-galactose-D-glucose 1-galactosyltransferase (EC 2.4.1.22).

phenolphthalein β -glucuronide were purchased from Calbiochem (Los Angeles, Calif., U.S.A.), UDP-[1- 14 C]-galactose was from New England Nuclear Corp. (Boston, Mass., U.S.A.), α -lactalbumin was from Pentex (Kankakee, Ill., U.S.A.) and *p*-nitrophenyl phosphate was from Sigma Chemical Co. (St Louis, Mo., U.S.A.). β -Galactosidase was kindly given by Dr Richard Newton (this Laboratory). Galactose dehydrogenase was prepared from *Pseudomonas saccharophila* G6 by the method of Cline & Hu (1965), who generously supplied a culture of the mutant strain.

Homogenization and fractionation techniques. Fresh mammary-gland tissue was immediately placed in ice, cut into thin strips and placed between paper towels to remove much of the milk. To decrease the tissue size to about 1 mm. for homogenization, the tissue was either cut by scissors or rapidly frozen in liquid N_2 and shattered by a blow. The pieces were washed in 10 vol. of ice-cold 0.25 M-sucrose in 1 mM-tris-HCl buffer, pH 7.5, filtered through one layer of cheesecloth and centrifuged in the Servall SS-34 rotor at 5° at 50 000 *g* for 160 min., or 8×10^6 *g*-min. [The term *g*-min. is used to denote gravitational force in *g* (obtaining at the bottom of the tube) times min., to facilitate comparisons of various centrifugal methods.] The washing was repeated twice to remove the last traces of milk as determined by lactose assay. Washed particles were then suspended in 5 vol. of 0.25 M-sucrose. Homogenization was performed by a hand-operated glass homogenizer (Aloe no. VC 96580, clearance 0.12–0.17 mm.) with two to five strokes.

Whole cells, nuclei and large fragments of connective tissue were sedimented from the homogenate by centrifugation at 10⁴ *g*-min. The sediment was termed *P*₁. Other particulate fractions were sedimented successively at 5×10^4 *g*-min. (*P*₂), 3×10^5 *g*-min. (*P*₃) and 8×10^6 *g*-min. (*P*₄). The terms 'mitochondrial' and 'microsomal' are avoided, for reasons to be made evident below.

Analytical methods. To assay lactose synthetase activity, preparations were incubated at 37° for 2 hr. in a medium containing 25 mM-tris-HCl buffer, pH 7.5, 25 mM-glucose, 0.5 mM-galactose or 0.5 mM-UDP-[14 C]galactose and 40 mM-MnCl₂. The reaction was terminated by boiling for 2 min. and centrifuging to clear. Lactose was measured in the supernatant by the radioactivity method described by Karimoto & Reithel (1965) by liquid-scintillation counting of the purified [14 C]lactose, or by an enzymic method (Coffey & Reithel, 1968a), by using the sequential action of β -galactosidase and galactose dehydrogenase without prior purification of lactose. In the enzymic assay, samples of the supernatant were incubated with β -galactosidase at room temperature for at least 2 hr. in 50 mM-imidazole, pH 7.0,

containing 10 mM-KCl and 10 mM-MgCl₂. Galactose dehydrogenase was then added, and the reduction of NADP⁺ was followed spectrophotometrically at 340 m μ . The total NADPH formed was equated to the total amount of lactose and galactose. Samples of the supernatant were treated with galactose dehydrogenase alone to determine free galactose.

Phosphodiesterase I (EC 3.1.4.1) was assayed by the method of Razzel (1961), with *p*-nitrophenyl thymidine 5'-phosphate. β -Glucuronidase (EC 3.2.1.31) was assayed at pH 4.8 with phenolphthalein β -glucuronide and 0.1% (v/v) Triton X-100 by the method of Gianetto & de Duve (1955). Succinate dehydrogenase, or more properly the 'succinoxidase complex', was assayed by the 2,6-dichlorophenol-indophenol method of Green, Mii & Kohout (1951), with 1.5 mM-EDTA added to the assay mixture. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951), with crystalline bovine plasma albumin as standard.

