

Further Observations on the Pathogenesis of Acne

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British Medical Journal, 1972, 3, 444-446

Summary

The composition of the lipid of the forehead skin surface and the sebum excretion rate were determined in 217 subjects and controls. Acne was associated with an increase in serum excretion rate and in the squalene and wax and sterol esters in surface lipid. The changes in sebum excretion rate and squalene were statistically significant only in women, but acne in men was associated with a significant decrease in the free fatty acid content or surface lipid.

The increased amounts of squalene and wax esters may lead to pilosebaceous obstruction in acne subjects. The increased sebum excretion rate in acne may ensure increased production of sebaceous triglyceride substrate available for lipolysis to irritant free fatty acids, which may then mediate the inflammatory changes of acne.

Introduction

There is some indirect evidence to suggest that the composition of sebum may be important in the pathogenesis of acne vulgaris. Firstly, free fatty acids injected into normal skin produce an inflammatory response similar to that of acne (Strauss and Pochi, 1965) and may also be comedogenic (Kligman *et al.*, 1970). Secondly, successful antibiotic therapy in acne is associated with a significant decrease in the percentage of free fatty acids in skin surface lipid (Freinkel *et al.*, 1965; Beveridge and Powell, 1969; Cotterill *et al.*, 1971a; Cunliffe *et al.*, 1972). Most observers, however, have not found any significant differences in the composition of skin surface lipid in acne subjects compared with controls, but factors such as age, sex, and site and method of collection which modify the composition (Nikkari, 1965; Greene *et al.*, 1970; Ramasastry *et al.*, 1970; Cunliffe *et al.*, 1971) have often been ignored.

We have taken samples of surface lipid from the forehead by absorbent papers in a large number of subjects. This technique enables the lipid composition to be determined together with the sebum excretion rate, which is important in the pathogenesis of acne vulgaris (Cunliffe and Shuster, 1969a). Another advantage is that the composition of samples of surface lipid obtained by absorbent paper is nearer that of pure sebum than of samples obtained by other commonly-used techniques (Cunliffe *et al.*, 1971).

Patients and Methods

We investigated 271 subjects (104 men and 167 women) aged from 16 to 60 years. Two independent observers assessed the severity of the acne in those suffering from it, and the subjects were then allocated to one of the following four groups.

Group 1.—Nine men and 27 women aged from 16 to 25 years who had either no acne lesions or subclinical acne (Burton *et al.*, 1971).

Group 2.—Fifty-seven men and 74 women aged from 16 to 25 years with acne, of whom 34 men and 48 women had mild acne with a few small papules and pustules, 15 men and 18 women had moderate acne, and 8 men and 8 women had severe acne with cysts and numerous large pustules.

Group 3.—Twenty-four men and 32 women aged from 26 to 60 years with both a previous history of acne and acne scarring.

Group 4.—Fourteen men and 34 women aged from 26 to 60 years with no past history of acne and no acne scarring on examination.

The sebum excretion rate on the forehead was determined by using absorbent papers according to the method of Strauss and Pochi (1961) as modified by Cunliffe and Shuster (1969b). The composition of the skin surface lipid was determined semiquantitatively by thin-layer chromatography for squalene, wax and sterol esters, triglycerides, free fatty acids, and cholesterol (Cotterill *et al.*, 1971b). This was done with minimum delay to obviate possible changes during storage (Downing, 1970). Samples were collected at the same time of day to avoid possible circadian variations (Burton *et al.*, 1970; Cotterill, 1972).

Statistical analysis was by an analysis of variance.

Results

FEMALES

Acne in females compared with controls matched for age (Fig. 1) was associated with a significant increase in the sebum excretion rate ($P < 0.005$) and in the percentages of squalene ($P < 0.025$) and wax and sterol esters ($P < 0.05$) in skin surface lipid. A previous history of acne was associated with an increased sebum excretion rate compared with controls

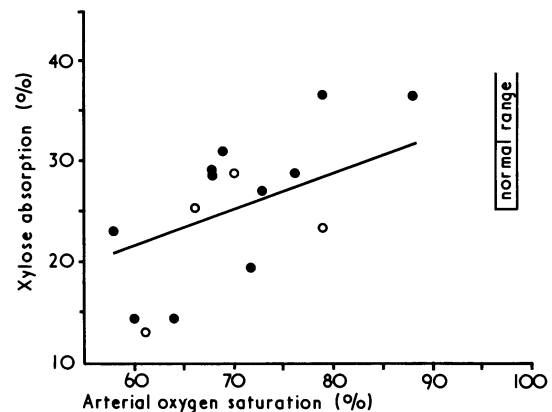


FIG. 1.—Sebum excretion rate and squalene and wax esters in skin surface lipid.

