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# LIPOLYSIS BY HUMAN SKIN SURFACE DEBRIS IN ORGANIC SOLVENTS*

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## ABSTRACT

Human skin surface debris collected from ether extracts of the scalp catalysed the hydrolysis of triolein, tristearin and methyl stearate, but not cholesteryl stearate or cetyl palmitate, when suspended in ether or hexane solutions of these lipids. The activity was slowly destroyed by heating at 120° C, but was not inhibited by addition of tetracycline.

Human skin surface lipids contain free fatty acids, apparently liberated from esters by lipases present either in the follicular epithelium, the micro-flora, or in the epidermis. There has been much interest in the origin of the free acids, since the production of free fatty acids in the follicular canal has been postulated as an important factor in the pathogenesis of acne vulgaris (1, 2). In this connection, it has been shown that freshly-synthesized human sebum does not contain free acids (4), and Reisner et al found that the constituent triglycerides can be hydrolyzed by pure cultures of *Corynebacterium acnes* (5), the principle organism present in the follicle.

Human skin surface lipids collected by extraction with ether normally contain 20–50% of triglycerides. We have observed that such extracts, containing epidermal debris, may become devoid of triglycerides when stored for long periods, even at −20° C. We now report that the sediment recovered from scalp extracts contains a lipase which is specific for triglycerides, is not readily denatured by heat, and is not inhibited by tetracycline.

### MATERIALS AND METHODS

*Collection of the surface debris.* Extracts were obtained from adult males by immersion of the scalp in a basin of ether. Prior to the initial extraction, the subjects had shampooed and rinsed thoroughly, and were prohibited from using hair dressings during the period of the collection.

The sediment was collected on a sintered glass filter, washed several times with ether, and allowed to dry on the filter before being collected and stored at −20° C.

*Assessment of lipolytic activity.* Since it appeared that the hydrolysis of esters was occurring in ether suspension, this unusual medium was investigated further. The substrates included pure samples of triolein, tristearin, cholesterol oleate (from Applied Science Laboratories), and cetyl palmitate, chosen to represent the triglycerides, cholesterol esters, and wax esters which occur in human skin surface lipid. Substrates (5 to 10 mg) in 5 ml of ether were added to weighed amounts of skin surface debris (25 or 50 mg), and shaken at room temperature in test tubes with Teflon-lined screw-caps. The time course of the reactions was studied by withdrawing 0.1 ml aliquots of supernatant solution at intervals. The aliquots were evaporated to dryness and the lipid residues stored at −20° C until analyzed.

For analysis, the lipid samples were dissolved in 0.2 ml of hexane and 3 $\mu$l of the solution was spotted on 6 mm lanes ruled in standard 20 × 20 cm thin-layer plates coated with a 250 $\mu$-thick layer of silica gel G, which had been developed in ether to remove lipid contamination and then activated by heating at 120° C for 30 minutes. The plates were developed in three successive solvent systems (hexane; benzene; hexane:ether:acetic acid, 70:30:1), as described previously (6, 7). The resolved lipids were then charred by spraying with 50% sulfuric acid and heating to 220° C. The chromatograms were quantitated by scanning with a photodensitometer (Photovolt Corp, model 530) attached to a stripchart recorder (Varicord model 42-B). Degree of lipolysis was assessed from the relative areas of the peaks of the hydrolysis products and starting material (5).

To support the assumption that the reaction is enzymic, incubations of triolein were performed without addition of surface debris, as well as after addition of the powder and immediate removal by centrifugation. Triolein, with and without debris, was also incubated after flushing the tubes with nitrogen, saturation of the ether with water, or heating the active powder at 100 or 120° C for

