

Membranes of *Saccharomyces cerevisiae*

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A crude small particle pellet, obtained from postmitochondrial supernatant fractions of *Saccharomyces cerevisiae*, contains about half the ergosterol and phospholipid of crude cell homogenates. Most of the phospholipid of this pellet is in a "heavy" fraction which, with the aid of electron microscopy, shows membranous elements in addition to discrete particles. The "heavy" fraction, upon treatment with deoxycholate, can be freed of membranes, or, with ribonuclease treatment, ribosomes can be removed, leaving relatively clean membranes. The "heavy" fraction resembles the microsomes of animal cells, but contains considerably less lipids, including phospholipids, thus suggesting a less well-developed intracellular membrane system.

Since the early observations of Agar and Douglas (1), several groups have presented photographic evidence for the existence of intracellular membranes in yeasts (6, 17, 18, 20). This evidence consisted of electron microphotographs of thin-sectioned yeast cells, showing membranous elements including the cytoplasmic membrane, nuclear and vacuolar membranes, membranes of mitochondria, and occasional membranous structures not directly related to any organized subcellular units. From these findings, it is not clear, however, whether the yeast cells used in these studies contained an endoplasmic reticulum characteristic of animal cells (15).

Preparations of yeast membranes have recently been obtained by the lysis of yeast protoplasts (3, 13). However, the usefulness of such preparations for analytical work is questionable as they appear to be extremely heterogeneous. Boulton, for example, showed that washed preparations of protoplast "ghosts" contained ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in addition to lipids and proteins (3). Furthermore, electron micrographs of this type of preparation attest to the heterogeneity of this material.

Studies in this laboratory have been concerned with the intracellular site(s) of lipid synthesis in *Saccharomyces cerevisiae*. Previous reports (8-12) on this organism have demonstrated that preparations from which mitochondria and heavier organelles have been removed by centrifugation incorporate acetate into a wide variety of lipids. In many ways, this active mitochondrial supernatant fraction appeared to resemble classical microsomal preparations [e.g., those of rat liver

(5)]. It is the purpose of this report to give details concerning the microsome-like nature of the particulate elements of the mitochondria-free yeast supernatant fractions.

MATERIALS AND METHODS

Information on the organism used in this study, *S. cerevisiae* strain LK2G12, its cultivation, harvesting, disintegration, and subsequent centrifugal fractionation, is given in earlier publications (8-11). After removal of the mitochondrial fraction, homogenates were centrifuged at $100,000 \times g$ for 90 min to obtain a crude "small" particle fraction. Crude "small" particles were further fractionated into "light" and "heavy" fractions by methods described previously (11).

Total lipids were extracted by the procedure of Bligh and Dyer (2). Thin-layer chromatography was used to characterize the lipid classes present; ergosterol was measured spectrophotometrically; phospholipid, by total phosphate analyses of the extracted lipids; protein, by the Lowry technique; and RNA, by the orcinol method. These methods are all described in earlier publications (8-11).

Choline-methyl- ^{14}C -chloride (specific activity, 2 mc/mole) was obtained from the New England Nuclear Corp., Boston, Mass. Radioactivity was determined in a Packard Tri-Carb liquid scintillation counter (model 3314).

For electron microscopy, particulate preparations were fixed for 2 hr in osmium tetroxide according to Caulfield (4). Subsequently, the fixed pellets were dehydrated and embedded in the plastic, Epon, and then were sectioned in a Porter-Blum MT-2 ultramicrotome to yield sections of 400 to 700 Å. The sections were picked up on grids and stained first with uranyl acetate (19) and then with lead hydroxide to increase contrast (7). The treated grids were then examined in a Hitachi microscope (model HU-11) at 75 kv.

