SOME OBSERVATIONS ON A CELL FREE LIPID SYNTHESIZING SYSTEM FROM *SACCHAROMYCES CEREVISIAE*[1]

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Previous reports (Klein *et al.*, 1954; Klein 1955), have shown that cells of *Saccharomyces cerevisiae*, when grown anaerobically and subsequently aerated, synthesize considerable quantities of cellular lipids from endogenous sources or from added glucose. More recently, it was found that active cell free extracts could be obtained from such cells (Klein and Booher, 1955). Brief communications containing descriptions of some of the properties of this system have appeared (Klein and Booher, 1955, 1956). The purpose of this paper is to report in greater detail some properties of these homogenates. In particular we wish to note the presence of two classes ("large" and "small" particles) of particulate matter and show their relation to lipid formation.

MATERIALS AND METHODS

Organism. *Saccharomyces cerevisiae* strain LK2G12 was used throughout this study. Stock cultures were kept at room temperature on agar slants containing medium I (Klein, 1955).

(1) Treatment of cells prior to breakage:—The growth from one slant was used to inoculate 1.7 L of medium II (Klein, 1955) in a 2-L Erlenmeyer flask. After incubation for 48 hr at 30 C, the cells were harvested, washed twice in ice cold water, and resuspended for aeration as described by Klein *et al.* (1954). Aeration, achieved either on a rotary shaker or by the passage of air through the suspension, was allowed to continue for 2.5 hr. The cells were then harvested, washed twice in ice cold water, and packed by centrifugation. At this stage, the cell paste could be stored at 4 C for several days.

(2) Preparation of active extract:[2]—In a larger mortar (15 cm in diameter) approximately 250 ml of dry ice are finely powdered. To this are added 30 g of small glass beads.[3] A portion of the cell paste is spread over the end of the pestle, which is immediately plunged into the dry ice-glass bead mixture and vigorously ground. The cells quickly freeze and drop off the pestle in the form of flakes. After all the cells have been added to the dry ice glass bead mixture, the contents are ground for about 15 min in order to fragment the broken cells. The resulting mass is allowed to thaw and to it are added 12 to 15 ml of 0.02 M phosphate buffer (pH 7.0–7.5). Two successive centrifugations for 10 min each at 0 C in the International PR-2 centrifuge at 1800 × G serve to remove intact cells and cell walls, yielding a crude homogenate that remains active for several weeks if kept in the frozen state. Microscopic examination of the extracts revealed a mass of isolated particles ranging in size from fragments barely resolvable using the oil immersion objective of a microscope to somewhat larger particles (0.4 μ in diameter). There was little or no clumping of these particles, although occasionally what appeared to be groups of such particles were seen.

(3) Fractionation of crude homogenate:—Studies revealed that, at forces of up to 15,000 × G for 30 min, most of the particulate matter could be sedimented, leaving an opalescent supernatant (supernatant III), which appeared optically empty under the oil immersion objective of a microscope. From supernatant III, a second peak of material sedimented at 25,000 to 60,000 × G, leaving a water-clear supernatant (supernatant IV), usually with a layer of fat at the top.

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[2] Other methods were used in attempts to obtain active homogenates. These included sonic vibration for various periods, grinding with alumina, grinding in a device described by Utter and Werkman (1942), and shaking in the Mickle apparatus. While many of these procedures resulted in considerably better cellular disintegration, the extracts proved to be less active in acetate incorporation than the method given in detail here.

[3] Superbrite glass beads, type 111, obtained from Minnesota Mining Co.

For routine purposes, therefore, supernatant III (free of the "large" particle fraction) was obtained by centrifugation in the high-speed attachment of an International centrifuge at 19,000 × G for 30 min, and supernatant IV (free of the "small" particle fraction) by centrifugation at 100,000 × G for 30 min in a Spinco[4] centrifuge, model L.

