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The Lactose Synthetase Particles of Lactating Bovine Mammary Gland

CHARACTERISTICS OF THE PARTICLES

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1. The conditions that promoted the solubilization of particulate lactose synthetase were effective for solubilizing the thiamine pyrophosphatase of the Golgi apparatus but differed from those effective for β-glucuronidase or acid phosphatase of lysosomes. 2. Lactose synthetase-containing particles did not bind Mg^{2+} or Cs^{+} ions, suggesting that they are not related to endoplasmic reticulum membranes. 3. Intact lactose synthetase and thiamine pyrophosphatase particles banded isopycnically at a density of 1·143 in a sucrose gradient. The dissociated 'A' sub-unit of lactose synthetase, UDP-galactose hydrolase, *p*-nitrophenyl phosphate acid phosphatase, alkaline phosphatase and phosphodiesterase I were associated with particles of a broad density range from 1·12 to 1·20. Lysosomal enzymes β-glucuronidase, arylsulphatase and β-glycerophosphate acid phosphatase were associated with particles of density 1·20, 1·175 and 1·15 respectively. 4. Rate-zonal sedimentation studies indicated that lactose synthetase particles have $S_{20,w}$ values exceeding 24000s, corresponding to spherical particles of diameter exceeding $5{\cdot}4 \times 10^{-5}$cm. 5. Electron micrographs of lactose synthetase particles purified over 20-fold revealed small spherical bodies (0·1–0·5μ) resembling lysosomes, the smaller of which were attached to membranes, and larger heterogeneous spherical or oval bodies (0·7–1·8μ) resembling lipofuscin secretory granules. 6. The relationship between lactose synthetase particles and the Golgi origin of secretion granules is discussed.

In the preceding paper we showed that more than half of the lactose synthetase (EC 2.4.1.22) activity of mammary gland can be recovered intact in subcellular particles co-sedimenting with lysosomes (Coffey & Reithel, 1968). The subcellular distribution of the intact enzyme was found to be similar to that reported for the total 'A' sub-unit by Brodbeck & Ebner (1966), but was substantially different from that of the dissociated 'A' sub-unit. It is likely that intact lactose synthetase and the dissociated 'A' sub-unit have different loci within the cell.

The results of patient work in many Laboratories have demonstrated that many functionally distinct constituents of the microsomal fraction can be separated by centrifugal methods owing to differences in size and density. Among these constituents are lysosomes, secretion granules, smooth and rough endoplasmic reticulum and the Golgi substance. These may be distinguished by the use of marker enzymes.

This paper entails a description of the lactose synthetase particles that have been partially purified by combined equilibrium-density (isopycnic) and rate-zonal centrifugation.

MATERIALS AND METHODS

Materials. β-Glycerophosphate, thiamine pyrophosphate and *p*-nitrocatechol sulphate were purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.). The sources of all other materials used are those cited in the preceding paper (Coffey & Reithel, 1968).

Methods of homogenization and centrifugation. Fresh mammary-gland tissue was frozen at −196°, shattered, washed and homogenized in 0·25M-sucrose in 1mM-tris–HCl buffer, pH 7·5, as described previously (Coffey & Reithel, 1968). Nuclei and whole cells were always removed by sedimentation in the Servall SS-34 rotor at 10^4g-min.

Isopycnic and rate-zonal centrifugations were performed with the Spinco model L preparative ultracentrifuge with the SW 25.2 rotor holding three tubes of 60ml. each. For isopycnic separations 55ml. linear sucrose gradients of 2·0–0·5M-sucrose in 1mM-tris–HCl buffer, pH 7·5, were prepared. Tris was added to minimize aggregation of particles (El-Aaser *et al.* 1966). Then 3ml. of homogenate was layered over the gradient and centrifuged at 25000rev./min. at 5° for at least 12hr. Fractions of volume 2·5 or 3·0ml. were withdrawn from the bottom. The density of each fraction was measured by pycnometry. Results with this method agreed closely with those obtained with a magnetic densitometer. All fractions were then diluted with an equal volume of distilled water and centrifuged at

50000rev./min. with the Spinco 50 rotor for 1hr. The pellets were suspended in a minimum volume of 0·25M-sucrose for enzyme assays. For rate-zonal centrifugation a gradient of 52ml. of 0·67–0·30M-sucrose in 1mM-tris–HCl buffer was formed over an underlay of 3·0ml. of 1·8M-sucrose. Then 3ml. of homogenate was placed on the gradient and centrifuged at 6000rev./min. at 5° for 1hr. Fractions of volume 4ml. were withdrawn from the bottom and the densities were measured as described above. Fractions were concentrated by centrifugation in the Spinco 50 rotor.