RESULTS

Localization of ergosterol and phosphatidylcholine in the crude small particle fraction. Phospholipids and sterols are characteristically found in membranes of mammalian organisms (16). Since ergosterol is the major sterol of this yeast and phosphatidylcholine is the major phospholipid (8), the quantity of these substances in the crude "small" particle fraction was estimated. For this purpose, two kinds of tests were performed. One consisted in fractionating the yeast homogenates, hydrolyzing each fraction (8), extracting the nonsaponifiable lipids, and then measuring the ergosterol content of the latter. The second procedure consisted in growing cells in the presence of ^{14}C -choline and then, after fractionation of the homogenates, assaying the extracted lipids for radioactivity. (Initial experiments with ^{14}C -choline, by use of thin-layer chromatography, proved that upon extraction of the total lipids virtually all the extracted radioactivity appears as phospholipid.)

In these experiments, both ergosterol and ^{14}C -phospholipid appeared to be about equally distributed between the mitochondrial fraction and the mitochondrial supernatant fraction (containing the crude "small" particle fraction). After removal of the mitochondria, the bulk of these substances is found in the "small" particle fraction (Table 1). Further fractionation of the crude small particles into "light" and "heavy" fractions (Table 2) reveals that the "heavy" fraction accounts for the main portion of radioactive phospholipid found in the crude "small" particles, thus suggesting that the "heavy" fraction, but not the "light" fraction, contains membranous material. In this connection, it should be mentioned that the "heavy" fraction has been found to contain the ribosomes, as well as acetyl-coenzyme A synthetase, and the fatty acid desaturating and squalene oxidocyclase systems (H. P. Klein and C. M. Volkmann, Proc. Meeting Am. Chem. Soc., 152nd, p. C-288), whereas the "light" fraction has been shown (12) to contain the fatty acid synthetase of this organism.

Electron microscopy of "heavy" fraction. Earlier attempts (10) to demonstrate the presence of membranes in the "heavy" fraction, with use of air-dried and shadowed preparations, were routinely unsuccessful. However, the analytical data above indicated that this fraction should contain membranes or membranous subunits. Accordingly, renewed attempts were made to search for such structures, this time, however, by use of the sectioning and staining procedures outlined in the Materials and Methods section. Figure 1 shows that, in addition to discrete particles, the

TABLE 1. *Distribution of Ergosterol and phospholipid in postmitochondrial yeast fractions*

Fraction	Phospholipid ^a	Ergosterol ^b
	counts/min	mg
Mitochondrial supernatant fraction.....	7.4×10^6	1.75
Crude "small" particles...	6.8×10^6	1.14
Soluble supernatant fraction.....	0.6×10^6	0.17

^a Cells were grown in medium containing labeled choline (4.5×10^7 counts/min, $4.3 \mu\text{moles}$ per liter). A 68.9-g (wet weight) amount of cells was resuspended to a total of 120 ml in 0.002 M tris(hydroxymethyl)aminomethane-0.002 M MgCl_2 , pH 7.6, and broken in a French pressure cell (10). An 80-ml amount of crude homogenate was further fractionated to yield fractions shown. Counts per minute shown represent ^{14}C -choline incorporated into lipids extracted in each fraction.

^b Measured spectrophotometrically.

TABLE 2. *^{14}C -choline incorporation into lipids of "light" and "heavy" fractions of crude small particles^a*

Expt	Crude particles (total counts/min)	"Heavy" fraction (total counts/min)	"Light" fraction (total counts/min)
1	14.0×10^6	8.5×10^6	0.06×10^6
2	23.0×10^6	15.0×10^6	1.10×10^6
3	65.0×10^6	51.5×10^6	0.87×10^6

^a Cells were grown and disrupted as described in Table 1 (different volumes of cell suspension were processed in each experiment); the resultant homogenates were centrifuged to yield "heavy" and "light" fractions (11), which were then extracted for total lipids.

"heavy" fraction contains numerous membranous elements. Figure 3 shows another preparation at higher magnification, in which double-layered membranous elements, having an overall diameter of approximately 120 to 130 A, are seen, together with discrete particles.