RESULTS

Validity of the enzymic assay. The assay of lactose synthetase activity by the enzymic procedure with β -galactosidase and galactose dehydrogenase is more specific than methods measuring UDP liberation. By omitting β -galactosidase the enzymic conversion of UDP-galactose into free galactose, here termed UDP-galactose hydrolase activity, was determined simultaneously. Measurements of lactose synthetase and UDP-galactose hydrolase activities by this method were comparable with, but slightly higher than, measurements by the more tedious radiochemical incorporation procedure, as shown in Table 1. In this and other Tables, specific activity is reported as units (m μ moles of lactose or galactose formed/min.)/mg. of protein under assay conditions detailed in the Materials and Methods section.

The more rapidly sedimenting fraction *P*_{2–3} contained more active lactose synthetase and less active UDP-galactose hydrolase than fraction *P*₄. Subsequent solubilization of lactose synthetase from acetone-dried powders by the method of Karimoto & Reithel (1965) was accompanied by 76% recovery from fraction *P*_{2–3} with a sixfold

Table 1. *Specific activities of enzymes in mammary-gland particles measured by enzymic and radiochemical methods*

Mammary-gland tissue was cut, washed, and homogenized and fractionated as described in the Materials and Methods section. Specific activity (m μ moles of product formed/min./mg. of protein) was assayed enzymically or radiochemically as described in the Materials and Methods section.

Assay method	...	...	Lactose synthetase		UDP-galactose hydrolase	
			Enzymic	Radiochemical	Enzymic	Radiochemical
Fraction						
<i>P</i> _{2–3}			0.45	0.38	3.50	2.88
<i>P</i> ₄			0.22	0.15	6.80	6.21

Table 2. *Specific activities in mammary-gland particles prepared by two methods of decreasing tissue size*

The mammary-gland tissue size was reduced by means of scissors or by shattering tissue frozen at -196° . Homogenization of washed tissue was performed as described in the Materials and Methods section.

Method of decreasing tissue size	Protein (mg./g. of washed tissue)	Lactose synthetase (μ mole/min./mg. of protein)	UDP-galactose hydrolase (μ moles/min./mg. of protein)	Succinate dehydrogenase (ΔE_{400} /min./mg. of protein)
Scissors	10.7	0.44	2.25	0.79
Frozen, shattered	10.7	0.39	2.56	0.87

purification in terms of soluble protein. Less recovery from the P_4 fraction was observed. About 10% of the UDP-galactose hydrolase was solubilized by the same technique.

Decrease of tissue particle size. Because of the large amount of connective tissue in mammary gland it is necessary to decrease the tissue particle size to about 1mm. before it can be homogenized in a tight-fitting glass homogenizer. This was done for the homogenate in Table 1 by the tedious procedure of cutting with scissors. In Table 2, this method was compared with an alternative procedure of freezing the tissue in liquid nitrogen and shattering it with a blow. In both cases the small sections of tissue were washed and homogenized as described in the Materials and Methods section. The fraction sedimenting between 10^4 and 10^5 g.-min. was used for assays of protein, lactose synthetase, UDP-galactose hydrolase and mitochondrial succinate dehydrogenase. The similarity of values for these components led us to adopt the simple method of freezing and shattering to decrease the tissue size in subsequent experiments.

Incubation time. In determinations of units of enzyme activity by sampling techniques it is desirable to withdraw samples before 10% of the substrate has been used. In our assay a 10% conversion of the substrate would give rise to E_{340} 0.155. The units of enzyme represented by this change would be 0.42 or 1.67 if the incubation time were 120 or 30min. respectively. Actual changes in E_{340} are not proportional to the time of incubation, as shown in Fig. 1. Enzyme units calculated for the 120min. incubation were about one-half those for the 30min. incubation. Since the change in E_{340} at 120min. was twice that of the 30min. incubation, the longer incubation time was always used in subsequent experiments. Specific activities reported in this paper should be multiplied by about 2 to compare our results with those of workers who employed the 30min. incubation.

Effects of UTP. The inhibition of soluble lactose synthetase by UTP was noted by Babad & Hassid (1966), whereas Watkins & Hassid (1962) and Karimoto & Reithel (1965) observed an enhancement of the particulate activity by UTP. Fig. 2

Fig. 1. Effect of incubation time on the calculation of enzyme units. Mammary-gland subcellular particles were prepared by shattering and homogenization as described in the Materials and Methods section. Particles sedimenting between 1×10^4 and 8×10^4 g.-min. were assayed for lactose synthetase (○) and UDP-galactose hydrolase (□) for the indicated times. Units of enzyme were calculated as μ moles of lactose or galactose formed from UDP-galactose/min./mg. of protein and converted into relative arbitrary units for comparison in the Figure.

