showed no impurities; infrared analysis proved that no *trans* isomer was present. I.V. 90.5 (theor. 89.9); acid value 198.7 (theor. 198.6); $n^{26/D} = 1.4585$ [Lit. $n^{20/D} = 1.4585$, 1.4599 (1)].

Preparation of Derivatives. Methyl oleate was prepared from our oleic acid (99+%, undistilled), and distilled at 168–170°C./2 mm. to give a clear, colorless product. The gas chromatogram showed no impurities. I.V. 85.8 (theor. 85.6); $n^{26/D} = 1.4510$, Lit. $n^{20/D} = 1.4522$ (1).

Oleoyl chloride was prepared by refluxing undistilled oleic acid, in dry benzene, with oxalyl chloride (11). The crude material was distilled at 169–170°C./4 mm. to give a clear, colorless product in 87% yield. Infrared analysis revealed no trace of oleic acid or other contaminants.

Reduction with lithium aluminum hydride of the methyl oleate made from oleic acid (both undistilled) gave oleyl alcohol in quantitative yield (12). The undistilled product had a saponification value of zero and an I.V. of 93.2 (theor. 94.5). When analyzed in the gas chromatograph as the acetate, no impurities were detected.

Summary

Oleic acid of 99–100% purity has been prepared in 36–43% yield from olive oil. The combination of two urea-adduct separations (at room temperature) and three acid soap crystallizations (at 3°C.) gives an oleic acid of high quality without recourse to fractional distillation or low-temperature solvent crystallization.

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REFERENCES

1. "Biochemical Preparations," vol. 2, pp. 100–104, John Wiley and Sons Inc., New York, 1952.
2. Schlenk, H., "Urea Inclusion Compounds of Fatty Acids," ch. 5 in vol. 2 of "Progress in the Chemistry of Fats and Other Lipids," Academic Press Inc., New York, 1954.
3. Schlenk, H., and Holman, R. T., J. Am. Chem. Soc., *72*, 5001–5004 (1950).
4. Swern, Daniel, and Parker, W. E., J. Am. Oil Chemists' Soc., *29*, 614–615 (1952).
5. Smull, J. G. (National Lead Co.), U. S. 2,858,324 (1958).
6. Meade, E. M., private communication.
7. Kairys, S., Meade, E. M., Munns, W. O., and Walder, D. A. (Canada Packers Limited), U. S. 2,895,976 (1959).
8. Ralston, A. W., "Fatty Acids and Their Derivatives," pp. 888–891, John Wiley and Sons Inc., New York, 1948.
9. Keppler, J. G., Sparreboom, S., Stroink, J. B. A., and von Mikusch, J. D., J. Am. Oil Chemists' Soc., *36*, 308–309 (1959).
10. Craig, B. M., and Murty, N. L., Can. J. Chem., *36*, 1297–1301 (1958).
11. Adams, R., and Uhlich, L. H., J. Am. Chem. Soc., *42*, 599–611 (1920).
12. Amos, A. A., and Ziegler, P., private communication.

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The Enzymatic Hydrolysis and Tissue Oxidation of Fatty Acid Esters of Sucrose[1]

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FATTY ACID ESTERS of sucrose have been employed (1) as emulsifiers for the oral administration of fat to dogs and humans. It was subsequently found that the equivalent of as much as 100 g. of fat as the sucrose fatty acid ester in a synthetic diet could be orally administered without the expected elevation of plasma turbidity or increase in the amount of fat excreted in feces (2). In an extension of these studies, humans were maintained for short periods on this material as the sole source of dietary lipid. In an attempt to determine whether some unusual mode of absorption of sucrose fatty acid might be involved, various modes of enzymic attack were studied. Quastel (3) reported that sucrose monostearate was hydrolyzed to glucose and fructose by surviving intestine at one-fourth the rate of sucrose hydrolysis; and York, Finchler, Osipow, and Snell (4) reported the hydrolysis of sucrose monolaurate by fructo-invertase. However Bourne (5) was unable to demonstrate hydrolysis of sucrose monostearate by α-amylase or by gluco- or fructo-invertase. Sucrose fatty acid esters were found by Isaac and Jenkins (6) to be capable of supporting oxidation by sewage.