Separation of membranes and ribosomes. In attempts to separate the membranes from ribosomes by simple differential centrifugation, the membranes, assayed with use of ^{14}C -choline as a tracer, sedimented somewhat more rapidly than the ribosomes (Fig. 2). (The rapid initial sedimentation of some of the ribosomal population suggests that approximately 17% is attached to membranes.) Although centrifugation studies of this kind indicate differences in sedimentation behavior between the membranes and ribosomes,

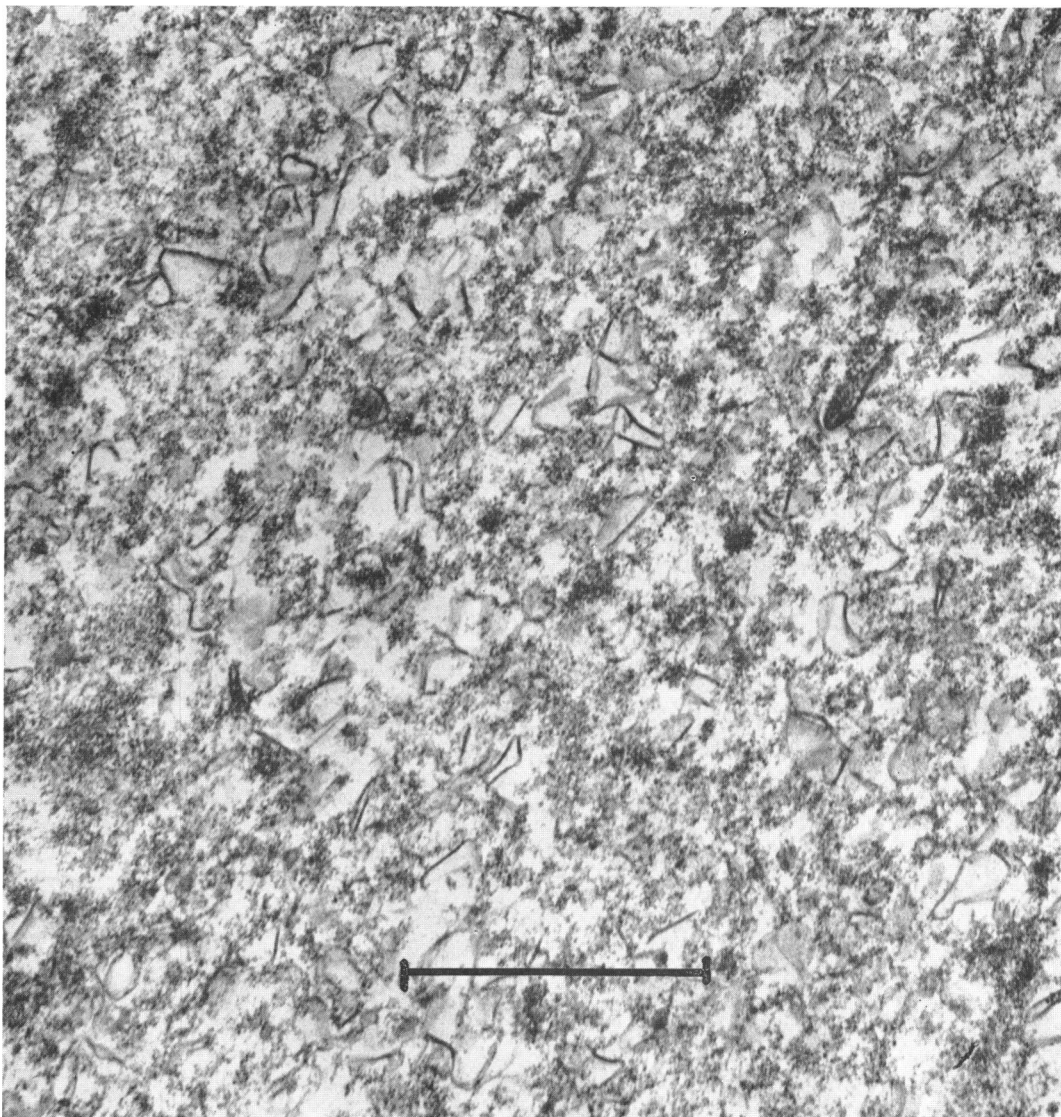


FIG. 1. *Electron micrograph of "heavy" fraction. Line indicates 1 μ .*

this technique did not yield clean preparations, for there was considerable cross-contamination of the two components in the final fractions obtained.