Analytical methods. Details concerning the incubation of extracts and their subsequent treatment to obtain non-saponifiable lipids and fatty acids have been presented (Klein, 1955; Klein and Booher, 1955). Radioactive samples with negligible self absorption were plated directly on metal discs and counted in a Tracerlab windowless gas flow counter. The values given in the tables are average figures for duplicate samples and are corrected for background. Total lipids were isolated using a method to be described (Parks and Klein, *unpublished experiments*). Assays for total lipids, total non-saponifiable lipids, total fatty acids, ergosterol and "hydrocarbons" were performed colorimetrically as described earlier (Klein, 1955). Isolation of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) from the two particulate fractions was performed using a modification of the procedure of Ogur and Rosen (1950). Estimation of these polynucleotides as well as of phospholipids was made from their phosphorus content (Fiske and SubbaRow, 1925). These determinations were checked colorimetrically using the orcinol method (Albaum and Umbreit, 1947) for RNA and the procedure of Seibert (1940) for DNA. Protein was determined by the biuret method (Gornall *et al.*, 1949); and polysaccharides were obtained and estimated by a modification of the methods of Trevelyan and Harrison (1951).

(1) Chemicals:—Carboxyl labeled acetate (sodium) was purchased from the Research Specialties Corp., Berkeley, Calif. For experiments on CO_2 fixation into lipids, radioactive barium carbonate, obtained from the Atomic Energy Commission, was converted to sodium bicarbonate. Diphosphopyridine nucleotide (DPN) (95% pure) and triphosphopyridine nucleotide (TPN) (95 to 100% pure) were purchased from Sigma Chemical Co.

[4] We are indebted to Drs. A. F. Brodie, Harvard Medical School, and P. L. Munson, Harvard Dental School, for the use of their centrifuges.

RESULTS

Incorporation of acetate into lipids of homogenate. Previous communications have shown that carboxyl labeled acetate is incorporated in these homogenates into fatty acids and non-saponifiable lipids (Klein and Booher, 1955, 1956). In an attempt to determine more precisely the distribution of radioactivity in the lipids of the crude homogenate, the following experiment was performed. After incubation, the total lipids were removed and made up to a known volume. One aliquot of this was counted and to another was added purified yeast lecithin.[5] The latter was precipitated with acetone, washed, reprecipitated, and counted directly on a steel planchet. A third aliquot was hydrolyzed and the total fatty acids and the non-saponifiable lipids were each made up to known volumes. Portions of these were counted, while to one portion of the non-saponifiable lipids was added purified ergosterol.[5] The latter was precipitated with digitonin, washed several times with ether, and counted. The "hydrocarbons" count was calculated by subtracting the radioactivity of ergosterol[6] from that of the total non-saponifiable lipids. Table 1 presents the results of these determinations, from which it is evident that acetate carbon has been incorporated into lecithin, ergosterol, "hydrocarbons," and probably into neutral fat.

Net synthesis of lipids. In many experiments so much acetate carbon was incorporated into lipids that net synthesis was indicated. Accordingly, larger amounts of extract were incubated with non-labeled acetate, and the total fatty acids and non-saponifiable lipids were measured before and after incubation. In table 2 are recorded analytical data from two such experiments, which included increases of up to 60 per cent under these conditions. Therefore, we are not dealing with preparations that merely reduce acetate molecules and exchange such 2-carbon residues for pre-existing counterparts in the lipids of the homogenates.

[5] Hanahan and Jayko (1952) found dipalmitoleyl-L-α-glycerylphosphorylcholine to be the most abundant phospholipid in *S. cerevisiae*. We are indebted to Dr. Hanahan for his generosity in making available for our use a sample of this compound as well as of purified ergosterol.

[6] It has previously been observed that ergosterol accounts for virtually all the sterol in this strain of yeast (Klein, 1955).

TABLE 1

*Distribution of acetate carbon in lipids of yeast homogenate**

Substance	Cpm × 10^{-4} Incorporated	Per Cent of Total
(1) Total lipids	45.0	100
(2) Total non-saponifiable lipids	18.6	41.4
(3) Total fatty acids	24.4	54.1
(4) Lecithin	16.7	37.1
(5) Ergosterol	5.8	12.8
(6) Hydrocarbons†	12.8	28.5
(7) Neutral fat‡	7.7	17.1

* Total cpm added = 3.34×10^6; 5.0 ml of crude homogenate incubated in air at 30 C for 4 hr with 10 μmoles of adenosine triphosphate and 15 μmoles of acetate. Total volume = 6.0 ml.