The present communication describes the effect of lipase, invertase, and liver and pancreatic extracts on various commercial preparations of sucrose fatty acid esters and the oxidation of these preparations by homogenates of liver and intestinal mucosa.

Experimental

Material. Sucrose fatty acid esters used were "Sequol 260"[2] (22% palmitic acid, 3.4% stearic acid, 22% oleic acid, 47% linoleic acid); sucrose monopalmitate[3] (89% palmitic acid, 4.3% stearic acid); sucrose monostearate A[4] (42% palmitic acid, 44% stearic acid, 5.4% oleic acid); sucrose monostearate B[5] (39% palmitic acid, 53% stearic acid, 3% oleic acid); sucrose di-,[6] tri-,[7] and tetralinoleate[8]; and the transesterification product[9] of sucrose and safflower oil.

Procedure. A 1% solution of each sucrose fatty acid ester was made up with 20 ml. 95% ethanol and 80 ml. glycerol. Each incubation vessel contained 50 μmoles sucrose ester, 70 μmoles Tris(hydroxymethyl)-aminomethane buffered at pH 8.1, 50 μmoles sodium taurocholate or sodium glycocholate, 100 μg. enzyme

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[2] Supplied by the Charles Pfizer Company and prepared as the transesterification product of sucrose and cottonseed oil.
[3] Sucrodet D-600, Berkeley Chemical Company, Lot No. S-187.
[4] Ottawa Chemical Company, Batch No. 7255 (8/10/55).
[5] Foster D. Snell, Batch No. 596.
[6] Colonial Sugars Company (7-L-184/5-22-59).
[7] Colonial Sugars Company (3-L-176/2-10-59).
[8] Colonial Sugars Company (5-L-175/5-22-59).
[9] Colonial Sugars Company (6-L-183/5-22-59).

in 0.1 ml. The total volume was 2.5 ml. In some tubes 50 μmoles Hormel triolein were present. Sources of enzymes were Viokase pancreatin (Viobin Laboratories), lipase (steapsin) and wheat germ lipase (Nutritional Biochemicals Corporation), invertase (Mann Biochemicals), α-amylase (Pentex Biochemicals), dog pancreatic juice from a jejunal fistula. Rat liver and intestinal mucosa were prepared as 10% (w/v) homogenates of frozen-dried tissue in 0.25M sucrose, containing 0.001M ethylene diamine tetracetic acid neutralized at pH 8.0. Incubations were carried out at 37°C. for 60 min. with 0.25-ml. samples being taken at 10-min. intervals for determination of ester groups by the method of Lipmann and Tuttle (7). Where reducing groups were determined, the method of Nelson (8) was used. After stopping the reaction by immersing the reaction vessel in boiling water, the reaction products were subjected to paper chromatography in the nitromethane/n-butanol/water-solvent system of Chargaff, Levine, and Green (9) and the color reagent of Horrocks and Manning (10). Control tubes contained no enzyme. In manometric experiments the flask center wells contained 0.2 ml. 30% CO_2-free KOH, and the side arm contained the enzyme.

Results

Figure 1 shows the results of studies of hydrolysis of "Sequol 260" by various enzyme preparations. Hydrolysis of the fatty ester bond by the liver homogenate was almost complete in 60 min. There was no detectable release of reducing groups by the liver preparation in this period of time. Substantial ester hydrolytic activity was noted with α-amylase although there was no release of reducing groups with this preparation. The ester hydrolytic activities of pancreatic juice, pancreatic lipase, Viokase (pancreatic), and invertase were all comparable and yielded between 23 and 33% hydrolysis in 60 min. The release of reducing groups by Viokase, pancreatic juice, and invertase was between 3 and 6% of the total available. Ester hydrolytic activity by wheat germ lipase was somewhat less than that of pancreatic lipase and yielded only 17% hydrolysis in 60 min. Paper chromatography in two solvents of the hydrolysis products of sucrose monostearate by pancreatic juice revealed sucrose as the only carbohydrate product.

Table I shows the effects of some cofactors and inhibitors on ester bond hydrolysis of "Sequol 260" by various enzyme preparations. An interesting difference is shown in the response of pancreatic and wheat germ lipase to the omission of taurocholate. Very little inhibition of the hydrolysis of "Sequol 260" because of the omission of taurocholate was noted with wheat germ lipase. However the hydrolysis of "Sequol 260" by pancreatic lipase was almost completely inhibited by omission of taurocholate.