Analytical methods. Lactose synthetase and UDP-galactose hydrolase were assayed by the enzymic procedure previously described (Coffey & Reithel, 1968). Acid (pH 4·8) and alkaline (pH 10·5) phosphatases were measured by the method of Linhardt & Walter (1963) with *p*-nitrophenyl phosphate as substrate. Alternatively, acid phosphatase was assayed with β-glycerophosphate at pH 5·0 (Appelmans & de Duve, 1955), orthophosphate being determined in the trichloroacetic acid supernatant by the method of Ames & Dubin (1960). β-Glucuronidase, succinate dehydrogenase and phosphodiesterase I were assayed as described previously (Coffey & Reithel, 1968). Arylsulphatase was assayed by the method of Roy (1958). All assays of the lysosomal enzymes (acid phosphatase, β-glucuronidase and arylsulphatase) were preceded by a 15min. incubation at 4° of the enzyme with 0·1% (v/v) Triton X-100.

Thiamine pyrophosphatase was assayed by an adaptation of the histochemical procedure described by Allen (1963). To a small centrifuge tube containing 0·2ml. of enzyme was added 0·2ml. of freshly prepared 50mM-cysteine in 0·3M-tris–HCl buffer, pH 9·5. The tube was flushed with N_2 to prevent formation of a green complex of cysteine and Mn^{2+}, capped and equilibrated in a water bath at 37°. At zero time 0·05ml. of a freshly prepared mixture of 40mM-thiamine pyrophosphate containing 50mM-$MnCl_2$ was added. The tube was again flushed with N_2, capped and incubated at 37°. After 1hr. the tube was placed in ice–water. Then 0·05ml. of 10% (w/v) $CaCl_2$ saturated with $Ca(OH)_2$ was added to ensure complete precipitation of inorganic phosphate. After 20min. at 0° the tube was centrifuged. Orthophosphate was determined in the sediment by the method of Ames & Dubin (1960). The phosphomolybdic acid complex was extracted with 2·5ml. of isobutanol to avoid high blank values. Reagent blanks and enzyme blanks were always assayed similarly, the sum of these being subtracted from each sample.

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). RNA was measured as ribose by the orcinol method of Mejbaum (1939) after precipitation from cold 80% (v/v) ethanol three times to remove sucrose.

Electron microscopy. Density-gradient fractions were pelleted in the Spinco 50 rotor, fixed in phosphate-buffered glutaraldehyde and OsO_4, dehydrated by increasing concentrations of acetone and embedded in Epon 812. Thin (approx. 800 Å) sections were stained with uranyl acetate and lead citrate, and examined with a Siemens IA electron microscope.

RESULTS

Microsomal membrane separation. Previous results (Coffey & Reithel, 1968) suggested that lactose synthetase is associated with large particles in the non-mitochondrial fraction. Dallner (1963) devised a technique utilizing density-boundary sedimentation in the presence of Cs^+ and Mg^{2+} ions to separate three elements of the non-mitochondrial fraction. An experiment with Dallner's method is reported in Table 1.

Before the separation the homogenate was centrifuged at 10^5*g*-min. to remove mitochondria. Over half of the total lactose synthetase, acid phosphatase, protein and RNA were thus sedimented; most of the UDP-galactose hydrolase, phosphodiesterase I and alkaline phosphatase remained suspended.

Of the material not co-sedimenting with mitochondria, over half of the lactose synthetase,

Table 1. *Density-boundary separation of microsomal elements*

Nuclei and mitochondria were removed from the 0·25M-sucrose homogenate by centrifugation in the Servall SS-34 rotor at 10^5*g*-min. The supernatant (4·6ml.) was made 15mM with respect to CsCl and layered over 7·3ml. of 1·3M-sucrose–15mM-CsCl. This was centrifuged in the Spinco 50 rotor at 50000rev./min. for 90min. Fraction II was sedimented through 1·3M-sucrose–15mM-CsCl, leaving a fluffy double layer at the boundary. The double layer was removed, diluted to 8·0ml. with 0·25M-sucrose, and M-$MgCl_2$ was added to give a final concentration of 10mM. This mixture was layered over 4·0ml. of 1·15M-sucrose–10mM-$MgCl_2$ and centrifuged at 50000rev./min. for 45min. Fraction Ib was pelleted. Fraction Ia was recovered from the boundary, diluted with distilled water and centrifuged. All pellets were suspended in a minimum volume of 0·25M-sucrose for the assays. The percentage of total units found in the mitochondrial supernatant is indicated for each constituent.