† Calculated as (2) − (5).

‡ Calculated as (3) − (4).

TABLE 2

Net synthesis of lipids in yeast homogenates

Experiment	Time	Total NSF*	Total FAF*
	min	*mg*	*mg*
1†	0	2.10	2.85
	240	3.18	4.25
		+1.08	+1.40
2‡	0	1.16	1.25
	240	1.66	2.03
		+0.50	+0.78

* NSF = non-saponifiable lipids; FAF = fatty acids.

† 4.0 ml of crude homogenate (19.3 mg of protein per ml) plus 75 μmoles of K acetate and 30 μmoles of adenosine triphosphate (ATP), incubated in air. Total volume = 4.6 ml.

‡ 5.0 ml of supernatant III (11 mg of protein per ml) plus 125 μmoles of K acetate and 50 μmoles of ATP, incubated in air. Total volume = 6.0 ml.

Effect of CO_2. During the incubation period it was customary to add KOH to the center wells of the "warburg" cups in order to obtain information on the rate of oxygen uptake (Klein and Booher, 1955). When KOH was omitted a significantly greater percentage of counts appeared in the fatty acids than before. Table 3 records the results of experiments in which the CO_2 content was varied. The data show that acetate incorporation appears to be diverted from fatty acids to the non-saponifiable fraction in those experiments in which CO_2 was absorbed from the atmosphere. Thus, by varying this factor, it becomes possible in these extracts to manipulate to some extent the biosynthetic route to be followed by the added acetate. These observations are inconsistent with earlier indications (for example, Maguigan and Walker, 1940; White and Werkman, 1948) that when yeast cells form lipids, the ratio of non-saponifiable lipids to total lipids was essentially fixed for each organism. Secondly, it is clear that these extracts are capable of incorporating acetate into lipids under anaerobic conditions.

In order to test the possibility that the enhancing effect of CO_2 on fatty acid formation might be the result of CO_2 fixation into a short chain acid which subsequently is lengthened by the addition of acetate residues, the following experiment was performed. Labeled sodium bicarbonate was incubated with an active extract in the presence and absence of non-labeled acetate, and the fatty acids and non-saponifiable lipids were assayed for radioactivity. As is seen in table 4, CO_2 fixa-

TABLE 3

Effect of gas phase on incorporation of acetate into lipids of yeast homogenate

Experiment	Gas Phase	Radioactivity Incorporated	
		NSF*	FAF*
		cpm	*cpm*
1	air	1,860	13,350
	air + 5% CO_2	1,740	14,000
	air (no CO_2)†	7,840	3,930
2	air (no CO_2)†	13,650	1,830
	O_2 (no CO_2)†	11,600	2,330
	N_2 (no CO_2)†	300	—
	100% CO_2	1,200	15,450

To 1.0 ml of homogenate was added 10 μmoles of adenosine triphosphate and 10 μmoles of K acetate (1.6×10^6 cpm). Incubation for 4 hr at 30 C. Total volume = 1.2 ml.

* For these designations, see table 2.

† In these cases, CO_2 was removed using 0.2 ml of 20% KOH and filter paper strips in the center wells of the warburg vessels being incubated.

TABLE 4

Incorporation of labeled CO_2 and acetate into lipids of yeast homogenate

Substrate	Total Radioactivity Added	Radioactivity Incorporated	
		NSF*	FAF*
	cpm	*cpm*	*cpm*
Acetate - 1 - C^{14}	5.4×10^5	9,600	23,500
$NaHCO_3$ - C^{14}	1.5×10^6	†	†
$NaHCO_3$ - C^{14} + acetate	1.5×10^6	†	†

In each case, to 1.0 ml of crude homogenate was added 10 μmoles of adenosine triphosphate and 3.2 μmoles of acetate and/or 1.0 μmoles of bicarbonate. Incubation in air for 4 hr at 30 C. Total volume = 1.2 ml.