TABLE I

The Effect of Cofactors and Inhibitors on Ester Bond Hydrolysis of "Sequol 260"

	% Available Ester Groups Hydrolyzed/60 min.				
	Wheat germ lipase	Pancreatic lipase	Viokase	Liver	Pancreatic juice
"Sequol 260"	16.8	26.5	29.0	98.0	35.5
"Sequol 260" taurocholate omitted	13.6	0.8	.....	.....	.....
Triolein	68.5	53.5	19.9	28.9	9.4
Triolein + "Sequol 260"	56.3	40.7	36.6	46.4	17.5
Sucrose + "Sequol 260"	.....	.....	.....	101.6	32.4
10^{-4}M TEPP + "Sequol 260"	.....	.....	.....	.....	35.2
3.7×10^{-2}M Cu^{++} + "Sequol 260"	.....	.....	.....	.....	96.6

It is suggested that taurocholate may exert more influence in altering the physical state of the enzyme than in affecting the substrate. Glycocholate was only about 55% as effective as taurocholate.

The presence of triolein appears to be inhibitory to the ester bond hydrolysis of "Sequol 260" by all enzyme preparations tested. Since the sum of ester bonds of "Sequol 260" and of triolein hydrolyzed is greater than the ester bonds hydrolyzed when both are present together, it appears that there may be competition for the active centers on the enzyme in question.

The presence of sucrose in the medium did not inhibit ester bond hydrolysis of "Sequol 260" by pancreatic juice or liver homogenates.

The presence of 10^{-4}M tetraethyl pyrophosphate did not inhibit ester bond hydrolysis, and 3.7×10^{-2}M cupric ions produced an activation of ester bond hydrolysis by pancreatic juice. These findings tend to rule out hydrolysis by pancreatic esterases or proesterases such as those described by Gjessing, Emery, and Clements (11).

Figure 2 shows the ester bond hydrolysis by pancreatic lipase of different preparations of sucrose fatty acid esters. It may be seen that the greatest hydrolytic activity is noted with sucrose trilinoleate, followed by the sucrose di- and tetralinoleates. The distribution

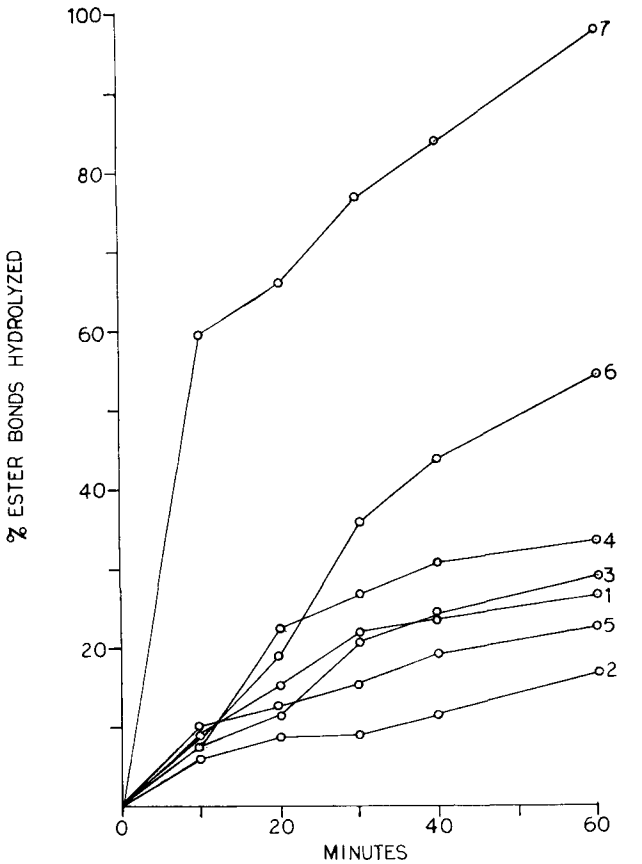

FIG. 1. Hydrolysis of "Sequol-260" by the following enzyme preparations: 1, pancreatic lipase; 2, wheat germ lipase; 3, Viokase; 4, pancreatic juice; 5, invertase; 6, α-amylase; 7, liver homogenate.

