Effect of Phenobarbital on the Volume of the Biliary Tree in the Rat

Chronic administration of phenobarbital to the rat has several effects on the liver; it increases the weight of the liver (KLAASEN[1], KLAASEN and PLAA[2], ROBERTS and PLAA[3]), causes a proliferation of the smooth endoplasmic reticulum (REMMER and MERKER[4]), and stimulates some enzymes involved in the metabolism of drugs (BARKA and POPPER[5], REMMER[6], KAPPAS and SONG[7]). Furthermore the bile flow increases (BERTHELOT et al.[8], PAUMGARTNER et al.[9]).

With respect to these effects of phenobarbital, it should also be of interest to know whether the biliary tree volume is influenced under these conditions. The present study was designed to answer this question.

Methods. Male Wistar rats weighing 200–400 g were maintained on a standard diet (Altromin) with water, and divided in 2 equal groups of 10 rats. The rats were given orally phenobarbital sodium 60 mg/kg (Merck, Darmstadt) or saline once daily for 4–6 days. 20 h after the last phenobarbital dose the animals were anaesthetized with urethane (1.25 g/kg i.p.). The common bile duct was cannulated. Body temperature was maintained between 36.5–37.5°C by placing the animals into a thermostat. The capacity of the biliary tree was measured by the method described by BARBER-RILEY[10] using BSP as dye-marker. At the end of the experiments the rats were sacrificed, the livers removed and the dry and wet weight determined. The data were analyzed using a group comparison Student's *t*-test; $P < 0.05$ was considered significant.

Results. Table I demonstrates the wet and dry weight of the liver in rats pretreated with phenobarbital (60 mg/kg daily for 4–6 days) in comparison with the controls. The wet and dry weight of the liver increased to 38 and 56%. This increase was highly significant ($P < 0.001$). The phenobarbital-treated rats had also a significantly greater bile flow than the controls, 71.1 vs. 50.3 µl/100 g rat/10 min. In contrast to the enlargement of the liver, the volume of the biliary tree calculated for 100 g body weight did not change after pretreatment with phenobarbital. These two effects of phenobarbital, enlargement of the liver on the one side and an unchanged absolute biliary tree volume on the other side, also explain that the relative biliary tree volume calculated for one gram liver wet or dry weight decreased significantly (Table II).

Discussion. Phenobarbital administration, under these experimental conditions, induced an increase in wet weight of the liver which was 38% and not far from that of the bile flow which was 41%. The dry weight of the liver increased more than the wet weight, 56 vs. 38%. This may be explained by a hypertrophy of the liver cells and an accelerated production of liver protein. In this connection an increased synthesis of Y-protein is now discussed (REYES et al.[11]). The absolute biliary tree volume, calculated for 100 g rat, using the method of BARBER-RILEY[10] and BSP as dye marker was interestingly not changed in phenobarbital-treated rats. The relative biliary tree volume, calculated for 1 g wet or dry liver weight decreased significantly as the liver weight was increased by phenobarbital. In this connection the question

[1] C. D. KLAASEN, J. Pharmac. exp. Ther. *168*, 218 (1969).
[2] C. D. KLAASEN and G. L. PLAA, J. Pharmac. exp. Ther. *161*, 361 (1968).
[3] R. J. ROBERTS and G. L. PLAA, Biochem. Pharmac. *16*, 827 (1967).
[4] H. REMMER and H. J. MERKER, Ann. N. Y. Acad. Sci. *123*, 79 (1965).
[5] T. BARKA and H. POPPER, Medicine *46*, 103 (1967).
[6] H. REMMER, Dt. med. Wschr. *92*, 2001 (1967).
[7] A. KAPPAS and C. S. SONG, Gastroenterology *55*, 731 (1968).
[8] P. BERTHELOT, S. ERLINGER, D. DHUMEAUX and A. M. PREAUX, Am. J. Physiol. *219*, 809 (1970).
[9] G. PAUMGARTNER, W. HORAK, P. PROBST and G. GRABNER, Naunyn-Schmiedebergs Arch. Pharmak. *270*, 98 (1971).
[10] G. BARBER-RILEY, Am. J. Physiol. *205*, 1122 (1963).
[11] H. REYES, A. J. LEVI, Z. GATHMAITAN and I. M. ARIAS, Proc. natn. Acad. Sci., USA *64*, 168 (1969).

Table I. Effect of chronically administered phenobarbital on wet and dry weight of the liver in the rat

	Wet weight of the liver (g/100 g rat) $\bar{x} \pm s$	Increase (%)	Dry weight of the liver (g/100 g rat) $\bar{x} \pm s$	Increase (%)
Controls $N = 10$	3.46 ± 0.29	–	1.03 ± 0.15	–
Phenobarbital group $N = 10$	4.79 ± 0.37	+38	1.61 ± 0.11	+56
P	< 0.001		< 0.001	

$\bar{x} \pm s$; mean value and standard deviation.

Table II. Effect of chronically administered phenobarbital on biliary tree volume in rats

	Volume of the biliary tree (µl/100 g rat) $\bar{x} \pm s$	Volume of the biliary tree (µl/g wet weight of the liver) $\bar{x} \pm s$	Volume of the biliary tree (µl/g dry weight of the liver) $\bar{x} \pm s$
Controls $N = 10$	19.3 ± 1.55	5.70 ± 0.40	19.8 ± 1.41
Phenobarbital group $N = 10$	19.5 ± 1.63	4.12 ± 0.31	12.1 ± 0.69
P	0.8 not significant	< 0.001	< 0.001

$\bar{x} \pm s$; mean value and standard deviation.

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).