* For these designations, see table 2.

† Less than 100 cpm.

tion into lipids was not detected. If this process occurs at all in these extracts, it cannot account for the effect observed on fatty acid synthesis. The role of CO_2 remains to be explained satisfactorily. In this respect it should be mentioned that Brady and Gurin (1950) noted similar stimulatory effects of CO_2 on lipid synthesis in liver slices.

Effect of fractionation on acetate incorporation. In a previous communication, it was shown that when all the particulate matter of the homogenate is sedimented and resuspended in phosphate buffer, neither this suspension nor the resulting supernatant (supernatant IV) incorporates acetate into fatty acids or non-saponifiable lipids; only a combination of these two fractions is active (Klein and Booher, 1956). However, if only the large particle fraction is removed by centrifugation, the suspension remains fully active. Table 5 illustrates this point and again demonstrates the inactivity of supernatant IV alone. In other experiments, when the two particulate fractions were separated and resuspended independently in aliquots of supernatant IV, the small particle fraction proved to be the more important in lipogenesis (table 6). The small amount of acetate incorporation in the presence of the large particle fraction may well be the result of contamination of this fraction by small particles.

Electron microscope studies[7] revealed the small particle fraction to contain essentially uniform, roughly spherical particles, 20 to 30 mμ in diameter when shadowed. Figure 1 shows the droplet pattern of a water suspension of this fraction. A corresponding photograph is given of the droplet pattern of a suspension of the total particulate material (figure 2). Figures 3 and 4 are photographs, obtained at greater magnification, of the small particle and total particle fractions, respectively.

We have been unable to obtain good electron micrographs of the large particles. As is apparent from figures 2 and 4, only the small particles seem

[7] The author is indebted to Mrs. Hilda D. Agar, Department of Microbiology, University of Washington, for assistance in obtaining these electron micrographs.

TABLE 5

Effect of removal of large particle fraction on acetate incorporation

Fraction Used	Radioactivity Incorporated	
	NSF*	FAF*
	cpm	*cpm*
Crude homogenate.....	28,500	90,700
Supernatant III.....	27,700	81,700
Supernatant IV.....	†	†

To 1.0 ml of each fraction was added 10 μmoles of adenosine triphosphate and 10 μmoles of K acetate (1.6×10^6 cpm). Incubation in air for 4 hr at 30 C. Total volume = 1.2 ml.

* For these designations, see table 2.

† Less than 100 cpm.

TABLE 6

Incorporation of acetate in the presence of various fractions

Fraction Used	Protein	Radioactivity Incorporated	
		NSF*	FAF*
	mg	*cpm*	*cpm*
Supernatant IV	12.6	—	—
Supernatant IV + large particle fraction	12.6 11.5	660	435
Supernatant IV + small particle fraction	12.6 <2.0	16,850	12,600

To 1.0 ml of supernatant IV were added, as indicated, particulate fractions suspended in 0.1 M phosphate buffer (pH 7.0), 10 μmoles of adenosine triphosphate and 10 μmoles of K acetate (1.6×10^6 cpm). Incubation in air for 4 hr at 30 C. Total volume = 1.5 ml.

* For these designations, see table 2.