Beginning with Palade and Siekevitz (14), numerous investigators have used deoxycholate or similar reagents to "solubilize" the membranes of animal microsomes in order to obtain ribosomal preparations. Accordingly, suspensions of the "heavy" fraction were treated briefly with 1% sodium deoxycholate (5) to obtain cleaner ribosomal preparations. As is evident from Fig. 3,

this treatment was very effective in eliminating the membranes from these suspensions. Furthermore, such preparations previously labeled with ^{14}C -choline showed a corresponding loss of phospholipid radioactivity after treatment with deoxycholate.

Treatment of the "heavy" fraction with ribonuclease, in attempts to obtain clean membrane preparations, appeared to aggregate and precipitate the ribosomes rather than to solubilize them at ribonuclease concentrations of about 0.1 mg/ml. Figure 4 shows the ultracentrifugal

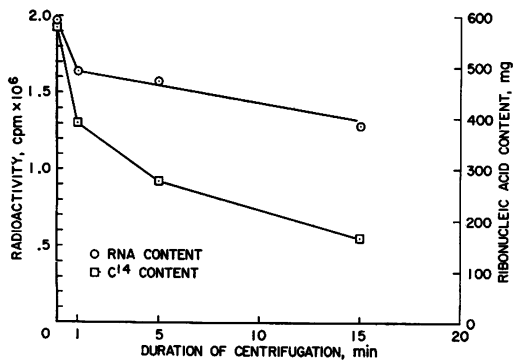


FIG. 2. The effect of centrifugation on the removal of membranes and ribosomes from a suspension of crude small particles. Crude small particles (1,080 mg of

patterns of a preparation of the "heavy" fraction before and after ribonuclease treatment. It is seen that the large 87S ribosomal component is completely removed by this procedure. Also evident in this figure is a minor peak which consists of a small amount of contaminating "light" particles. [The latter have been shown to be insensitive to ribonuclease treatment (12)]. In the figure, the heavier membranous component cannot be esti-

protein), obtained from cells grown with ¹⁴C-choline, were resuspended to 54 ml in 0.002 M tris(hydroxymethyl)aminomethane-0.002 M MgCl₂ (pH 7.6). Samples were centrifuged at 100,000 × g for intervals shown, after which the supernatant fraction from each tube was removed and assayed for RNA and radioactive phospholipid.