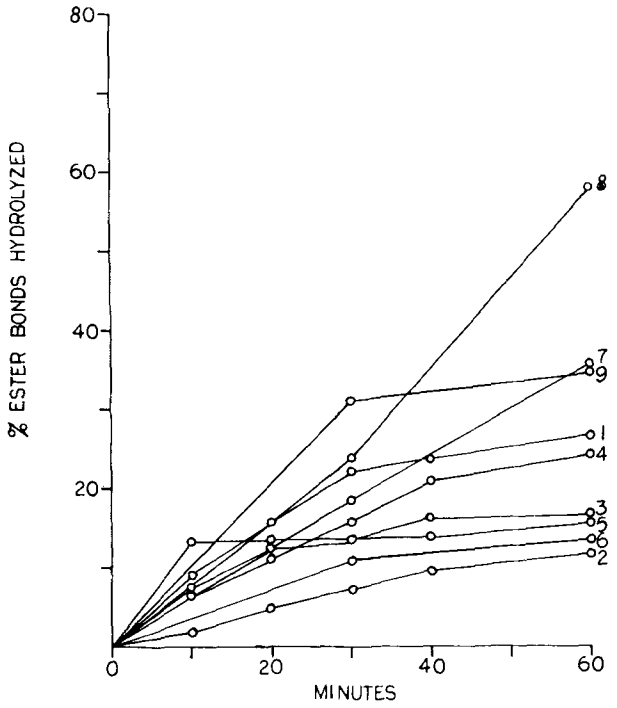

FIG. 2. Hydrolysis by pancreatic lipase of the following sucrose fatty acid esters: 1, "Sequol 260"; 2, sucrose monostearate A; 3, sucrose monostearate B; 4, recrystallized sucrose monostearate B; 5, sucrose monopalmitate; 6, sucrose-safflower oil transesterification product; 7, sucrose dilinoleate; 8, sucrose trilinoleate; 9, sucrose tetralinoleate.

of fatty acids on these preparations is uncertain. They may be arranged on the trilinoleate in such a way that the molecule more closely resembles a triglyceride than do the di- or tetralinoleates. The greater activity of pancreatic lipase on unsaturated fatty acid sucrose esters than on esters with saturated fatty acids is in agreement with the observations of Gomori (12) and Ravin and Seligman (13). The hydrolysis of some of the sucrose monostearate preparations by pancreatic lipase is less active than that of the sucrose linoleate preparations. It would have been expected that sucrose monopalmitate hydrolysis would have been more active than that of sucrose monostearate. However all of the sucrose ester preparations tested except "Sequol 260" and the sucrose linoleates contained appreciable proportions of residual solvents. The inhibitory action of residual solvents on pancreatic lipase activity is partially revealed by comparing the difference in activity between the sucrose monostearate B (Curve 3) and the recrystallized sucrose monostearate B (curve 4), in which much of the solvent was removed.

Since it was not demonstrated that the sucrose fatty acid esters were cleaved at the glycosidic linkage by intestinal or pancreatic enzymes, their oxidation by intestinal mucosa was considered as a possible mechanism involved in absorption. Figure 3 shows the oxygen consumption of mucosal tissue as determined with various substrates. The values plotted represent the difference between that obtained with the sucrose ester and sucrose plus the corresponding fatty acids. The best oxygen uptake measured would only account for 0.09% of the substrate or 0.1% of the fatty acid. It may be seen that "Sequol 260" was the best substrate, closely followed by the sucrose linoleate preparations. The other sucrose monostearate preparations may offer some inhibition of their own oxidation because of the presence of residual solvent.

Figure 4 shows a similar study of oxygen consumption by liver tissue with various substrates. The pattern is considerably different from that of intestinal mucosa. Much greater oxidation was noted, but there was three times as much oxidation with "Sequol 260" and sucrose dilinoleate as with any other substrate. The least oxidation occurred with those preparations containing the most residual solvent.

Discussion

Pancreatic lipase appeared to be the principal enzyme occurring in the normal digestive process which exhibited activity with the sucrose fatty acid esters as substrates. No appreciable hydrolysis of the glycosidic linkage occurred since there was no detectable release of reducing groups nor were there any glucose or fructose esters found by paper chromatography. The presence of the fatty acid may have sterically hindered the attack on the glycosidic linkage.