Fig. 2. Inhibition of enzymes by UTP. Mammary-gland particles were prepared and assayed for lactose synthetase (○) and UDP-galactose hydrolase (□) as described for Fig. 1, in the presence of the indicated concentrations of UTP.

shows that the initial enhancement of particulate lactose synthetase by 1.3mM-UTP was accompanied by complete inhibition of UDP-galactose hydrolase. Since both enzymes compete for the substrate it is possible that no enhancement of

particulate lactose synthetase would occur if UDP-galactose hydrolase were absent.

Addition of α -lactalbumin. The stimulatory effect of α -lactalbumin on particulate lactose synthetase is shown in Fig. 3. Intact lactose synthetase or 'AB' activity is considered by us as that portion of activity observed in the absence of additional α -lactalbumin. Total 'A' component is considered as the maximum activity in the presence of added α -lactalbumin. The difference between these values represents the dissociated 'A' component, and is denoted simply as 'A'.

Addition of 1mg. of α -lactalbumin/ml. was sufficient to achieve maximum lactose synthetase activity in this case, in which about 25% of the enzyme was intact. In some cases the extent of dissociation may be greater than 75%, so 2.5mg. of α -lactalbumin/ml. was always added in subsequent experiments to ensure measurement of the total activity.

Fig. 3. Effect of α -lactalbumin on enzymes. Mammary-gland particles were prepared and assayed for lactose synthetase (O) and UDP-galactose hydrolase (□) as described for Fig. 1. α -Lactalbumin was added as indicated.

UDP-galactose hydrolase was greatly inhibited by α -lactalbumin. This inhibition appeared to be linear with α -lactalbumin concentration, and could be due to tight binding of UDP-galactose to the protein.

Cell disruption. The procedure of freezing and shattering the tissue to decrease the particle size for homogenization was suspected to disrupt the cells as well. This was confirmed by the experiment reported in Table 3.

The shattered particles were washed and sedimented as usual at 10^6g -min.; they were then suspended in 0.25M-sucrose and resedimented at 10^4g -min. The fraction remaining suspended in 0.25M-sucrose consisted of subcellular particles released by freezing and shattering alone. The sediment was resuspended in 0.25M-sucrose, homogenized by three strokes with the glass homogenizer and centrifuged at 10^4g -min. This additional homogenization disrupted most of the remaining whole cells, as further homogenization of the 10^4g -min. sediment released negligible amounts of protein.

Comparison of sucrose and salt media. The preparation of particles with a high recovery of intact lactose synthetase was attempted. In Table 4 a comparison of two homogenizing media revealed that the use of potassium chloride gave particles retaining a higher percentage of intact enzyme than did sucrose. Washing the particles by repeated sedimentation did not alter the percentage of intact activity.

Subcellular distribution of lactose synthetase. A study of the subcellular distribution of lactose synthetase is recorded in Table 5. The sedimentation forces were those used by other workers to differentiate the nuclear-whole cell (P_1), mitochondrial (P_2), lysosomal (P_3) and microsomal (P_4) fractions. Each particulate fraction was pelleted twice, the supernatants being combined for the subsequent fraction. The final supernatant (S_4) was concentrated against Ficoll (Pharmacia,

Table 3. *Tissue disruption by freezing and shattering*

Particles were released from mammary-gland tissue by freezing at -196° and shattering, and by subsequent homogenization as described in the text. Results are means of two experiments. The notations 'AB' for intact enzyme and 'A' for the dissociated 'A' component are described in the text.

Particles released by	Lactose synthetase (units/g. of washed tissue)		Protein (mg./g. of washed tissue)
	'AB'	'A'	
Shattering	7.6 ± 1.4	23.9 ± 5.5	16.7 ± 1.7
Subsequent homogenization	1.4 ± 0.1	6.9 ± 2.2	4.5 ± 0.1
Total	9.0 ± 1.5	30.8 ± 7.7	21.2 ± 1.8
% released by shattering	84 ± 2	78 ± 2	79 ± 1

Uppsala, Sweden), and residual lactose was removed by dialysis for 12 hr. against 50 mM-tris-hydrochloric acid buffer, pH 7.5. Under these dialysis conditions less than 10% of the α -lactalbumin diffused through the membrane.