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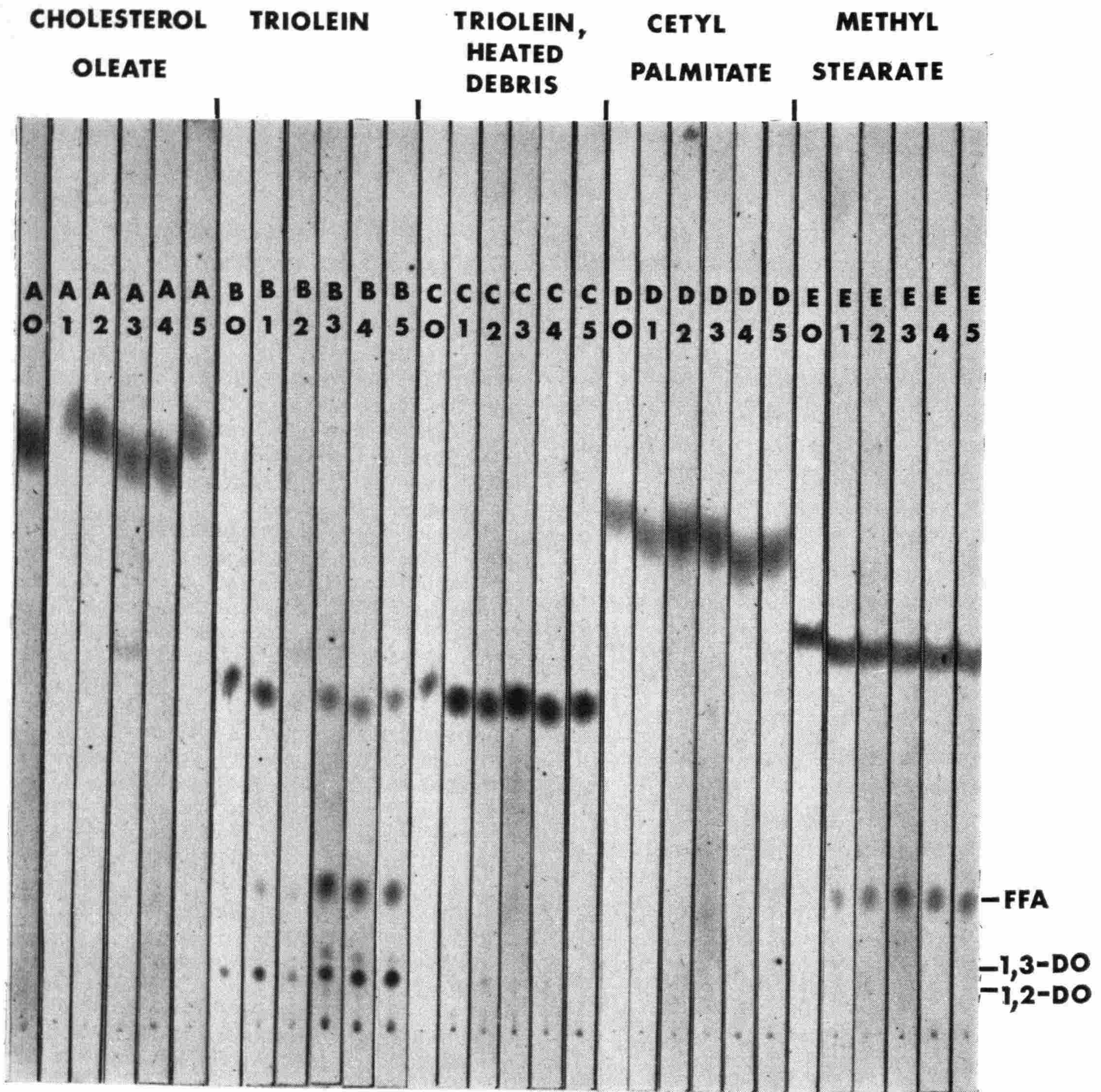


FIG. 1. Appearance of charred thin-layer chromatograms of lipids subjected to the lipolytic action of skin surface debris in ether. Across: A, cholesterol oleate; B, triolein, C, triolein with debris heated at 120° C; D, cetyl palmitate; E, methyl stereate. Numbers refer to days of incubation. Down: FFA: free fatty acids; 1,3-DO: 1,3-diolein; 1,2-DO: 1,2-diolein.

varying lengths of time. The effect of tetracycline base was assessed in incubations in which the ether medium contained 1 mg/ml of the antibiotic.

Several incubations of triolein were performed in which ether was replaced by hexane as the medium.

## RESULTS

Figure 1 shows the appearance of a typical series of chromatograms. From this it is apparent that the lipolytic activity was limited to triglycerides and methyl esters. Quantitation of the chromatograms allowed the rate of reactions to be followed. Typical rate curves for the hydrolysis of triolein in ether are shown in Figure 2a. Rather more diglyceride was produced than free fatty acid. Figure 2b shows the rate curves for hydrolysis of triolein in hexane, where the concentration of diglyceride produced always remained at a low level. In both of these cases, 50 mg of the epidermal debris was used. When a high concentration of tetracycline was included in an incubation in ether, the rate of hydrolysis was similar in incuba-