The best hydrolytic activity was obtained with esters having a high preponderance of unsaturated fatty acids. The assessment of hydrolytic activity may have been partially obscured by transesterification reactions. However addition of free fatty acids and glycerol to the systems did not result in an inhibition of hydrolysis or in net increase in the amount of ester bonds present.

Since cupric ions and tetra-ethyl pyrophosphate were not inhibitory to hydrolysis, there was probably little esterase activity contributing to the hydrolysis of sucrose esters. The stimulation of hydrolysis observed with cupric ions may have been caused by the removal of reaction products by the formation of copper soaps of the liberated fatty acids.

Oxidation of the sucrose fatty acid esters by both intestinal mucosa and liver homogenates was greater than that of sucrose and the corresponding fatty acid alone. Although this may have resulted from a primary gradual release of sucrose and fatty acid by hydrolytic enzymes at the site of oxidation, the extent of oxidation was considerably less than the extent of hydrolysis. In addition, the oxidative activity with

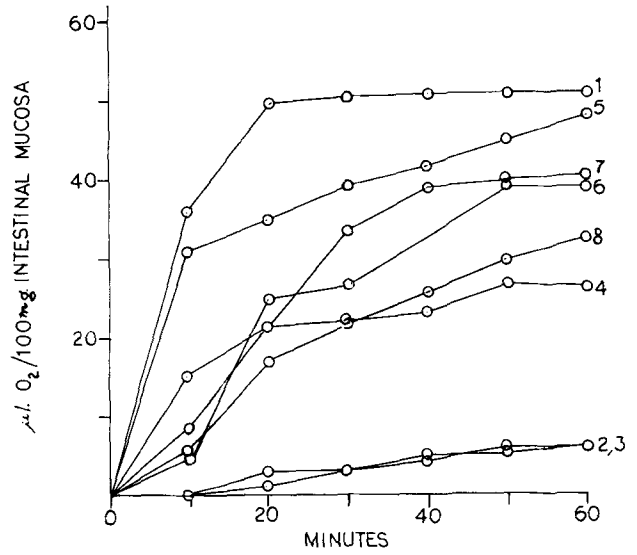

FIG. 3. Oxygen uptake by rat intestinal mucosa with the following sucrose fatty acid esters: 1, "Sequol 260"; 2, sucrose monostearate B; 3, recrystallized sucrose monostearate B; 4, sucrose monostearate A; 5, sucrose dilinoleate; 6, sucrose trilinoleate; 7, sucrose tetralinoleate; 8, sucrose-safflower oil transesterification product.

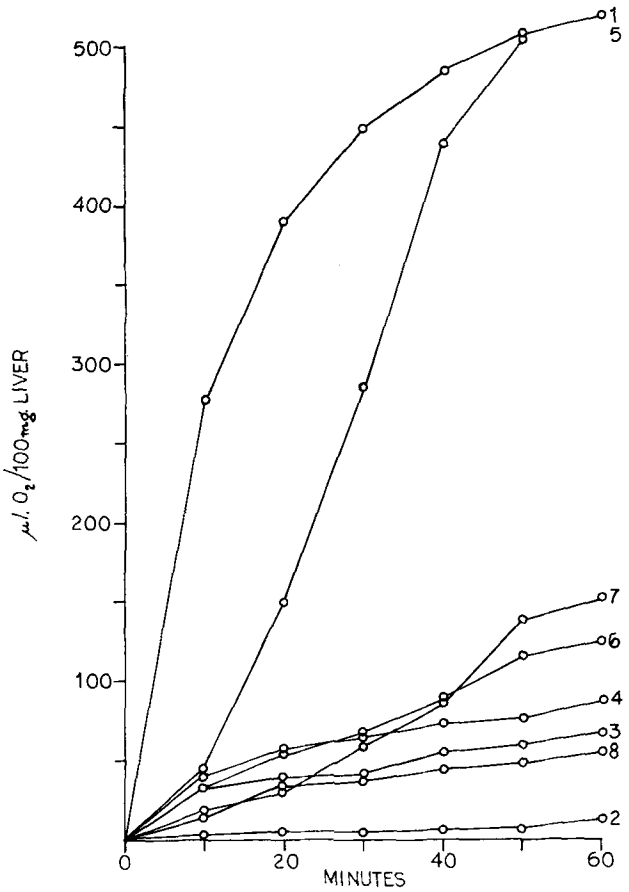

FIG. 4. Oxygen uptake by rat liver homogenates with the following sucrose fatty acid esters: 1, "Sequol 260"; 2, sucrose monostearate B; 3, recrystallized sucrose monostearate B; 4, sucrose monostearate A; 5, sucrose dilinoleate; 6, sucrose trilinoleate; 7, sucrose tetralinoleate; 8, sucrose-safflower oil transesterification product.