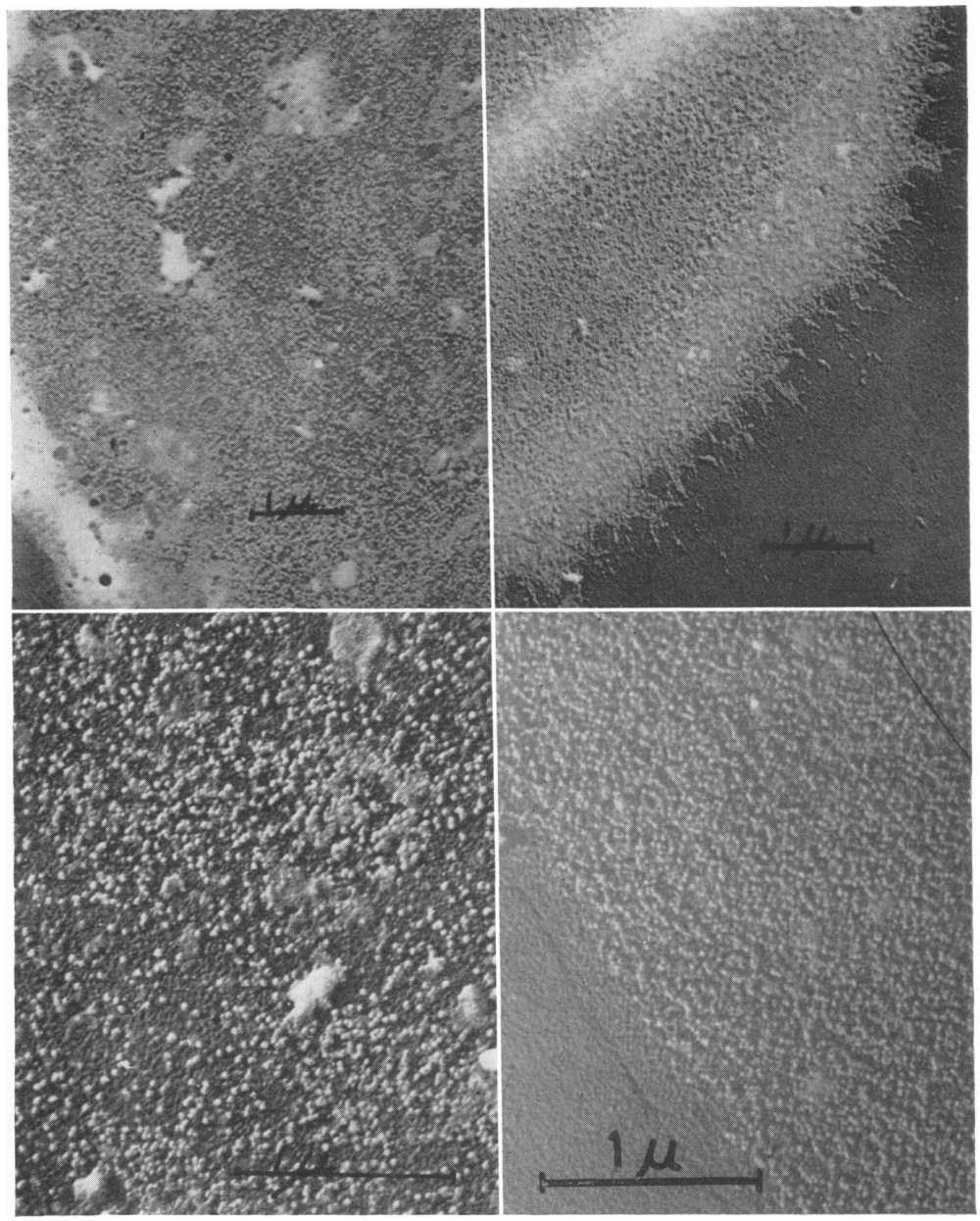

Figure 1 (*above, right*). Edge of droplet of the small particle fraction. For photography, the material in this fraction was resuspended in distilled water and sprayed on formvar coated copper grids with an all glass pressure spray gun. The droplets were then air dried, shadowed with a thin film of palladium at a 1:5 angle, and viewed in an RCA model EMU-2b electron microscope.

Figure 2 (*above, left*). Edge of droplet of the total particulate matter. Procedures similar to those for figure 1, except that shadowing was done at an angle of 1:6.

Figure 3 (*below, right*). Edge of droplet of the small particle fraction. Procedure similar to those for figure 1, except that photographs were taken at greater magnification.