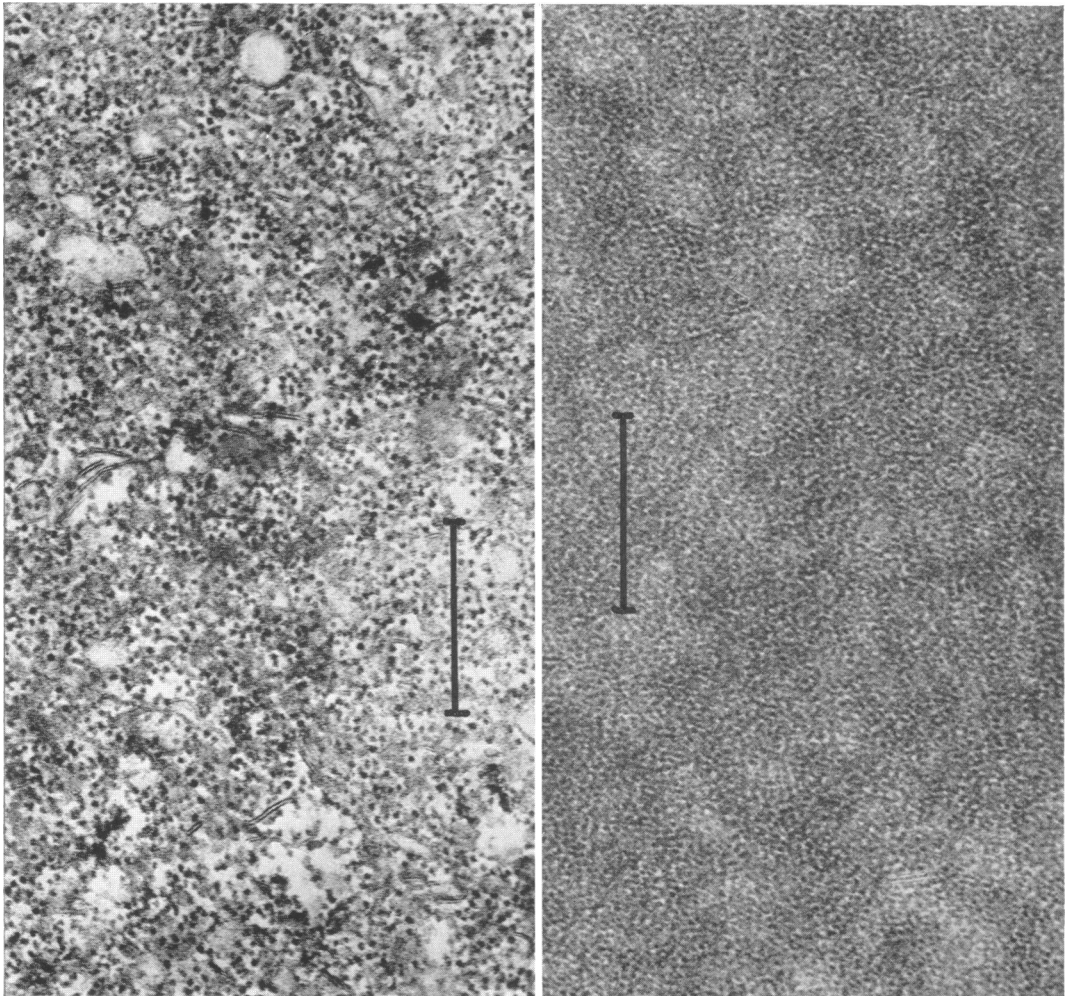


FIG. 3. "Heavy" fraction before (left) and after (right) treatment with deoxycholate. Line indicates 0.5 μ .