	Lactose synthetase		UDP-galactose hydrolase	Phosphodi-esterase I	*p*-Nitrophenyl phosphatases		Protein	RNA
Fraction	(mµmole/ min./mg.)	(%)	(%)	(%)	Acid (%)	Alkaline (%)	(%)	(%)
Ia	0·58	58	51	68	66	24	20	24
Ib	0·72	15	29	9	13	53	4	10
II	0·07	27	20	23	21	23	76	66

Table 2. *Effects of various treatments on particulate enzymes*

The homogenate was prepared as described in the Materials and Methods section, and the material sedimenting between 1×10^4 and 8×10^6g-min. was treated as indicated. Samples were homogenized with the Potter–Elvehjem homogenizer (fitted with a Teflon pestle, mechanically driven at about 1000rev./min.) for 4min. at 4°, or frozen at −20° and thawed at 20° ten times, or dialysed for 18hr. against 20mM-tris–HCl buffer, pH 7·4, or treated with detergents at the indicated concentration for 1hr. at 0°. Samples were then sedimented at 8×10^6g-min. The pellet and supernatant were assayed separately for intact lactose synthetase (AB) or for the dissociated 'A' subunit (A), thiamine pyrophosphatase (TPP), β-glycerophosphate acid phosphatase (AP) and β-glucuronidase (β-G) as described in the Materials and Methods section. The percentage solubilization was calculated from the sum of the sedimented and soluble activities for all enzymes except thiamine pyrophosphatase, which was inhibited by detergent. For this enzyme the percentage solubilization was calculated as 100 minus % of control activity found in the sediment after an additional washing to remove detergent.

	% solubilized				
	Lactose synthetase				
Treatment	AB	A	TPP	AP	β-G
Potter–Elvehjem	11	3	1	4	88
Frozen and thawed	10	10	0	13	18
Dialysed	0	6	1	23	22
0·02% (v/v) Triton X-100	0	8	0	12	28
0·1% (v/v) Triton X-100	14	35	20	—	54
0·6% (v/v) Triton X-100	73	38	64	51	71
2·0% (v/v) Triton X-100	100	43	76	57	96
1·0% (w/v) Digitonin	67	41	53	21	84

phosphodiesterase I and acid phosphatase occurred in fraction Ia. This fraction binds neither Cs^+ nor Mg^{2+} ions. It is characterized by vesicles containing negligible activities of most known liver microsomal enzymes, and appears to have a different origin and function *in vivo* from the Mg^{2+}-binding smooth vesicles of fraction Ib (Dallner, 1963). Fraction Ib had most of the alalkaline phosphatase activity and much of the UDP-galactose hydrolase, but very little of the other enzymes, RNA or protein. Though the specific activity of lactose synthetase was higher in this fraction than in fraction Ia the total amount was low, and so it was concluded that intact lactose synthetase is not primarily associated with endoplasmic reticulum. Fraction II, supposed to consist of Cs^+-binding ribosomes and attached membranes, contained most of the RNA and protein, but very little of the enzymes tested.

Having established the non-identity of lactose synthetase particles with endoplasmic reticulum, we considered the possibility that the particles may belong to the lysosome or Golgi groups. The particles were treated by various methods known to disrupt lysosomal membranes. The resulting solubilization of lactose synthetase was compared with that of thiamine pyrophosphatase, acid phosphatase (with β-glycerophosphate as substrate) and β-glucuronidase. The results are shown in Table 2.

The Potter–Elvehjem-homogenizer treatment effected only slight solubilization of lactose synthetase, thiamine pyrophosphatase and acid phosphatase, whereas most of the β-glucuronidase was solubilized. Repeated freezing and thawing resulted in a decrease of lactose synthetase activity and a slight solubilization of all enzymes except thiamine pyrophosphatase. Dialysis against 20mM-tris–hydrochloric acid buffer, pH 7·5, solubilized small amounts of acid phosphatase and β-glucuronidase, but did not affect lactose synthetase or thiamine pyrophosphatase.

Detergents solubilized a considerable amount of lactose synthetase, as well as the other enzymes. However, the effect on lactose synthetase and thiamine pyrophosphatase was much less than that on β-glucuronidase and acid phosphatase, indicating that the former are either non-lysosomal or that they are more tightly bound to the membrane than lysosomal enzymes. The amounts of intact lactose synthetase and thiamine pyrophosphatase solubilized by Triton X-100 appeared to be proportional to the concentration of detergent, but this cannot be expressed with precision owing to the activation of the lactose synthetase and the inhibition of the thiamine pyrophosphatase. The solubilization of sub-unit 'A' of lactose synthetase was notably different from that of the intact enzyme. The different effects of detergent and the Potter–Elvehjem homogenizer on acid phosphatase and β-glucuronidase indicate that these enzymes may not belong to the same particle.