Figure 4 (*below, left*). Portion of droplet of the total particulate matter. Procedures similar to those for figure 2, except that photographs were taken at greater magnification.

to retain any structural integrity during the photographic procedure, which includes a period of air drying. One assumes that if large particles of definite structure are present in the intact cells they are disorganized either during cell breakage and centrifugation or subsequently while the preparations are being dried for microscopy. The former possibility seems less likely, since observations concerning oxygen uptake and cytochrome content would seem to indicate these activities to be concentrated in the large particle fraction, with little or no leakage into supernatant III.

Some properties of the large particle fraction. Removal from the crude homogenate of the large particle fraction resulted in drastic reductions in several activities despite the fact that acetate incorporation remained essentially unchanged. Table 7 presents data on the rate of oxygen uptake. In this experiment, removal of the large particle fraction resulted in a 73 per cent reduction in respiration without affecting acetate uptake into lipids. Further evidence bearing on the localization in this fraction of respiratory activity is given in table 8 which shows that the crude whole homogenate carries out a cyanide sensitive oxidation of *p*-phenylenediamine, and that removal of the large particle fraction results in a loss of this activity. In addition, suspensions of this fraction alone also give this presumptive test for cytochrome oxidase.

Spectroscopic examination at room temperature, using a Zeiss hand spectroscope, of suspensions of the large particle fraction in the presence of a few crystals of sodium hydrosulfite revealed a strong absorption band at 550 to 562 mμ, with weaker bands at 580 to 585 and 525 to 534 mμ.[8] These bands could be seen also in the crude homogenates; but samples of supernatant III, if they showed any absorption at all, revealed only a weak band in the region of 550 to 562 mμ.

TABLE 7

Effect of removal of large particle fraction on oxygen uptake

Fraction Used	Q_{O_2}*	Radioactivity Incorporated in Total Lipids
		cpm
Crude homogenate	8.0	44,000
Supernatant III	2.2	44,400

To 1.0 ml of homogenate or supernatant III were added 10 μmoles of Na succinate, 2 μmoles of $MgCl_2$, 1 mg of diphosphopyridine nucleotide, 0.5 mg of triphosphopyridine nucleotide, 10 μmoles of adenosine triphosphate and 2 μmoles of K acetate (1.6×10^6 cpm). Incubation in air for 4 hr at 30 C. Total volume = 1.2 ml.

* μL of O_2 per hr per mg of protein.

TABLE 8

"Nadi" reactions of various fractions of yeast homogenate

Fraction	0.001 M KCN	Reaction*
Crude homogenate	−	Positive
	+	Negative
Superantant III	−	Negative
	+	Negative
Supernatant IV	−	Negative
	+	Negative
Large particle fraction in 0.02 M phosphate buffer	−	Positive
	+	Negative

* To 0.5-ml fraction (pH 7.2) is added 0.5 ml of 0.5% *p*-phenylene diamine and KCN added as indicated. Positive test = development of blue color within 4 min; negative = no color development within 60 min at 30 C.

On the basis of these properties it is not unreasonable to conclude that the large particle fraction is analogous to mitochondrial preparations. Additional support for this conclusion was obtained through the use of Janus green B (Lazarow and Cooperstein, 1953). This dye, when added to the crude homogenate (final concentration of dye, 1:100,000), became bound to the large particle fraction as shown by its sedimentation with this fraction at 19,000 × G. Furthermore, suspensions of this fraction, but not of supernatant III, rapidly reduced this concentration of dye in the presence of 0.1 M sodium succinate, a property of animal mitochondria previously noted by Potter *et al.* (1951).

Composition of particulate fractions. Chemical analyses were performed on both particulate fractions to ascertain their composition and to see whether any differences could be detected. For

[8] The full cytochrome complement is not present in these cells. When cultivated under aerobic conditions, cells yield a large particle fraction with strong absorption bands at 548 to 555 mμ and at 560 to 565 mμ with weaker bands at 520 to 525, 530 to 535, and 600 to 605 mμ.