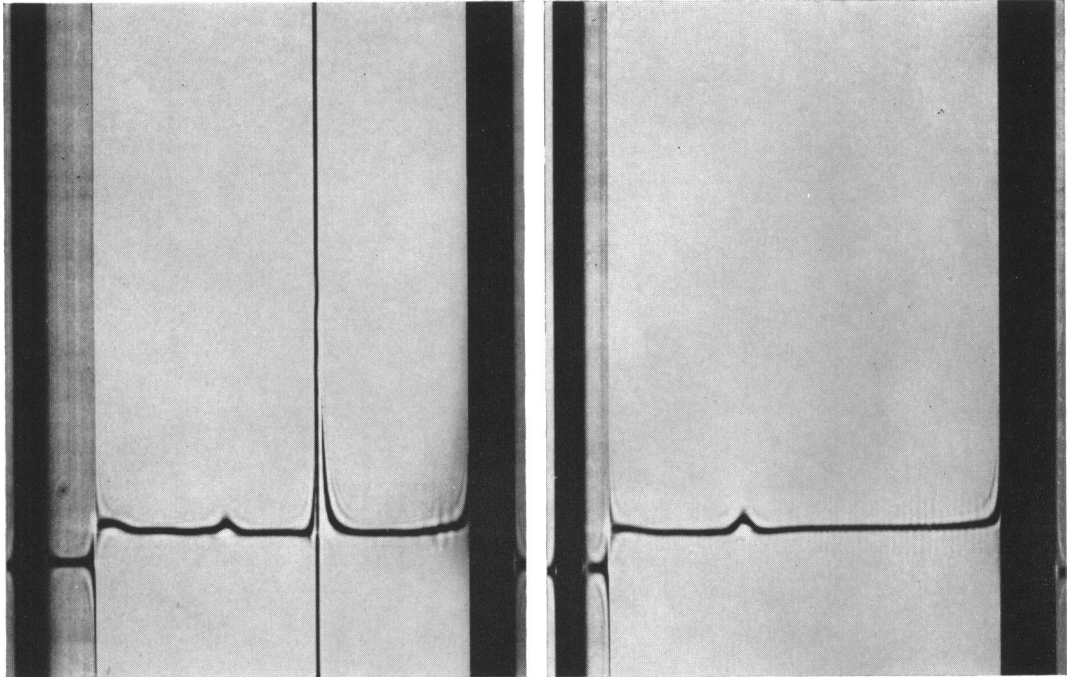


FIG. 4. Ultracentrifugal patterns of "heavy" fraction before and after ribonuclease treatment. Fractions were examined in the Spinco ultracentrifuge, model E. Photographs were taken 8 min after reaching 42,040 rev/min. Left "heavy" fraction (5 mg of protein per ml); right, same material after treatment with ribonuclease and removal of precipitate (see text).

mated, since they sediment rapidly under these conditions and, by the time these pictures were taken, they had sedimented to the bottom of the centrifuge tubes. After treatment with ribonuclease, the aggregated ribosomes can easily be removed by sedimenting them at $25,000 \times g$ for 30 min. The resultant supernatant fluid can then be centrifuged at $100,000 \times g$ for 60 min to obtain relatively clean membranes. The pellet obtained, when sectioned and examined in the electron microscope, shows abundant membranous vesicles, with very few contaminating particles (Fig. 5).

DISCUSSION

The experiments detailed above, together with earlier observations (12), indicate that the post-mitochondrial homogenates of *S. cerevisiae* contain both membranes and ribosomes. Both of these structures, furthermore, are associated with the "heavy" fraction of crude "small" particles obtained from such homogenates. Superficially, therefore, the "heavy" fraction resembles classical microsomes of animal tissues. However, several differences between the two systems should be

noted. To begin with, analyses for total lipids have shown that a wide variety of animal microsomes contain 30 to 50% lipids (16). By contrast, the "heavy" fraction of this organism consists largely of protein and RNA, with lipids accounting for 10% or less of the total dry weight (smaller amounts of glycogen are also present). Furthermore, animal microsomes are particularly rich in phospholipids, which account for almost as much of the dry weight as do the proteins (16). Although the ^{14}C -choline data presented here demonstrate that phospholipid is indeed present in the "heavy" fraction of *S. cerevisiae*, this fraction contains only about 3 to 5% phospholipid.

In contrast to animal microsomes, which must be chemically or mechanically treated (5) prior to separation of membranes and ribosomes, the results given here (see Fig. 2) suggest that, for the most part, membranes and ribosomes are not bound together in a common superstructure. Indeed, when "heavy" fraction preparations are examined in the ultracentrifuge, a very small fraction of the material sediments out very quickly, leaving behind the main sedimenting