Isopycnic centrifugation. Isopycnic centrifugation

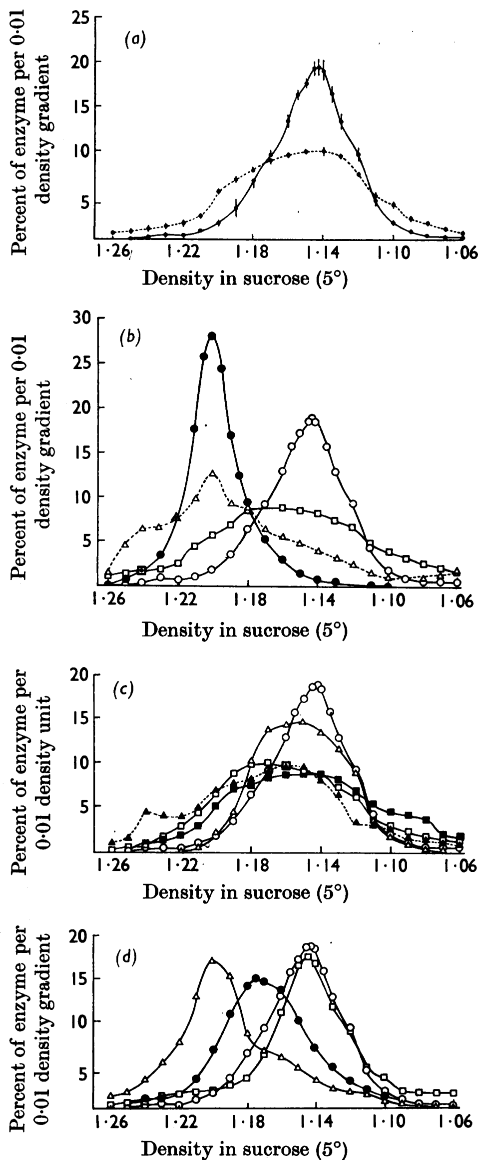


Fig. 1. Isopycnic centrifugation. Conditions of centrifugation are described in the Materials and Methods section. Curves represent the means, calculated as described in the text, for the numbers of experiments (n) indicated in parentheses. Vertical bars represent the S.E.M. (a) ——, Intact lactose synthetase ($n=7$); ------, dissociated 'A' sub-unit ($n=4$). (b) ○, Intact lactose synthetase ($n=7$); ●, succinate dehydrogenase ($n=5$); □, UDP-galactose hydrolase ($n=6$); △, protein ($n=5$). (c) ○, Intact lactose synthetase; △, β-glycerophosphate acid phosphatase ($n=2$); ▲, p-nitrophenyl phosphate acid phosphatase ($n=3$); □, phosphodiesterase I ($n=5$); ■, alkaline phosphatase ($n=3$). (d) ○, Intact lactose synthetase; □, thiamine pyrophosphatase ($n=3$); △, β-glucuronidase ($n=2$); ●, arylsulphatase ($n=2$).

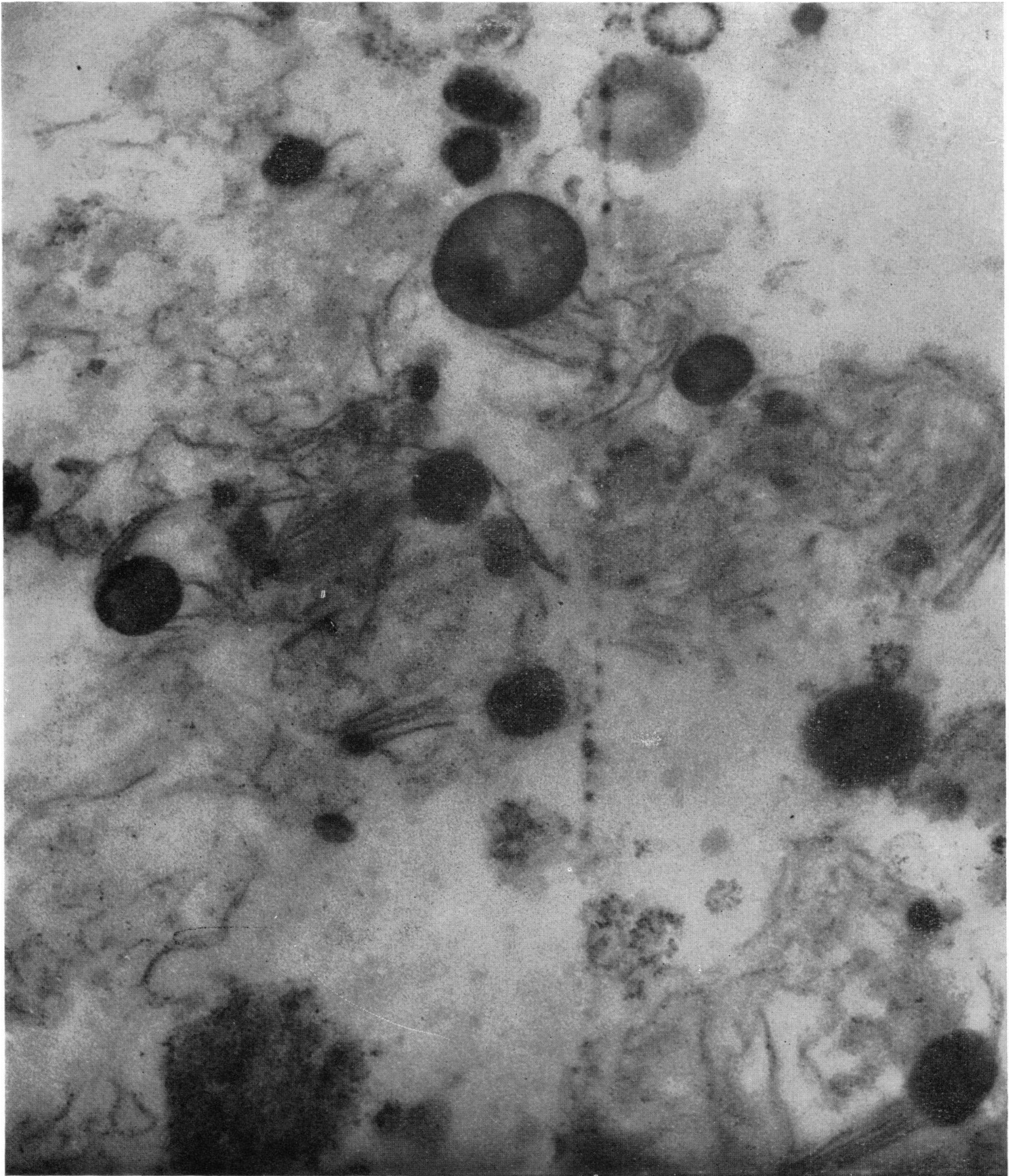
has been used on a large scale to purify subcellular particles, and on a smaller scale to establish the density of particles. Fig. 1 shows the results of several isopycnic centrifugations for ten enzymes and for protein.