Over half of the total intact enzyme ('AB') was found in fraction P_3 , and this fraction provided the highest percentage intact recovery. The specific activity of the enzyme in fraction P_3 was 2.4 times that of the homogenate. The remainder of intact lactose synthetase was distributed between fractions P_1 , P_2 and P_4 , with very little in the soluble phase. The dissociated 'A' protein was found largely in the nuclear-whole cell fraction P_1 , with a considerable amount in the soluble fraction and smaller amounts in fractions P_2 , P_3 and P_4 .

Enzyme activity in the tissue washings was regarded as being derived from both soluble cellular and milk proteins. The activity of intact lactose synthetase was greater in the washings than in the entire homogenate. This result was not unexpected

since over half of the wet weight of lactating mammary tissue is due to milk (Folley & Greenbaum, 1947), which contains a high content of the intact enzyme. The differences between the soluble fraction S_4 and the tissue washings are great enough to permit the assumption that most of the enzyme in the washings is extracellular in origin.

If we consider the sum of fractions P_3 and P_4 as microsomal, the distribution of intact lactose synthetase is very similar to that given by Brodbeck & Ebner (1966b) for the total 'A' protein, but is quite different from our calculated values of dissociated 'A' protein. The specific activity for intact lactose synthetase in fraction P_3 is nearly twice that obtained by Brodbeck & Ebner (1966a) for microsomes derived from VirTis-homogenized preparations. Considering that the latter authors used the 30 min. incubation, the specific activity of intact enzyme in fraction P_3 is more than twice that of VirTis-homogenized microsomes. These differences prompted a comparison of our homogenizing method with the VirTis-homogenizer method used by Brodbeck & Ebner (1966b). To follow the effects of homogenizing methods on the subcellular distribution, three marker enzymes were also measured.

For this comparison, reported in Table 6, fresh tissue was frozen in liquid nitrogen, shattered, washed and suspended in 5 vol. of 0.25 M-sucrose. Samples were homogenized by three strokes with the hand-driven glass homogenizer, by 30 sec. in a VirTis 45 overhead homogenizer with the Variac setting at 80, or by 1 min. with the Branson Sonifier at 100 w at 0° under nitrogen. Each homogenate was separated into five subcellular fractions as described for Table 5. The results are expressed for each fraction as percentages of the sum of all

Table 4. *Lactose synthetase ('AB') activities in particles prepared from 0.25 M-sucrose and 0.14 M-potassium chloride.*

Tissue was frozen at -196° , shattered, washed in 10 vol. of 0.25 M-sucrose or 0.14 M-KCl (both buffered at pH 7.5 with 1 mM-tris) and homogenized by three strokes with the glass homogenizer. Particles were sedimented between 1×10^4 and 8×10^6 g-min.

	Medium	...	Sucrose	KCl
Sp. activity			0.28	0.49
Units/g. of washed tissue			7.5	9.3
% of intact enzyme			25	34

Table 5. *Subcellular distribution of lactose synthetase*

The tissue was frozen at -196° , shattered, washed, homogenized by one stroke with the glass homogenizer and fractionated by differential centrifugation at the forces indicated. Each fraction was pelleted twice, the supernatants being combined for the subsequent fraction. The supernatant after pelleting fraction P_4 is termed S_4 . The combined washings were treated with $(\text{NH}_4)_2\text{SO}_4$; enzyme precipitated between 30% and 80% saturation was assayed. Specific activity and units/g. are defined in Tables 2 and 3.

Fraction	Lactose synthetase								% intact
	Protein		'AB'			'A'			
	(mg./g.)	(%)	(sp. activity)	(units/g.)	(%)	(units/g.)	(%)		
P_1 (10^4 g.-min.)	22.8	42	0.06	1.4	14	13.9	44	9	
P_2 (5×10^4 g.-min.)	3.6	7	0.31	1.1	11	4.2	13	21	
P_3 (3×10^5 g.-min.)	12.4	22	0.44	5.4	55	4.5	14	55	
P_4 (8×10^6 g.-min.)	10.4	19	0.16	1.7	17	2.8	9	38	
S_4	5.5	10	0.06	0.3	3	6.5	20	4	
Total	54.7			9.9		31.9			
Washings				14.6		8.7			