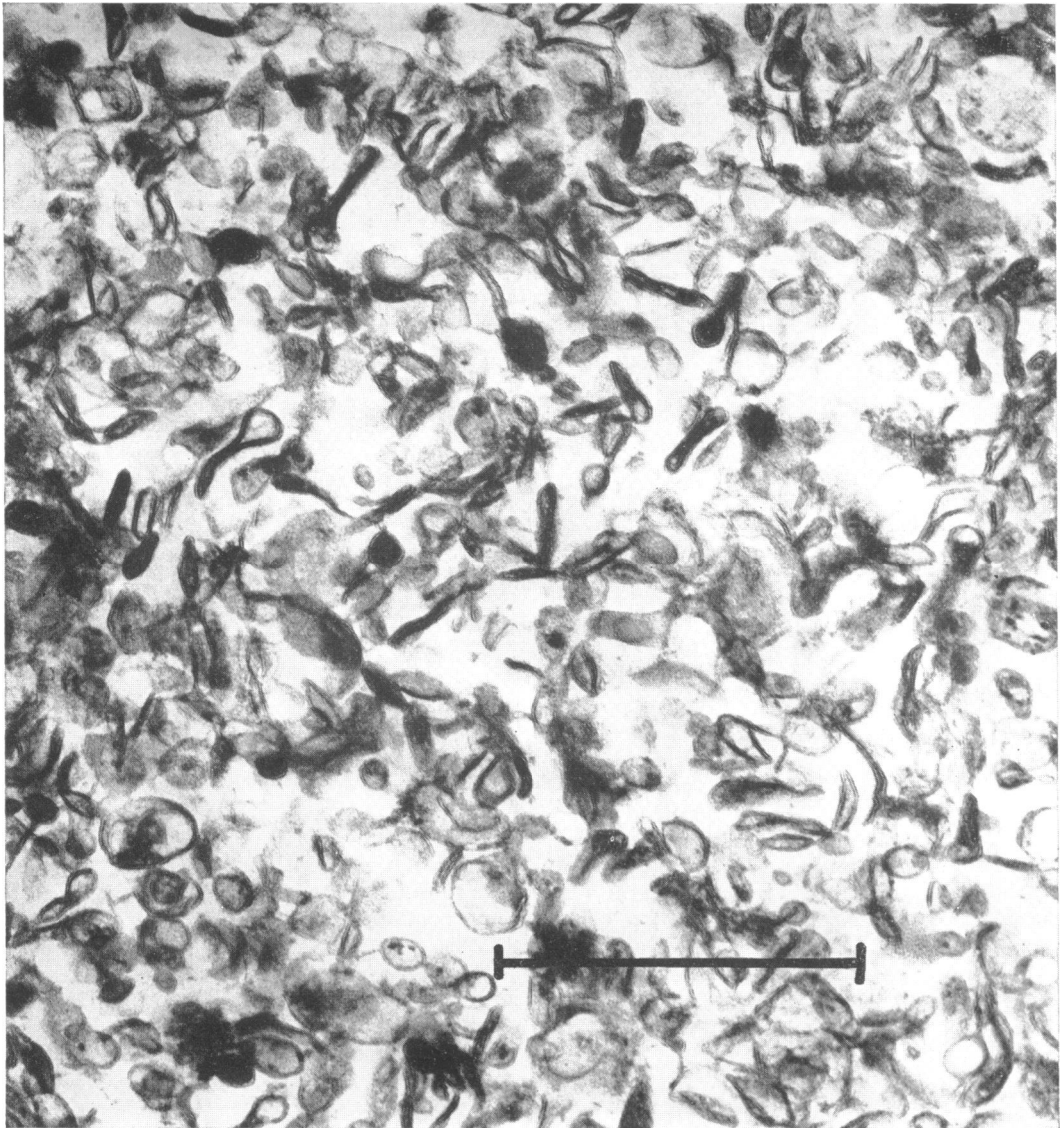


FIG. 5. Electron micrograph of sectioned pellet remaining after removal of ribosomes with ribonuclease treatment. Line indicates 1 μ .

boundary, the 87S ribosomal peak (10). These properties, along with the chemical composition discussed above, indicate a considerably less extensive and, perhaps, simpler membrane system in these yeast cells than is found in animal cells. Until additional work is carried out, it would appear premature to ascribe to this yeast a typical microsomal component.

Little is known about enzymatic activities associated either with the membranous or ribosomal portions of the "heavy" fraction. Our own in-

vestigations have centered primarily around enzymes involved in the synthesis of lipids. In a separate report (H. P. Klein and C. M. Volkmann, Proc. Meeting Am. Chem. Soc., 152nd, p. C-288), we have presented evidence that acetyl-coenzyme A synthetase and squalene oxidocyclase are associated with the membranes, whereas the fatty acid desaturase system is associated with the ribosomes. It would be of interest in this connection to look for enzymes, such as glucose-6-phosphatase, that are tightly bound to

the membranes of microsomes (16) in animal tissues.

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