The results were plotted as percentages of the total enzyme found in each fraction (corrected to percentage per 0·01 density gradient) versus the density gradient. Points corresponding to the intersection of the curves with each 0·01 gradient axis were averaged for two to seven experiments and replotted as a single curve.

In Fig. 1(a) the isopycnic distributions of intact lactose synthetase and the dissociated 'A' sub-unit are graphed as the means of seven and four experiments respectively. The peak of lactose synthetase occurred at a sucrose density of 1·143. Intact enzyme was limited to a much narrower range of densities than was the 'A' sub-unit. The specific activity of intact lactose synthetase at the peak was 4·5 times that of the starting material, based on protein estimation. The curve for intact enzyme is repeated in Figs. 1(b), 1(c) and 1(d) for ease in comparing it with curves for other enzymes.

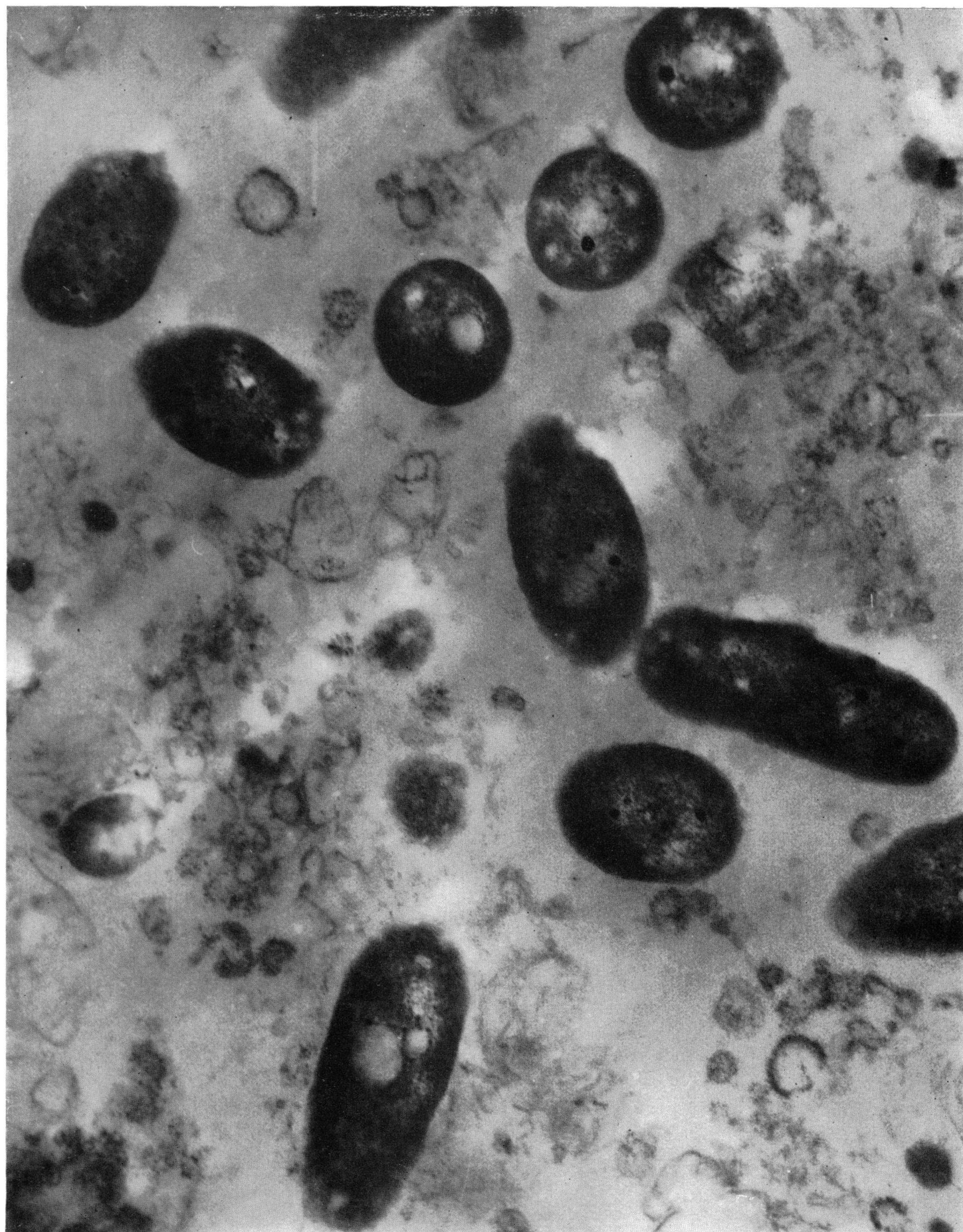
In Fig. 1(b) the mitochondria were located by succinate dehydrogenase at a density of 1·20. This value is in agreement with literature values for mitochondria from liver (Barber, Rankin & Anderson, 1966; Anderson *et al.* 1966) and kidney (Allen & Beard, 1965). UDP-galactose hydrolase activity was distributed over a broad range of densities, with a peak between 1·14 and 1·18. Protein distribution was similar to that of succinate dehydrogenase, but the peak was much broader. The distribution of RNA (not shown) was measured in two experiments and found to be very similar to that of protein, except that more RNA was found at densities greater than 1·2 than protein. Fig. 1(c) shows that the density distribution of acid phosphatase depends on the substrate. When β-glycerophosphate was used the peak at density 1·15 was much sharper than when p-nitrophenyl phosphate was