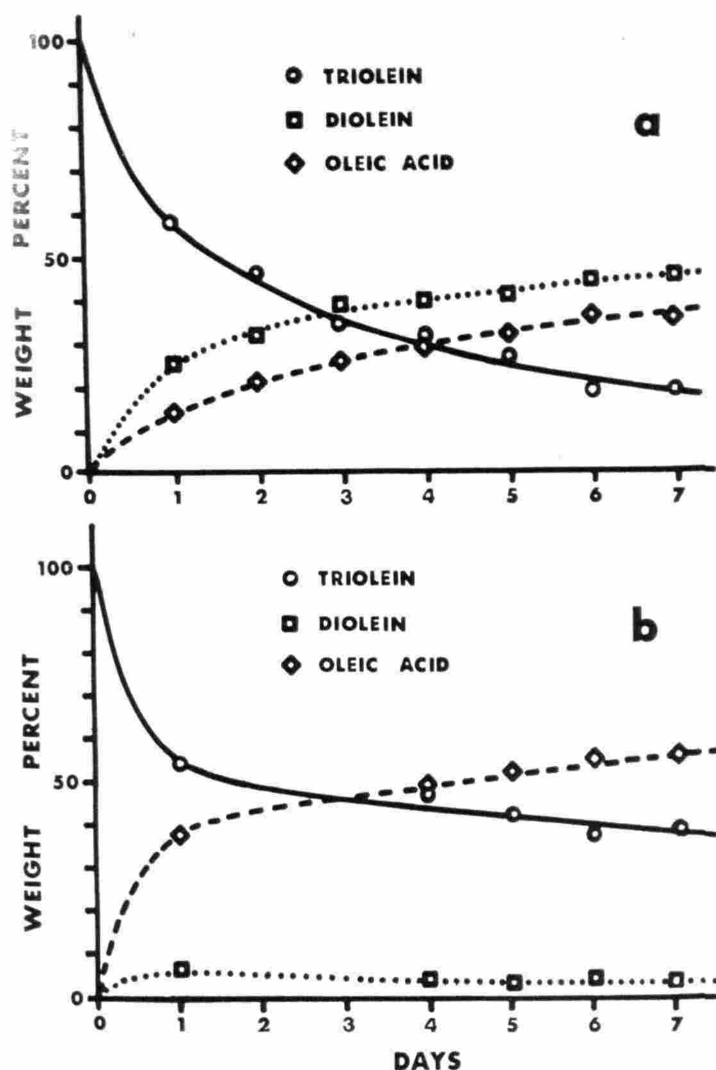


FIG. 2. Rate curves for conversion of 5 mg of triolein to oleic acid and diolein by 50 mg of epidermal debris: (a), in 5 ml of ether, and (b), in 5 ml of hexane.

tions, both with and without antibiotic, containing 25 mg of epidermal debris (Fig. 3).

The observations which have been made regarding the enzymic activity under the conditions studied may be summarized as follows:

i) The enzyme was active when suspended in ether, and to a lesser extent when dispersed in hexane.

ii) The activity was only slowly destroyed when the air-dried powder was heated at 100° C in a sealed tube and most of the activity was retained after three hours. Heating for two hours at 120° C completely eliminated activity.

iii) In ether, triolein and tristearin were converted principally to free fatty acids and 1,2-diglycerides, with only traces of 1,3 diglycerides and monoglyceride. Methyl stearate was slowly attacked. Cholesterol stearate and cetyl palmitate were unchanged. In hexane, the triglycerides were converted principally to free fatty acids, with only a small proportion of diglycerides and traces of monoglycerides accumulating.

iv) Apparently water present in the air-dried skin surface debris or the "anhydrous" ether allowed the hydrolysis to proceed. Under the conditions used, sufficient water for