Table 6. *Enzyme distributions in three kinds of homogenates*

A tissue particle suspension was prepared as described for Table 5, divided into three portions and treated by homogenizing procedures as described in the text: G, glass homogenizer; V, VirTis homogenizer; S, Sonifier. The homogenates were then fractionated as described for Table 6. Results are given as percentages of the total units. Total mg. of protein or units of enzyme/g. of washed tissue are indicated below each column. The sum of enzyme units in the separated fractions did not differ significantly from the total units in the homogenate for any of the enzymes. Units of lactose synthetase, UDP-galactose hydrolase and succinate dehydrogenase are described in Table 2. Units of β -glucuronidase and phosphodiesterase I are arbitrary changes in E_{550} and E_{400} respectively.

Fraction	Lactose synthetase												UDP-galactose hydrolase						Succinate dehydrogenase						β -Glucuronidase						Phosphodiesterase I								
	Protein						'AB'			'A'			G			V			S			G			V			S			G			V			S		
	G	V	S	G	V	S	G	V	S	G	V	S	G	V	S	G	V	S	G	V	S	G	V	S	G	V	S	G	V	S	G	V	S						
P_1 (10^4 g.-min.)	32	2	2	9	1	14	33	1	0	37	29	22	55	1	0	45	3	5	35	6	21	7	35	4	2	35	6	21	7	35	4	2	35	6	21	7			
P_2 (5×10^4 g.-min.)	7	28	10	17	32	2	13	29	3	8	2	6	27	60	0	14	21	15	9	22	19	15	9	22	31	48	33	8	49	41	15	55	49	15	55	49	15	55	
P_3 (3×10^5 g.-min.)	28	23	24	54	27	6	16	20	21	12	12	7	17	24	23	15	11	9	22	31	48	33	8	49	15	55	49	15	55	49	15	55	49	15	55	49	15	55	
P_4 (8×10^5 g.-min.)	23	35	42	13	40	78	16	19	31	18	19	14	1	15	77	6	17	23	31	48	33	8	49	15	55	49	15	55	49	15	55	49	15	55	49	15	55		
S_4	10	12	22	7	0	0	22	31	45	25	28	51	0	0	0	20	48	48	6	8	33	8	49	15	55	49	15	55	49	15	55	49	15	55	49	15	55		
Total	51	52	50	4.7	1.6	0.4	14	18	16	17	16	19	7.1	6.8	7.0	14	14	14	7.0	14	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15		

fractions. This sum did not differ significantly from the total units in the homogenate for any of the enzymes. The total units are indicated at the bottom of each column.

Less disruption of whole cells was accomplished by the hand-driven glass homogenizer than by the other methods, as evidenced by the large amount of protein and all enzymes in the nuclear-whole cell fraction P_1 . Intact lactose synthetase ('AB') and dissociated 'A' protein distributions after use of the glass homogenizer were similar to those in Table 5. Recovery of intact activity in glass-homogenized fractions was 2.9 and 11.7 times that of the VirTis-homogenized and ultrasonically treated fractions respectively. The use of the VirTis homogenizer resulted in considerable dissociation of lactose synthetase, and ultrasonic treatment almost completely dissociated it. Less than one-quarter of the total lactose synthetase activity could be reconstituted by adding the soluble fraction obtained by ultrasonic treatment to the particulate fractions. The distributions of the dissociated 'A' component after VirTis-homogenizer and ultrasonic treatments are similar to those of β -glucuronidase.

Over half of the mitochondrial succinate dehydrogenase was found in fraction P_1 of the glass-homogenized preparation, the remainder in fractions P_2 and P_3 . The VirTis homogenization completely removed succinate dehydrogenase from fraction P_1 , leaving most of it in fraction P_2 , with significant amounts found in fractions P_3 and P_4 . Ultrasonic treatment broke all mitochondria, so that most of the succinate dehydrogenase appeared in fraction P_4 . Lysosomal β -glucuronidase also appeared in fraction P_1 of the glass-homogenized preparation, and was evenly distributed between fractions P_2 and P_3 . Considerable solubilization of this enzyme occurred by glass homogenizing, but much more solubilization as well as redistribution into smaller particles was accomplished by the VirTis homogenization and ultrasonic treatment. The last two methods did not seem to damage either succinate dehydrogenase or β -glucuronidase, since the total units were similar for all three preparations. This was not true for phosphodiesterase I, which lost 10% and 25% of its activity as a result of the VirTis-homogenization and ultrasonic treatments respectively. Ultrasonic treatment solubilized significant amounts of this enzyme. UDP-galactose hydrolase distributions were similar to those for phosphodiesterase I, except that high amounts of UDP-galactose hydrolase were found in the nuclear-whole cell fraction P_1 for all homogenates. UDP-galactose hydrolase activity might be considered to have both nuclear and microsomal origins, since the P_1 fractions from VirTis-homogenized and ultrasonically treated preparations had virtually no intact cells.