used. The latter activity had a distribution similar to those of phosphodiesterase I and alkaline phosphatase. In Fig. 1(d) the correspondence of thiamine pyrophosphatase distribution to that of lactose synthetase is marked. Lysosomal activities of β-glucuronidase and arylsulphatase were distributed differently. The peak of β-glucuronidase occurred at a density of 1·20, similar to values for liver lysosomes (Wattiaux, Wibo & Baudhuin, 1963), whereas the peak of arylsulphatase was at 1·175.

The above results indicate that intact lactose synthetase was associated with structures characterized by thiamine pyrophosphatase as the Golgi substance rather than with lysosomes. The dissociated 'A' sub-unit probably adheres to endo-



EXPLANATION OF PLATES 1 AND 2

Electron micrographs of mammary-gland particles possessing a high activity of lactose synthetase. Particles were purified over 20-fold with respect to intact lactose synthetase by combined isopycnic banding and rate-zonal sedimentation as described for Figs. 1 and 2. The particles were prepared for electron microscopy as detailed in the Materials and Methods section and photographed at magnifications of 44000 (Plate 1) and 28000 (Plate 2).



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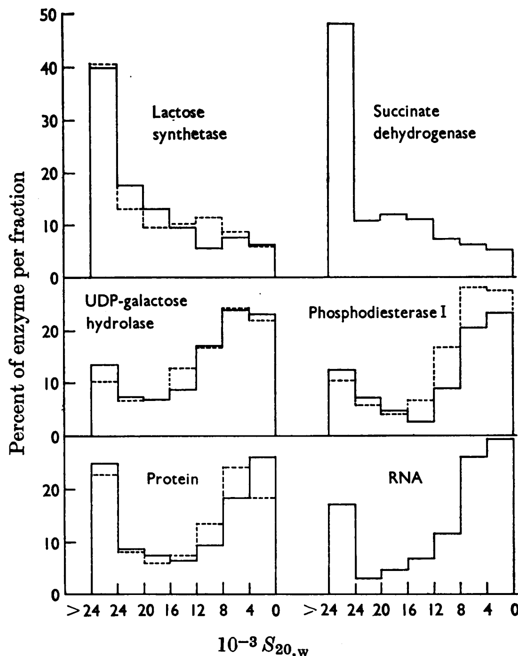

Fig. 2. Rate-zonal sedimentation. Conditions of centrifugation are described in the Materials and Methods section. Bars indicate the means of three experiments for lactose synthetase, two experiments for other enzymes. Solid lines represent sedimentation of the entire homogenate (minus nuclei). Broken lines represent sedimentation of particles banded isopycnically between sucrose density 1·11 and 1·17.

plasmic reticulum membranes, judged by its coincidence with the curves for alkaline phosphatase and phosphodiesterase I.

