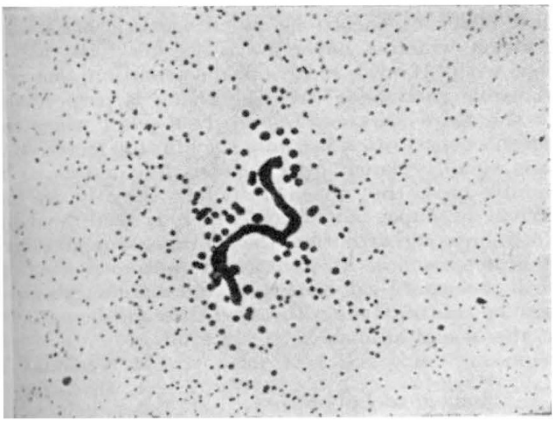
Unsaturated Fatty Acids in Cotton Wool Plugs

DURING a study of a diphtheroid bacterium (not yet identified, but temporarily labelled *Q*) it has been found that the slight growth on nutrient agar can be abolished by adding 0·2 per cent charcoal to the agar. Growth on charcoal-agar could be restored by adding excess of oleic, linoleic or linolenic acids. Experiments in synthetic media showed that the organism requires these unsaturated fatty acids as growth factors.

It was noted that 'satellitism' of colonies of *Q* occurred on charcoal-agar plates around a fibre of cotton wool accidentally dropped on the surface (see accompanying illustration). It appeared that the cotton fibre was supplying the necessary fatty acid growth-factor. 20 gm. of the ordinary non-absorbent cotton wool used in the laboratory for plugging all sterile glassware were therefore extracted for 3 hr. in a soxhlet with methanol, yielding 24·7 mgm. (more than 0·12 per cent) of petrol-soluble lipoid.



GROWTH STIMULATION, BY A COTTON FIBRE, OF MICRO-COLONIES OF A BACTERIUM (*Q*) REQUIRING OLEIC ACID; MEDIUM NUTRIENT AGAR CONTAINING 0·2 PER CENT CHARCOAL. × 25

This phenomenon was at once linked with the observation that a greasy film developed on the inside of flasks and tubes, previously acid-cleaned, plugged with cotton wool and sterilized by treatment for 1½ hr. in the oven at 180°. This greasy film, which was itself invisible, was only detected because it rendered the surface of the glass completely hydrophobic. It did not appear when plugs made of the extracted cotton wool were used. The bacteriostatic properties of the fatty distillate from cotton wool plugs has previously been reported by Wright[1] for pneumococci and by Drea[2] for tubercle bacilli, but the importance of their observations has not perhaps been fully appreciated.

When the fat-extracted cotton wool was used for plugging flasks in experiments with *Q*, the organism completely failed to grow in the absence of oleate; whereas previously, with ordinary plugs, quite considerable, though variable, growth had occurred in the basal synthetic medium alone.

It was thus possible, using *Q* as the test organism, to make a rough assay of the growth-promoting activity of the fatty extract from cotton wool, which was found to be 25–50 per cent that of oleic acid. (I should be mentioned here that pure linoleic and linolenic acids have exactly the same growth-promoting activity for *Q* as oleic acid, while the two saturated acids, palmitic and stearic, are quite inactive.) It was calculated that each cotton wool plug contained 0·5–1·0 mgm. of oleate-equivalent lipoid. Further, by measuring the average growth of *Q* in 5 ml. of basal medium without oleate in 50 ml. flasks plugged with ordinary cotton wool and sterilized in the usual way by hot air at 180° for 1½ hr., the oleate-equivalent quantity of lipoid distilled off the plugs and afterwards dissolved in the medium amounted to about 10 μgm. per flask. The same figure was arrived at by assaying the activity of petroleum-ether washings from samples of dry flasks, similarly plugged and sterilized. This amount was quite enough to give considerable growth of *Q*, which responds visibly to a concentration of 0·5 μgm. oleic acid per ml., and also to cause marked inhibition of organisms, such as *H. pertussis*, which are highly sensitive to unsaturated fatty acids. A strain of *H. pertussis* was found to grow heavily in broth with 0·01 per cent albumen in flasks with extracted cotton wool plugs, but failed completely to grow in the same medium in flasks with ordinary plugs.

Long-chain unsaturated fatty acids such as oleic, linoleic or linolenic (which may be derived from cotton wool in its natural state or after washing with soap solutions) have remarkable activity both as growth-promoters[3–7] and growth-inhibitors[2,3,8,9] of a wide variety of micro-organisms, down to a concentration of 1 μgm./ml. or less. These substances may thus be responsible for erratic results in growth experiments on sensitive micro-organisms where cotton wool is used for plugging tubes afterwards sterilized in an oven at high temperatures. To avoid this difficulty, Wright[1] suggested using a particular grade of absorbent cotton wool, and sterilizing by autoclave followed by drying at 120°. The fatty acid content of absorbent cotton wool, however, depends largely upon its method of preparation and may be higher than that of the non-absorbent variety. It is clearly preferable to attempt to get rid of the easily liberated fat by extraction with an organic solvent such as methanol (as described) or, even better, to use some substitute such as glass, 'Cellophane' or aluminium caps as recommended by Drea[2]. A more promising substitute which is being tested here at the moment is a very fine glass wool (Fibreglass, Ltd.). This is unfortunately treated with a mixture containing oleic acid in the process of manufacture, but can easily be subjected to fat-extraction and/or treated with concentrated acid and so obtained as chemically clean as the flasks themselves.

M. R. POLLOCK
(Leverhulme Research Fellow)
Medical Research Council,
Unit for Bacterial Chemistry,
Lister Institute,
London, S.W.1.
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