must be discussed in as much the measured volume of the biliary tree in phenobarbital-treated rats represent the true values of the biliary tree. Using various dye-markers (BSP, rose bengal, indocyanin green) SICOT et al.[12], showed that the values of the biliary tree volume may differ to some extent. Thus they are lower with BSP than with rose bengal and still lower with indocyanin green. Therefore the measured values of the biliary tree in these experiments are only valuable for BSP as dye-marker. Beside these differences in biliary tree volume according to different dye-markers, the biliary tree volume is also dependent of the bile flow and the dye-transit time through the hepatocyte. Thus an increased bile flow will result in an overestimation of the biliary tree volume when the dye-marker transit time remains unchanged, as is the case if the choleresis is increased by sodium dehydrocholate infusion. In our experiments such an overestimation may have been compensated by a quicker hepatic transport of BSP as a consequence of an increase of Y-protein (REYES et al.[11]).

Zusammenfassung. Nach Vorbehandlung von Ratten mit Phenobarbital (60 mg/kg, 4 bis 6 Tage lang) zeigten Gallefluss und Lebergewicht eine signifikante Zunahme. Die Absolutwerte des Gallengangsvolumens blieben dabei unbeeinflusst, während seine Relativwerte signifikant absanken.

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17 May 1972.

[12] C. SICOT, A. RICHARD and J.-P. BENHAMON, Revue fr. Études clin. biol. *13*, 270 (1968).

The Effect of Lipase Activity on the Fat Content of *Staphylococcus aureus*

The presence of a lipolytic enzyme in *Staphylococcus aureus* was described as early as 1901[1] and since then has drawn the attention of various investigators[2,3]. As pointed out by SMITH and WILLET[4], normal human skin has an abundance of lipophylic bacteria, among them *Staphylococcus aureus*. These bacteria are able to split various triglycerides, phospholipids and Tween (Polyoxysorbitan-fatty acid). FRITSCHE[5] showed that fatty acids from the media are incorporated into the bacteria, thereby increasing their fat content.

Various investigators[6,7] found a correlation between the resistance of the bacteria to antibiotics and its fat content. In order to clarify the role of the lipase on the lipoidal material, 2 strains of *Staphylococcus aureus*, 1 Tween 80 positive and the other Tween 80 negative, were used. They were grown on various media, and the quantity of lipids was compared.

Materials and methods. Staphylococcus aureus 111 was isolated from clinical material. The bacteria did not hydrolyze Tween 80 (polyoxisorbitan monoleate) and was designated Tween 80 negative (Tw). This strain was streaked on a Sierra agar plate[8] containing Tween 80, and incubated for 4 days at 37°C. Following this incubation period lipolytic activity could be detected by the appearance of a precipitate of calcium oleate around some of the colonies. From these colonies the Tween 80 positive strain was isolated. The two strains were identical in all of the biochemical properties tested with the exception of their lipolytic activity.

Table I. Lipolytic activity of *Staphylococcus aureus* (111) on various substrates.

Substrate	Tween 80 positive strain	Tween 80 negative strain
Glycerol tributyrate	+	+
Glycerol trilaurate	+	+
Glycerol trimyrisitate	+	+
Glycerol tripalmitate	+	±
Glycerol tristearate	+	−
Glycerol trioleate	+	−
Glycerol trilinoleate	+	−
Tween[a] 20(-monolaurate)	+	+
Tween[a] 40(-monopalmitate)	+	±
Tween[a] 60 (monostearate)	+	−
Tween[a] 80 (monooleate)	+	−
Tween[a] 85 (trioleate)	+	−

[a] Polyoxylethylene sorbitan.

Lipolytic activity on triglycerides was detected using Spirit blue agar (DIFCO)[4]. Triglycerides (Sigma Chem. Co, St. Louis, Mo.) were incorporated into this sterile medium at a concentration of 1% as following. The fat was emulsified into the medium by an ultrasonic waver (Branson Sonifier model S-125) and plate containing the emulsified fat media were poured. The two strains were streaked and incubated for 2 days at 37°C. Lipolysis was indicated by a clear zone in the emulsion around the colonies. Lipolytic activity against Tween as a substrate was examined on Sierra agar plates containing various Tweens. Lipolysis was indicated by a heavy precipitation of calcium fatty acid salt in the vicinity of the colonies after 2 days of incubation at 37°C.

The cell lipids were extracted from bacteria grown in the various media at 37°C for 48 h in a New Brunswick controller environment shaker (200 rpm). They were harvested by centrifugation, washed twice with distilled water, and lyophilized. The lipids were extracted with chloroform methanol 2:1 for 3 h[9]. The extract was filtered using a sintered glass funnel No. 4 and the residue reextracted again. The pooled filtrates were dried by evaporating under a stream of N_2 and in vacuum over P_2O_5. The dry lipid fraction was expressed as % of the total dry weight of the bacteria.

Glycerol oleate 1-^{14}C or oleic acid 1-^{14}C (Radiochemical Center Amersham, England) were incorporated into Tween 20 and added to nutrient broth as indicated in Table III. The two strains of *Staphylococcus aureus* were grown on the two media for 48 h in a New Brunswick controlled environmental incubator shaker (200 rpm). The bacteria were harvested by centrifugation, washed

[1] C. EIGKMAN, Zentbl. Bakt I *29*, 841 (1901).
[2] G. T. STEWART, Ann. N. Y. Acad. Sci. *128*, 132 (1965).
[3] W. M. O'LEARY and J. T. WELD, J. Bact. *88*, 1356 (1964).
[4] R. F. SMITH and N. P. WILLELT, J. gen. Microbiol. *52*, 441 (1968).
[5] D. FRITZSCHE, Zentbl. Bakt. Hyg. I Abt. Orig. A. *217*, 483 (1971).
[6] J. K. DUNNICK and W. M. O'LEARY, J. Bact. *101*, 892 (1970).
[7] W. B. HUGO and R. G. STRENTON, J. gen. Microbiol. *42*, 133 (1966).
[8] G. SIERRA, Antonie van Leeuwenhoek *23*, 15 (1957).
[9] S. G. WILKINSON, Biochim. biophys. Acta *164*, 148 (1968).