TABLE 9

*Composition of particle fractions**

	Small (4 Determinations)	Large (2 Determinations)
Protein	60.00	71.00
Ribonucleic acid (RNA)	7.10	4.40
Deoxyribonucleic acid (DNA)	0.50	0.20
Phospholipid	4.20	13.30
Polysaccharide	0.60	2.00
Ergosterol	0.60	1.70
Protein:RNA	8.40	16.20
Protein:phospholipid	14.30	5.30

* Figures refer to per cent of dry weight.

these determinations, the large particle fraction was washed once in 0.85 per cent NaCl solution, and the small particle fraction once in distilled water. No further purification of these preparations was done. Consequently the analytical data given in table 9 represent chemical information on these fractions *as we have used them in these experiments*. The most interesting differences appear in the RNA and phospholipid figures, the small particle fraction being about twice as rich in RNA as the large particle fraction, on a protein basis, and the large particle fraction almost three times as rich in phospholipid.

DISCUSSION

It is apparent that the system described is suitable for use in studies on the detailed mechanisms involved in lipid synthesis. Certainly the enzymatic equipment for the formation of a variety of these substances must be present. One interesting question that arises is whether these preparations synthesize ergosterol under anaerobic conditions. Bucher and coworkers (*personal communication*) observed that oxygen was a requisite for the formation of cholesterol by liver homogenates, and Bloch and his collaborators (Tchen and Bloch, 1956) showed that molecular oxygen is necessary in this process in the formation of the 3-hydroxy group, which is found also in ergosterol. The yeast preparations discussed in this paper clearly incorporate acetate into the non-saponifiable fraction under anaerobic conditions. If oxygen is involved in the final stages of ergosterol synthesis, the non-saponifiable matter produced in the absence of air may be a ready source of ergosterol precursors.

These studies appear to prove that the large particle fraction—necessary for maximal respiration, and containing cytochromes and cytochrome oxidase—is not primarily involved in lipogenesis. This pattern is analogous to that found by Bucher's group (Bucher *et al.*, 1954; Bucher and McGarrahan, 1955) for the rat liver system. However, it should be pointed out that our experiments do not preclude the possibility that this fraction is active *in vivo*. It is possible that the method of cell disintegration results in the loss or inactivation of key enzymes or coenzymes from these larger structures. This contention is somewhat weakened by the fact that addition of soluble supernatant to this fraction does not restore the capacity to form lipids. Furthermore, whatever is supplied by the small particles evidently survives the disintegration procedures. Nevertheless, it must be noted that in a recent report Corwin *et al.* (1956) found virtually all the lipogenic activity of a yeast homogenate in a particulate fraction obtained at 11,500 × G, quite in contrast to the results given here. These two contradicting results may be reconciled if future studies show the small particles to be aggregated in the intact cell under certain conditions. In this regard we have encountered, on a few occasions, homogenates which, when fractionated, lost all activity upon removal of the large particle fraction. But in these cases, the resulting supernatant (supernatant III) when centrifuged for 30 min at 100,000 × G *gave no small particle pellet* (*unpublished experiments*).

Concerning the relationship between the two particle fractions, there exists the possibility that the small particles represent fragments of the larger structures. In this case, one would expect to find in electron photographs considerable variation in the size of the particles. Also, differential centrifugation studies should reveal a gradation of sedimentable matter. Neither of these conditions is found; the photographs reveal uniform small particles (figures 2 and 4) and the particulate matter sediments in two peaks. Furthermore, the analytical data show certain differences in chemical composition. These findings therefore make it highly unlikely that the small particles are fragments of larger ones.

SUMMARY

Methods were given for the preparation of homogenates of *Saccharomyces cerevisiae* capable of incorporating acetate-1-C^{14} into fatty acids, ergosterol, lecithin, hydrocarbons and, probably, neutral fat. When CO_2 was absorbed from the atmosphere, such extracts incorporated significantly less acetate into fatty acids than in air, and higher counts appeared in the non-saponifiable lipids. Using non-labeled acetate, it was found that these extracts carry out net synthesis of fatty acids and non-saponifiable lipids.

Fractionation of the homogenate revealed the small particle fraction to be more active in lipogenesis in this system than the large particle fraction. The latter is necessary for maximal respiration of these extracts, and appears to contain cytochrome pigments and cytochrome oxidase.