DISCUSSION

The present results indicate that particulate lactose synthetase activity in mammary gland is more highly organized than previously stated. By choosing gentle methods of homogenization, particles with over 50% yield of intact enzyme can be prepared. Such particles have a specific activity about twice that previously reported. Whether the differences in specific activity observed among various particle preparations are related to the functional state of the tissue cannot be decided as yet. Large changes in the activities of many enzymes occur throughout the lactation cycle (Slater, Greenbaum & Wang, 1963), and have been noted for UTP- α -D-glucose 1-phosphate uridylyltransferase and UDP-glucose 4-epimerase (Heitzman, 1967).

The discrepancy between the present results and those of Brodbeck & Ebner (1966b) can be ascribed to differences in terminology. They sedimented mammary-gland mitochondria at 5×10^4 g-min. and considered as microsomes all particles sedimented by greater forces. Judged by the distribution of succinate dehydrogenase, 5×10^4 g-min. did not sediment all mammary-gland mitochondria. Other workers have used even higher forces to sediment mammary-gland mitochondria: 2.4×10^5 g-min. (Pynadeth & Kumar, 1964) and 3.2×10^5 g-min. (Smith & Dils, 1964). Smith, Easter & Dils (1966) found 80% of the rabbit mammary-gland succinate dehydrogenase to be sedimented at 5×10^4 g-min. and Slater & Planterose (1960) sedimented only 60% of rat mammary-gland succinate dehydrogenase by 5.2×10^4 g-min. The last-named authors compared this value with that of 80% for rat liver mitochondria. They also found that mammary-gland mitochondria underwent size changes during the lactation cycle, whereas liver mitochondria did not. Thus it can be misleading to consider as 'microsomes' all particles not sedimented by 5×10^4 g-min.

The force of 3.2×10^5 g-min. is considered sufficient to sediment liver lysosomes from 0.25M-sucrose (Tappel, Sawant & Shibko, 1963). Most of the lactose synthetase particles were sedimented by this force. Considering β -glucuronidase as a marker of lysosomes (Greenbaum, Slater & Wang, 1960) and phosphodiesterase I as a marker of endoplasmic reticulum (Smith *et al.* 1966), the sedimentation characteristics of the lactose synthetase particles seem to place them in the former category. However, the particles do not belong to β -glucuronidase-characterized lysosomes, as demonstrated in the next paper (Coffey & Reithel, 1968b).

The apparent subcellular distribution of enzymes was shown to vary with the method of homogenization. The VirTis homogenizer caused much greater

disruption of the particles than the hand-driven glass homogenizer, as well as a different distribution. Watkins & Hassid (1962) obtained better yields of particulate lactose synthetase by using the Potter-Elvehjem homogenizer than by using the Waring Blendor. In his review Mathias (1966) recommended the use of the Potter-Elvehjem and the Dounce homogenizers, as well as hydraulic presses, and discouraged the use of homogenizers such as the Waring Blendor. The cavitation effects of the VirTis homogenizer are similar to those of the Waring Blendor. Mammary-gland lysosomes were found to be extremely fragile by Greenbaum *et al.* (1960).

A wide variety of conditions have been shown to solubilize the 'B' sub-unit, or α -lactalbumin, to a much greater extent than the 'A' sub-unit (Brodbeck & Ebner, 1966b). This is consistent with our findings that VirTis homogenization and, to a greater degree, ultrasonic treatment disrupt most of the intact enzyme. These methods do not provide intact soluble enzyme, though in our hands nearly half of the 'A' sub-unit was rendered soluble. Apparently the natural complement of α -lactalbumin, once dissociated from the 'A' sub-unit, does not easily recombine with the 'A' sub-unit.

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