various substrates did not correspond to the hydrolytic activity of these substrates with lipase.

The results reported offer little explanation for the intestinal absorption of fatty acid esters without a corresponding increase in serum turbidity on the basis of a hydrolytic mechanism. Thus it will probably be necessary to seek for a physico-chemical or physiological mechanism for this phenomenon.

Summary

Various preparations of sucrose fatty acid esters were hydrolyzed by wheat germ or pancreatic lipase, pancreatin, pancreatic juice, α-amylase, invertase, or liver homogenates to yield sucrose and free fatty acids as products. The greatest activity was observed with the liver homogenate.

None of the enzymes studied cleaved the glycosidic linkage as indicated by the lack of appearance of reducing groups and by paper chromatography of the products.

The greatest hydrolysis by pancreatic lipase was observed with sucrose esters having a greater preponderance of unsaturated fatty acids, namely, sucrose trilinoleate, sucrose dilinoleate, sucrose tetralinoleate, and "Sequol 260" (69% unsaturated fatty acids).

Sodium taurocholate was required for hydrolysis by pancreatic lipase but not by wheat germ lipase. Sucrose ester was inhibitory to the hydrolysis of triolein by all lipolytic preparations. Tetra-ethyl pyrophosphate and cupric ions were not inhibitory to the hydrolysis of sucrose ester.

Sucrose fatty acid esters supported respiration by rat liver homogenates and to a much lesser extent by rat intestinal mucosa. The rate of oxidation was greater than that observed with sucrose and the corresponding fatty acid. The greatest activity was observed with esters of fatty acids having a greater preponderance of unsaturated fatty acids, namely, "Sequol 260," sucrose di-, tri-, and tetralinoleate.

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REFERENCES

1. Turner, D. A., Am. J. Digestive Dis., *3*, 682–707, 594–640 (1958).
2. Turner, D. A., progress reports to the Sugar Research Foundation on Project No. 109.
3. Quastel, J. H., unpublished data.
4. York, W. C., Finchler, A., Osipow, L., Snell, F. D., J. Am. Oil Chemists' Soc., *33*, 424–426 (1956).
5. Bourne, E. J., progress reports to the Sugar Research Foundation on Project 125.
6. Isaac, P. C. G., and Jenkins, D., Chemistry and Industry, 976–977 (1958).
7. Lipmann, F., and Tuttle, L. C., Biochim. Biophys. Acta, *4*, 301–309 (1950).
8. Nelson, N., J. Biol. Chem., *153*, 375–380 (1944).
9. Chargaff, E., Levine, G., and Green, C., J. Biol. Chem., *175*, 67–71 (1948).
10. Horrocks, R. H., and Manning, G. B., Lancet, *256*, 1042–1045 (1949).
11. Gjessing, E. C., Emery, R., and Clements, J. P., J. Biol. Chem., *234*, 1098–1101 (1959).
12. Gomori, G., Proc. Soc. Exptl. Biol. Med., *72*, 697–700 (1949).
13. Ravin, H. A., and Seligman, A. M., Arch. Biochem. Biophys., *42*, 337–354 (1953).

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Relationship Between the Apparent Bulk Density of a Bleaching Clay and Its Oil Retention

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OIL RETENTION of bleaching clay is an important economic factor that is often ignored when calculating the comparative cost of clays. The principal reason is difficulty in obtaining an accurate plant oil-retention figure, and thus in many comparative-cost evaluations between two clays the economics are based upon bleaching power and laid-down clay cost alone. A more complete comparison should include consideration of oil retention as well (2).

The importance of oil retention may be realized by the fact that with oil at 10¢ per pound, every 1% difference in oil retention between two clays, clay-as-