Table II. Lipids in cells of *Staphylococcus aureus* after growth on various media

Medium of growth	% of lipids calculated for the dry weight of the bacteria	
	Tween 80 positive strain	Tween 80 negative strain
Nutrient broth	5.88 ± 1.29	5.29 ± 1.1
Nutrient broth + 1% Tween 20 [a]	14.28 ± 0.38	13.37 ± 0.17
Nutrient broth + 1% Tween 80 [b]	13.16 ± 0.70	8.37 ± 2.50

[a] Polyoxyethylene sorbitan monolaurate. [b] Polyoxyethylene sorbitan monooleate.

Table III. Incorporation of Oleic acid 1-C^{14} by *Staphylococcus aureus* grown on various media

Nutrient broth supplied with Tween 20 containing	Strain	Radioactivity dpm/ml of medium	Radioactivity (dpm/ml washed bacterial suspension)	Radioactivity uptake by the bacteria (%)
Glycerole trioleate 1-C^{14}	Tween positive	21485	2799	13
	Tween negative	22908	199	0.8
Oleic acid 1-C^{14}	Tween positive	29029	6876	23.6
	Tween negative	30950	7057	22.8

twice with *tris* buffer pH 7.5 and resuspended to original volume. For determination of the radioactivity, samples of 0.5 ml of the washed bacteria were solubilized by adding 0.5 ml of a 3% solution of butanol and transfered to scintillation vials. As controls, 0.5 ml samples of uninoculated media were used. 10 ml of a scintillation mixture (1 g 1.4 *bis* (5phenyl Oxazolyl-2benzene, 500 g methanol and 100 ethylene glycol) were added to each and the radioactivity was determined in a Packard Tri Carb Scintillation Spectrophotometer.

Results. The lipolytic activity of the Tween 80 positive and Tween 80 negative strains of *Staphylococcus aureus* were compared by using 7 different triglycerides and 5 different tweens as substrates. The results illustrated in Table I show that the enzyme produced by the Tween 80 positive strain hydrolyzed all the substrates tested. On the other hand, the Tween 80 negative strain was able to hydrolyze only the substrates containing shorter fatty acids up to 16 carbons. There were no differences in the ability of the various strains to split the fatty acids, whether they bound to glycerol or to polyoxysorbitan. In equal small amounts of lipids were found when the two strains were grown on media lacking lipids (Table II).

When the strains grew on the same media supplemented with Tween 20, which is hydrolyzed by both strains, there was a significant increase in the extractable fat without differences between the two strains. However, when the media was supplemented with Tween 80 which is hydrolyzed only by the Tween 80 positive strain, a significant increase in the fat extracted from this bacteria was observed. A low increase in the amount of fat was found in the Tween 80 negative strain and may be contributed to the contamination of the Tween 80 with free fatty acids[10].

There were no significant differences in the incorporation of oleic acid 1-C^{14} into the two strains of the bacteria when grown on media containing this fatty acid. However, when the bacteria were grown on a media containing glycerol trioleate 1-C^{14} there was a profound difference (of 1:20) in the amount of radioactivity measured in the two strains. This experiment suggested that once the fatty acid was available there were no differences in uptake between the two strains.

Discussion. Differences in the ability of various strains of the same bacterium to split various Tweens were shown by GONZALES[11] in the case of *Hallobacterium*. Our work with *Staphylococcus aureus* shows that the greater ability of one strain, Tween 80 positive, to hydrolyze lipids is related only to a small number of lipids, while other ones containing short and medium (up to 16) chain fatty acids could be split by both strains. As to the possible physiological role of the staphylococcal lipase our work shows that the bacteria utilized the free fatty acids resulting from the enzyme action for increasing their fat content. Such a mechanism for increasing fat content of the bacteria could contribute to higher resistance to deteriorating agents as was shown for phenol and antibiotics[12]. The use of labelling compounds suggests that there are no differences in the uptake of fatty acids by the two strains of *Staphylococcus aureus*.

Résumé. Deux souches de *Staphylococcus aureus*, l'un positif pour Tween 80 et l'autre négatif pour Tween 80 ont la propriété de décomposer des substrates lipidiques. Tous deux peuvent libérer des esthers d'acides gras contenant 16 atomes de carbone (longs et courts). Après l'action du *Staphylococcus aureus* positif pour Tween 80, des esthers contenant des longs acides gras sont aussi libérés. Dans ces conditions, la quantité totale de lipide bactérien devient plus élevée. Ces deux staphyloques incorporent de la même manière les acides gras libérés.

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Department of Life Sciences, Bar-Ilan University, Ramat-Gan (Israel), 25 May 1972.

[10] M. W. STINSON and M. SOLOTROVSKI, Bact. Proc. *20*, 83 (1969).
[11] C. GONZALEZ and C. GUTIEREZ, Can. J. Microbiol. *16*, 1165 (1970).
[12] W. B. HUGO and I. FRANKLIN, J. gen. Microbiol. *52*, 365 (1968).