Short Communications

A particulate fraction of yeast and its relation to lipid synthesis

In previous communications[1,2], we have reported on the incorporation of 1-^{14}C-acetate into the lipids of a yeast homogenate. The present report concerns the "anatomical" nature of these cell-free extracts.

Cells of *Saccharomyces cerevisiae*, strain LK2G12, were grown under anaerobic conditions, washed, aerated, and ruptured with dry ice and sand as described earlier[1-3]. Centrifugation of the extract at 100,000 × *g* for 30 minutes sedimented all the particulate material leaving a crystal-clear supernatant with a film of fatty substance at the top. The fatty layer was discarded and, after removal of the soluble portion, one part of the sedimented material was resuspended in distilled water, sprayed on "Formvar"-coated grids for electron microscopy, and air dried. The grids were shadowed with palladium at a 1:6 angle and viewed at an electronic magnification of 900 × in an RCA model EMU-2b electron microscope. A typical field (Fig. 1) showed the grids to be covered, almost exclusively, by uniform particles of the order of 20–30 millimicrons in diameter. Irregular structures of larger dimensions were also present.

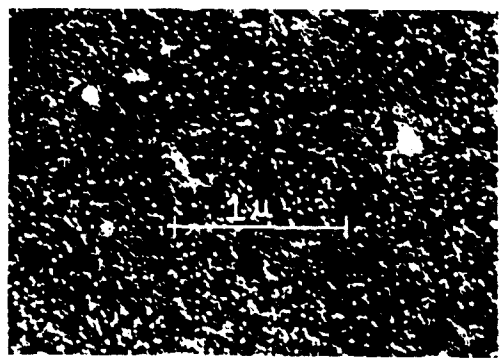

Fig. 1. Electron micrograph of particulate material from *S. cerevisiae*. See text for details.

Two additional aliquots of this particulate material were resuspended in ten volumes of phosphate buffer (0.1 *M*, pH 7) and supernatant fluid, respectively. A final aliquot was resuspended in five volumes of supernatant fluid. All three suspensions, as well as samples of the original homogenate and of the supernatant fluid alone, were then incubated with labelled acetate and subsequently assayed[1] for radioactive fatty acids and non-saponifiable lipids. Table I presents the results of this experiment, from which it is evident that incorporation into lipids occurred only in the presence of both the particulate and soluble fractions. Furthermore, when the concentration of particulate material was doubled, the radioactivity of the lipids was markedly increased, thus indicating that, under these conditions, the particles contain some limiting factor(s).

In other experiments, the particulate matter was fractionated by differential centrifugation and each fraction was tested for its ability to incorporate acetate into lipids in the presence of supernatant fluid. The greatest mass was sedimented after 30 minutes at 1500 to 6500 × *g*. However, the material sedimented after 30 minutes at 25,000 to 60,000 × *g* was at least 50 times more active, on a weight basis, in lipogenesis. Electron microscopy revealed the lighter, more active material to be comprised entirely of small particles identical to those shown in Fig. 1.

TABLE I

INCORPORATION OF LABELED ACETATE INTO LIPIDS BY YEAST FRACTIONS

*Fraction tested**	*Total c.p.m. incorporated*	
	Fatty acids	*Non-saponifiable lipids*
Whole homogenate	22,200	9,350
Clear supernatant	**	**
Particles in buffer (1:10)	**	**
Particles in supernatant (1:10)	21,000	7,000
Particles in supernatant (1:5)	32,600	19,300

* Fractions (1.0 ml) to be tested were shaken in Warburg vessels at 30° C in air for 4 hours. In addition, each vessel contained 2 μM ATP and 3.2 μM acetate ($5.4 \cdot 10^5$ c.p.m.) in a total volume of 1.2 ml.

** Less than 100 c.p.m.

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[1] H. P. KLEIN AND Z. K. BOOHER, *Proc. Soc. Exptl. Biol. Med.*, 89 (1955) 43.
[2] H. P. KLEIN AND Z. K. BOOHER, *Bacteriol. Proc.*, (1955) 136.
[3] H. P. KLEIN, *J. Bacteriol.*, 69 (1955) 620.

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Morphological changes accompanying thermal denaturation of tobacco mosaic virus

The inactivation of tobacco mosaic virus (TMV) at high temperature was described in the last century by MAYER[1], who noted that the sap of mosaic-diseased plants became non-infectious after exposure to temperatures of 80° or more. Later, with purified preparations of the virus, the thermal inactivation was found to be accompanied by denaturation and precipitation of the nucleoprotein[2]. However, when the virus is heated in salt solution at 100°, the nucleoprotein complex dissociates and the protein precipitates, leaving the ribonucleic acid (RNA) in suspension[3]. The mechanism of this dissociation is somewhat difficult to understand in view of the recent observation that the RNA lies within a tube of protein in the rod-shaped virus particles[4]. In the present note, the effects of heating TMV are described as seen in electron micrographs and from these observations a mechanism is proposed for the thermal release of virus RNA in salt solution.

The method of treatment was as follows: A suspension containing the purified TMV at a concentration of about 0.5 mg/ml was drawn into the lower part of a thin-walled glass capillary tube bent into the shape of a U. The tube was dipped into a hot-water bath for a timed interval, and then removed and emptied into a volume of distilled water sufficient to dilute the tube contents about 30-fold. Immediately after this dilution the material was sprayed upon electron-microscope grids and shadowed with uranium.

In Fig. 1 are shown the results of heating TMV in pyrex-distilled water. The same morphological changes occur over the entire temperature range from 80° to 98° (though the rate at which they occur is strongly dependent on temperature). The first visible change is a swelling at one or both ends of the rod. As heating continues the swelling takes the form of a terminal ball which increases in diameter as the attached rod becomes shorter. Finally the rod is completely

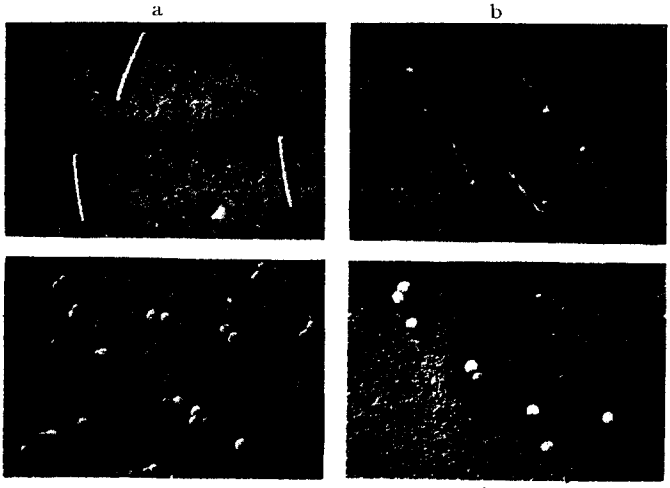

Fig. 1. The effect of heating tobacco mosaic virus in distilled water. Although different temperatures were used for the samples shown here, the entire conversion of virus rods to balls occurs at any of the temperatures used. These representative fields were chosen merely to illustrate stages in the conversion. The larger balls shown in d have approximately the same volume as the original virus rods. × 50,000. (a) untreated; (b) 15 seconds at 85°; (c) 10 seconds at 90°; (d) 10 seconds at 98°.