for *S. aureus*. In contrast chlorhexidine, benzalkonium chloride and EDTA in the presence of methyl cellulose and polyvinyl alcohol in a wetting solution did not sterilize either organism within 24 h.

Only four solutions containing thiomersal sterilized both organisms in less than 24 h; chlorhexidine was present in two of these and chlorbutol in the third. Besides being slow acting preservatives, the mercurials were reported by Brown (1968) and Richards & Reary (1972) to be inhibited by EDTA, yet all eleven solutions containing thiomersal contained EDTA.

It appears that there is a large variation in the antibacterial activity of commercial contact lens solutions. Although these results were obtained using high inocula of resistant organisms, it would seem prudent to require contact lens solutions to comply with standard challenge tests.

Department of Pharmacy,
Heriot-Watt University,
79 Grassmarket,
Edinburgh EH1 2HJ.

R. J. McBride
Mary A. L. Mackie

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The production of extracellular lipids by *Pseudomonas aeruginosa* NCTC 2000 in stationary liquid media containing macrogols

Lipids are not normally found among the products that accumulate in the growth medium when cells of *Pseudomonas aeruginosa* NCTC 2000 are grown in stationary nutrient broth culture. When small amounts of magrogols were incorporated in the media some lipid material was obtained from the cell-free culture using an extraction procedure with n-butanol. The amounts of lipid material partitioning into the organic phase were determined and the lipid classes identified.

The cells were grown at 37° for five days in 1 litre flasks containing 100 ml of Oxoid nutrient broth No. 2 to which was added 1% w/v of macrogols. The media became viscous after five days due to slime production. The cells and slime were removed by homogenization, centrifugation and precipitation of the slime by the addition of cold ethanol (Brown, Foster & Clamp, 1969). The lipid material was recovered by partition into n-butanol (Bradley & Khan, 1972).

The amount of extracellular lipid obtained was estimated by comparison with a series of calibration curves prepared with palmitic acid, triolein and a pseudomonas glycolipid, using the method of Amenta (1970). The lipid material was separated into two fractions, glycolipids and neutral lipids by separation chromatography on thin layers of silica gel G (HF 254 + 366 Merck) using development with solvent I (diethyl ether–benzene–ethanol–glacial acetic acid: 40:50:2:0·2 v/v) followed by drying and redevelopment in the same direction with the solvent II (diethyl ether–hexane; 6:94 v/v) (Freeman & West, 1966). The more polar glycolipids remained at

the origin whilst the neutral lipids migrated. These were: monoglycerides, diglycerides, triglycerides, free fatty acids and steryl esters. The use of solvent III (chloroform–acetone–water, 30:60: 2 v/v) separated the glycolipids with spots corresponding to digalactosyl diglyceride, steryl glucoside and ceramide monohexoside.

The more highly polar glycolipids were resolved with solvent IV (chloroform–methanol–ammonia 30% w/v–water, 65:30:5:2·5 v/v) which separated the following spots: digalactosyl diglyceride, trigalactosyl diglyceride, steryl glucoside, ceramide monohexoside and ceramide dihexoside. The regions of the silica gel containing the adsorbed glycolipids were carefully collected, mixed with the acidified dichromate reagent and the colour developed under standard conditions was determined at 430 nm (Amenta, 1970). The amount of glycolipid material present in the chloroform solution loaded on the chromatoplates was estimated by reference to the glycolipid standard after adjustment for absorption resulting from the silica gel adsorbent.

The glycolipid material was estimated in terms of the palmatic acid and triolein reference standards also, and by subtracting these values, which gave similar results, from the values obtained for total lipid materials, the amount of neutral lipid material was obtained.

The macrogols were also extracted and as they interfered with the determination of lipids procedure, they were removed by washing from an ethereal solution of the lipids with acidified water. The amounts of lipid obtained are shown in Table 1.

The yield of extracellular lipids from the media containing the macrogols is small for magrogol 300 to negligible quantities for cetomacrogol 1000 and Carbowax 1540. The amounts being produced decline as the molecular weight of the macrogols increases and this was correlated with the intensity of growth. *Ps. aeruginosa* NCTC does not grow well under conditions of oxygen starvation encountered in stationary culture over five days and both lipid and slime are produced according to environmental conditions. The optimum conditions for extracellular lipid production under stationary culture occur after 48 h growth in the presence of molecules of molecular weight 200–300 containing carbon and oxygen (Bradley & Khan, 1974).

When the media containing the macrogols was enriched with glucose the amounts of lipid material obtained increased to 1·5 times in the case of macrogol 300, 8 times with cetomacrogol 1000 and 6 times with Carbowax 1540 that produced when glucose alone was present.

With glucose-macrogol 300 the additional lipid probably resulted from metabolism of the macrogol. Nobel & Savin (1966) reported the multiplication of *Pseudomonas aeruginosa* in creams containing cetomacrogol with the inference that the organism is utilising this agent.

Table 1. *Extracellular lipids produced by* P. aeruginosa *NCTC 2000 grown in stationary liquid media for 5 days at 37°.*

Nutrient broth medium containing:	Total lipids (mg litre^{-1})	Glycolipids (mg litre^{-1})	*Neutral lipids (mg litre^{-1})
1% Glucose..	500	300	200
1% Macrogol 300	300	150	150
1% Cetomacrogol 1000	120	50	70
1% Carbowax 1540	20	very low	very low
1% Macrogol 300 +1% glucose ..	750	400	350
1% Cetomacrogol 1000 +1% glucose ..	4100	1500	2600
1% Carbowax 1540 +1% glucose ..	2800	1200	1600

* Obtained by subtracting the value for glycolipid from the total lipid value.

With the glucose-cetomacrogol 1000 and glucose-Carbowax 1540 combinations where the lipid yields increased six- to eight-fold, the amount of slime produced fell from about 1100 mg litre^{-1} in the glucose medium to about 800 mg litre^{-1} in the glucose-cetomacrogol 1000 medium and to about 500 mg litre^{-1} in the glucose-Carbowax 1540 medium.

These results suggest a possible two stage reaction. Since the cetomacrogol 1000 and Carbowax 1540 molecules are too large for normal cell uptake and metabolism as shown by low yields of lipid, the first lipid is produced from the glucose metabolism and this aids the transport of these large molecules into the cell for subsequent metabolism.

The non-ionic surface-active properties of the cetomacrogol 1000 and Carbowax 1540 may possibly enhance the growth stimulating properties of the early glycolipid production from the glucose metabolism. This glycolipid has surface-active properties (Hisatsuka, Nakahara & others, 1971) and in combination with those of the cetomacrogol 1000 and Carbowax 1540 could react with the lipids being produced and prevent a possible product feed-back inhibition.

An alternative explanation is based on peroxide formation by cetomacrogols. Autoxidation of glycols and peroxidation of the ethylene oxide groups of cetomacrogols in aqueous solution has been shown to occur (Lloyd, 1963; Azaz, Donbrow & Hamburger, 1973). Peroxides in the presence of glucose, a reducing substance, would release molecular oxygen into solution. In the static cultures used here this would improve the supply of oxygen to growing cells, a factor known to be important in lipid formation (Bradley & Khan, 1974).

Department of Pharmacy,
Manchester Royal Infirmary,
Oxford Road,
Manchester M13 9WL, U.K.

T. J. BRADLEY
N. H. KHAN

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