Chemical analysis of the two particulate fractions showed major differences in their content of phospholipid and ribonucleic acid.

REFERENCES

ALBAUM, H. G. AND UMBREIT, W. W. 1947 Differentiation between ribose-3-phosphate and ribose-5-phosphate by means of the orcinol-pentose reaction. J. Biol. Chem., **167,** 369–376.

BRADY, R. O. AND GURIN, S. 1950 The biosynthesis of radioactive acids and cholesterol. J. Biol. Chem., **186,** 461–469.

BUCHER, N. L. R., GROVER, J. W., AND KINGSTON, R. 1954 Incorporation of C^{14} from acetate into cholesterol by liver cell fractions. Federation Proc., **13,** 19–20.

BUCHER, N. L. R. AND MCGARRAHAN, K. 1955 Rat Liver microsomes in cholesterol biosynthesis. Federation Proc., **14,** 187.

CORWIN, L. M., SCHROEDER, L. J., AND MCCULLOUGH, W. G. 1956 Studies of lipid synthesis in cell-free yeast extracts. Federation Proc., **15,** 512–513.

FISKE, C. H. AND SUBBAROW, Y. 1925 The colorimetric determination of phosphorus. J. Biol. Chem., **66,** 375–400.

GORNALL, A. G., BARDAWILL, C. J., AND DAVID, M. M. 1949 Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., **177,** 751–766.

HANAHAN, D. J. AND JAYKO, M. E. 1952 The isolation of dipalmitoleyl-L-α-glycerylphosphorylcholine from yeast. A new route to (dipalmitoyl)-L-α-lecithin. J. Am. Chem. Soc., **74,** 5070–5073.

KLEIN, H. P., EATON, N. R., AND MURPHY, J. C. 1954 Net synthesis of sterols in resting cells of *Saccharomyces cerevisiae*. Biochim. et Biophys. Acta, **13,** 591.

KLEIN, H. P. 1955 Synthesis of lipids in resting cells of *Saccharomyces cerevisiae*. J. Bacteriol., **69,** 620–627.

KLEIN, H. P. AND BOOHER, Z. K. 1955 Synthesis of lipids in cell-free extracts of yeast. Proc. Soc. Exptl. Biol. Med., **89,** 43–44.

KLEIN, H. P. AND BOOHER, Z. K. 1956 A particulate fraction of yeast and its relation to lipid synthesis. Biochim. et Biophys. Acta, **20,** 387–388.

LAZAROW, A. AND COOPERSTEIN, S. J. 1953 Studies on the mechanism of Janus Green B staining of mitochondria. I. Review of the literature. Exptl. Cell Research, **5,** 56–69.

MAGUIGAN, W. H. AND WALKER, E. 1940 Sterol metabolism of micro-organisms. I. Yeast. Biochem. J. (London), **34,** 804–813.

OGUR, M. AND ROSEN, G. 1950 The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. Arch. Biochem., **25,** 262–276.

POTTER, V. R., RECKNAGEL, R. O., AND HURLBERT, R. B. 1951 Intracellular enzyme distribution; interpretation and significance. Federation Proc., **10,** 646–653.

SEIBERT, F. B. 1940 Removal of the impurities, nucleic acid and polysaccharide, from tuberculin protein. J. Biol. Chem., **133,** 593–604.

TCHEN, T. T. AND BLOCH, K. 1956 Role of oxygen and reduced pyridine nucleotides in the biogenesis of cholesterol. Abstr. Am. Chem. Soc., 130th Meeting, 56C-57C.

TREVELYAN, W. E. AND HARRISON, J. S. 1951 Studies on Yeast metabolism. I. Fractionation and microdetermination of cell carbohydrates. Biochem. J. (London), **50,** 298–303.

UTTER, M. F. AND WERKMAN, C. H. 1942 Dissimilation of phosphoglyceric acid by *Escherichia coli*. Biochem. J. (London), **36,** 485–493.

WHITE, A. G. C. AND WERKMAN, C. H. 1948 Fat synthesis in yeast. Arch. Biochem., **17,** 475–482.