Rate-zonal sedimentation. After the density of the lactose synthetase particles had been established it became possible to estimate their size by rate-zonal sedimentation. Such experiments were designed to distribute particles with sedimentation coefficients ($S_{20,w}$) under 24000s throughout the tube. Larger particles were prevented from complete sedimentation by the dense sucrose underlay.

[Sedimentation coefficients were estimated from the equation

$$S_{20,w} = \frac{dx/dt}{\omega^2 x}\left(\frac{\eta_{T,m}}{\eta_{20,w}}\right)\left(\frac{\rho_p - \rho_{20,w}}{\rho_p - \rho_{T,m}}\right)$$

given by Bishop (1966). The term $\eta_{T,m}/\rho_p - \rho_{T,m}$ was established at the centre of each fraction. This estimate becomes inaccurate for particles reaching the 1·8M-sucrose layer. Calculations with the above equation agreed closely with those obtained by use of the Tables published by McEwen (1967).]

About half the lactose synthetase and succinate dehydrogenase reached the dense sucrose underlay, corresponding to $S_{20,w}$ greater than 24000s. Calculation of the diameter from the sedimentation equation $S_{20,w} = 10^{13}(2r^2)(\rho_p - \rho_{w,20})/9\eta$ (de Duve & Berthet, 1954) yields values exceeding 0·5μ for spherical particles of density 1·143. The distributions of UDP-galactose hydrolase and phosphodiesterase were quite different; most of these activities were associated with particles with $S_{20,w}$ under 12000s. Thiamine pyrophosphatase (not shown) sedimented like lactose synthetase in an experiment in which only two fractions were taken because of the small amount of material. In this experiment the ratio of lactose synthetase units in particles of $S_{20,w}$ greater than 24000s to all particles of smaller $S_{20,w}$ values was 1·38. This ratio for thiamine pyrophosphatase was 1·25.

The possibility of aggregation of the lactose synthetase particles with mitochondria was ruled out by another rate-zonal centrifugation with particles previously banded isopycnically between sucrose densities of 1·11 and 1·17. This material included over 80% of the intact lactose synthetase activity, but excluded over 90% of the succinate dehydrogenase. The results are indicated in Fig. 2 by broken lines. The distributions of lactose synthetase, UDP-galactose hydrolase and phosphodiesterase I were quite similar to those obtained in the presence of mitochondria.

Electron microscopy. Electron micrographs of lactose synthetase particles purified over 20-fold to a specific activity of 4·0 by combined isopycnic and rate-zonal sedimentation are shown in Plates 1 and 2. These were photographed from different areas of the same section of the pellet. Plate 1 shows particles resembling lysosomes or protein droplets, with diameters of 0·1–0·5μ. The smaller of these are associated with membranous strands. Plate 2 shows heterogeneous large particles, ranging from 0·7 to 2·0μ in diameter. The periphery of the oval bodies is rather diffuse, but the spherical bodies seem to be limited by a membrane. A few large light areas and many electron-opaque spots are seen in both the spherical and oval bodies.

DISCUSSION

The use of marker enzymes to define the subcellular occurrence of an enzyme has been common for liver tissue but has not been extensively applied to mammary gland. Mammary-gland tissue contains both parenchymal epithelial and adipose cell types, as well as blood cells, a large amount of connective tissue and milk particles.

Smith, Easter & Dils (1966) found that phosphodiesterase I served as a marker for mammary-gland microsomes. The liver microsomal marker enzyme, glucose 6-phosphatase, was found to be absent from mammary gland both by Smith *et al.* (1966) and by us. Alkaline phosphatase is generally regarded as a microsomal enzyme. By the Dallner technique we have established that lactose synthetase is not primarily associated with either of the endoplasmic reticulum membranes known to bind cations. This was also found to be true for phosphodiesterase I, but alkaline phosphatase was found to be associated with the Mg^{2+}-binding membranes. The evidence indicates that the latter enzyme is a better marker for mammary-gland endoplasmic reticulum than is phosphodiesterase I.