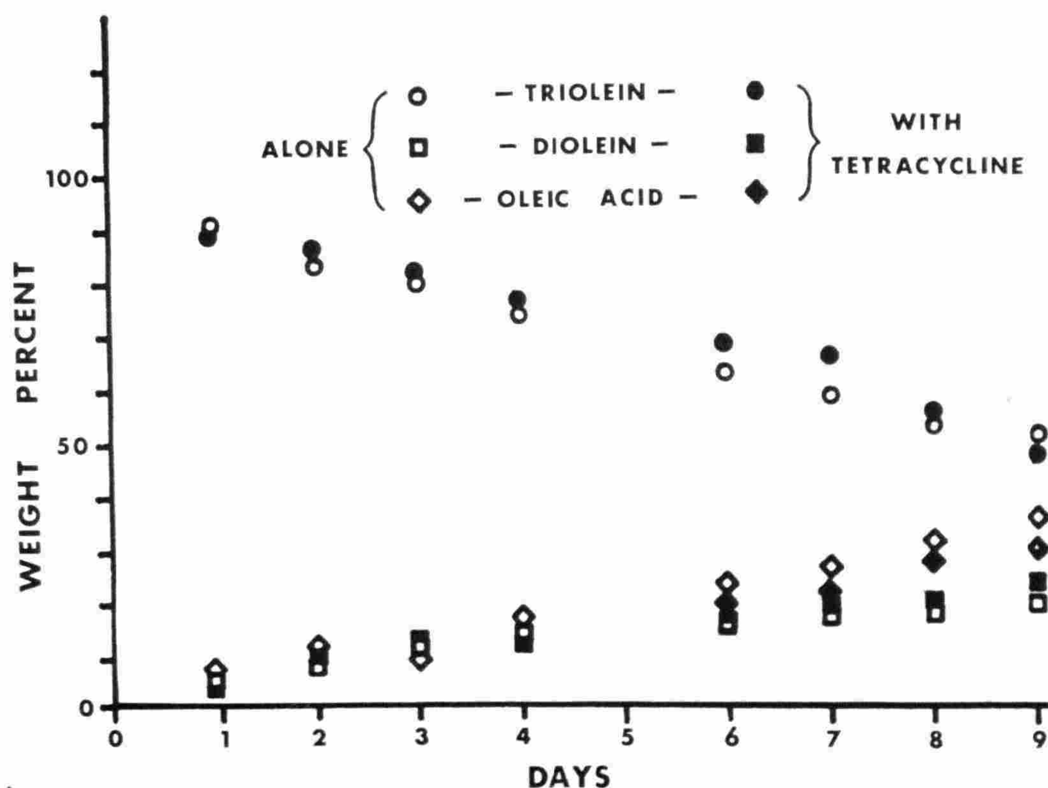


FIG. 3. Rate curves for conversion of 5 mg of triolein to oleic acid and diolein by 25 mg of epidermal debris in 5 ml of ether, with and without addition of 5 mg of tetracycline base.

complete conversion of triglycerides to free fatty acids and glycerol would have been provided by 3% of water by weight in the active powder or 0.03% water in the solvent. Addition of water as a saturated solution in the ether did not enhance the rate of reaction. Water did not appear to be the limiting factor in termination of the reaction in diglycerides, since in hexane, which would contain less water than ether, the diglycerides, which are partial hydrolysis products, were always much lower than in ether.

v) The reaction was not affected by addition of 1 mg of tetracycline per ml of solvent, more than enough to produce a saturated solution.

vi) The reactions catalysed by epidermal debris proceeded similarly under nitrogen as in air.

vii) Freshly-collected skin surface debris behaved similarly in all respects to that which had been stored for long periods.

viii) No hydrolysis occurred when incubations were carried out in the absence of epidermal debris.

## DISCUSSION

The conclusion that the hydrolysis is enzymic rather than merely chemical is based on the observation that the presence of the epidermal debris is essential, that its activity can be destroyed by heating, and that the hydrolysis occurs only with specific esters.

Although it may appear to be unwise to draw conclusions from observations made in such apparently unphysiological media as ether and hexane, it is conceivable that the hydrolysis of triglycerides which occurs in the human pilosebaceous follicle may also be proceeding in a largely non-aqueous environemnt. Sebum arriving at the skin surface is a clear oil rather than an emulsion, even when forcibly expressed (8), and its polarity more closely resembles hexane than ether. It may be significant that the relative proportions of free fatty acids, diglycerides and triglycerides present in sebum more closely resemble those produced in hexane than in the ether medium.

It has been proposed that the value of tetracyclines in the management of acne vulgaris is due to the reduction of free fatty acid production in the follicle (2), either by reduction of follicular microorganisms or by direct inhibition of bacterial lipases. In studying these possibilities Shalita and Wheatley have established that high concentrations of tetracycline inhibit hydrolysis of triglycerides by hog pancreatic lipase (9).

The enzymic reaction studied in the present investigation differs from pancreatic lipase in being resistant to tetracycline, and also in being unable to catalyse the hydrolysis of wax esters, which are split by the pancreatic enzyme (10). It is, therefore, undesirable to use pancreatic lipase as a model for investigation of cutaneous systems. The present study indicates a means of obtaining a lipase from the skin surface which may be used for further investigations. However, there is as yet no indication whether this enzyme is of epidermal or bacterial origin, and it would be unwise to conclude that it is the only lipase present in the skin.

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