β-Glucuronidase was regarded as a marker for mammary-gland lysosomes by Greenbaum, Slater & Wang (1960), who reported negligible activities of acid phosphatase in their preparations. The solubilization of β-glucuronidase by use of the Potter–Elvehjem homogenizer far exceeded that of lactose synthetase. This indicates that the lactose synthetase particles may not be classed with β-glucuronidase-characterized lysosomes. No evidence for latency of activity of lactose synthetase activity was found by physical methods such as repeated freezing and thawing, hypo-osmotic conditions or extensive homogenizing. The membrane of lactose synthetase particles may thus be considered as permeable to the substrates glucose and UDP-galactose.

By histochemical experiments Allen (1963) and Gluszcz (1966) established that thiamine pyrophosphatase activity was restricted to the Golgi substance. The correspondence of lactose synthetase activity with that of thiamine pyrophosphatase in our solubilization studies indicates that the two enzymes may belong to the same particles. This was corroborated by results from isopycnic sedimentation. Early work by Schneider & Kuff (1954) indicated that the Golgi substance has a much lower density than other microsomal elements. The sucrose density corresponding to the peak of activity for both lactose synthetase and thiamine pyrophosphatase was 1·143. The narrow range of density in which most of the activity of these enzymes was found distinguished them from phosphodiesterase I and alkaline phosphatase. The last two enzymes were associated with particles in the broad density range 1·12–1·20, similar to the values for rat liver microsomes (El-Aaser *et al.* 1966).

The densities of lysosomes characterized by β-glucuronidase, arylsulphatase and acid phosphatase varied from 1·20 to 1·15. These results are consistent with findings of other workers that indicate heterogeneous populations of lysosomes. Bowers, Finkenstaedt & de Duve (1967) have reported the discovery of three populations of rat spleen lysosomes. One of these, termed 'L15' (density of 1·15), was extremely sensitive to mechanical injury, and was said to be 'apparently incomplete', as it did not possess all the acid hydrolases that characterized the 'L19' and 'L30' populations. Romeo *et al.* (1966) found a bimodal density distribution of heart lysosomes. Additional evidence for the heterogeneity of lysosomes with regard to enzymic components (Sellinger, Beaufay, Jacques, Doyen & de Duve, 1959), latency (Slater, Greenbaum & Wang, 1963), widely different pH optima and membrane binding properties (Mahadevan, Nduaguba & Tappel, 1967; Tappel *et al.* 1967) do not permit us to rule out the concept of a lysosome-like particle for lactose synthetase.

Since lactose synthetase is very active in milk it would be reasonable to suppose that it is secreted within a vehicle such as the 'protein droplets' described by Wellings, DeOme & Pitelka (1960). In electron micrographs of lactating mouse mammary gland they found numerous dense bodies with diameters from 0·2 to 0·5 μ in close association with Golgi membranes of alveolar epithelial cells. The smallest of these droplets appeared inside vacuoles of the Golgi apparatus at the cell apex. During lactation the Golgi apparatus is greatly hypertrophied, and often occurs as stacks of membranes. At this time the protein droplets pass into the lumen and lose their single unit membrane. They suggested that these droplets originate in, or are condensed by, the Golgi apparatus, in a manner similar to that of zymogen granules of pancreatic acinar cells. Miyawaki (1965) defined similar dense bodies from non-lactating mouse mammary gland as lysosomes, with a single limiting membrane and diameters of 0·2–0·8 μ. These were seen by electron micrography to give a positive histochemical test for iron and acid phosphatase, and occurred in proximity to the well-developed Golgi cisternae and vesicles. Enlarged dense bodies composed of aggregates of ferritin-like grains, granular and membranous areas and large round or oval bodies also contained acid phosphatase and insoluble lipids or lipofuscin. These complex bodies appear similar to the largest heterogeneous dense bodies in our micrographs. El-Fiky (1967) found that acid phosphatase occurs in mouse mammary-gland lysosomes topographically identical with the Golgi apparatus. He stated that the Golgi substance occurs in the form of granules or crescent-like structures.

In a study of the secretory mechanism of mouse mammary gland, Hollman (1959) described two types of secretory particles, termed granules and droplets. In his Figs. 4 and 5 the larger (1–2 μ) sudanophilic droplets are clearly heterogeneous and nearly identical with the large heterogeneous bodies seen in our micrographs, whereas the smaller

(0·1–0·3 μ) granules are seen within the Golgi vesicles and appear to be similar to the smaller bodies associated with membranes in our pictures.

Though the results do not permit conclusions to be drawn about the origin of the lactose synthetase particles, it may be suggested that they are secreted by the Golgi substance in a fashion often suggested for secretory vacuoles and lysosomes. Excellent reviews of the findings leading to this concept are given by Novikoff (1963) and Strauss (1967). The correspondence of lactose synthetase activity and thiamine pyrophosphatase activity implicates the Golgi substance, and the size and appearance of the particles are similar to lysosomes and secretory granules.

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