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($P < 0.001$). The changes in the percentages of squalene and wax and sterol esters in surface lipid found in women with acne were not found in those with a previous history of acne (Fig. 1). There was no significant variation in the composition of skin surface lipid with the menstrual cycle.

MALES

Acne in males was associated with an increase in the sebum excretion rate and in the percentage of squalene in the skin surface, but these changes were not statistically significant (Fig. 1). Analysis of the wax and sterol ester percentages in relation to the severity of acne (Fig. 2) showed that these were higher with severe acne than with mild acne ($P < 0.025$).

Acne in males was associated with significantly less free fatty acid in surface lipid compared with controls matched for age ($P < 0.01$) (Fig. 3), and acne in increasing severity was associated with a progressive decrease in free fatty acids in

surface lipid (Fig. 2). There were reciprocal changes in triglyceride content (Figs. 2 and 3). These biochemical changes were not found in men with a previous history of acne (Fig. 3).

There were no significant differences in the cholesterol content in the groups studied.

Discussion

The present results confirm the earlier observations of Cunliffe and Shuster (1969a) that acne is associated with seborrhoea and that seborrhoea is present in subjects with a previous history of acne. Our investigations, however, show that whereas the sebum excretion rate remains raised in persons who have had acne compared with controls the biochemical differences no longer exist. It may be concluded that the changes in the composition of the surface lipid of the forehead found in acne subjects can occur independently of changes in the sebum excretion rate and, furthermore, that these biochemical changes may be important in the pathogenesis of acne.

The composition of skin surface lipid in man is unique, as is his susceptibility to acne. Human surface lipid is particularly rich in both triglycerides and their hydrolysis products and squalene, which are present in negligible amounts in other animal species (Nicolaidis *et al.*, 1968).

One of the earliest clinical and histological features of acne is obstruction of the pilosebaceous unit (Van Scott and MacCardle, 1956). All constituents of sebum in rabbits are comedogenic but squalene and some free fatty acids are most active in this respect, while wax esters have some activity, especially when they constitute more than 10% of the contents (Kligman *et al.*, 1970). Thus the higher percentages of squalene and wax esters in skin surface lipid in acne patients may be significant if the changes which initiate comedone formation in the rabbit can be shown also to occur in man.

We have shown that the percentages of free fatty acids in surface lipid are decreased in patients with acne, and significantly so in men. This may seem paradoxical in view of the known comedogenic properties of free fatty acids (Kligman *et al.*, 1970), their inflammatory properties (Strauss and Pochi, 1965; Kligman *et al.*, 1970), and the decrease in surface lipid fatty acids with successful long-term treatment with antibiotics (Beveridge and Powell, 1969; Cotterill *et al.*, 1971a; Cunliffe *et al.*, 1972). But our data are only semi-quantitative, and analysis by gas liquid chromatography may show detailed differences in the free fatty acid content of surface lipid in patients with acne and those without.

These fatty acids are derived from sebaceous triglycerides by the lipolytic action of *Corynebacterium acnes* (Freinkel and Shen, 1969), which is found in the pilosebaceous duct. Possibly the increased rate of sebum excretion in patients with severe acne may give less time for the lipolytic enzymes to act on the triglyceride substrate, but it should not be forgotten that collected sebum comes from many pilosebaceous units, whereas only a few glands need to be obstructed to produce severe acne.

The increased rate of sebum excretion in subjects with acne may ensure a greater production of sebaceous triglyceride substrate for subsequent lipolysis to free fatty acids. Under appropriate conditions, and in particular behind a blocked pilosebaceous gland, lipolysis of triglyceride is almost complete (Nicolaidis *et al.*, 1970). Thus in subjects with acne the increased amounts of squalene and perhaps wax esters may be responsible for initiating the obstruction to the pilosebaceous unit. Subsequent lipolysis of the abundant sebaceous triglyceride proximal to this obstruction may lead to the production of relatively large amounts of free fatty acids, which after leaving the sebaceous gland may induce inflammatory changes. These biochemical changes would ensure both the initiation and subsequent development of the inflammatory lesion characteristic of acne.

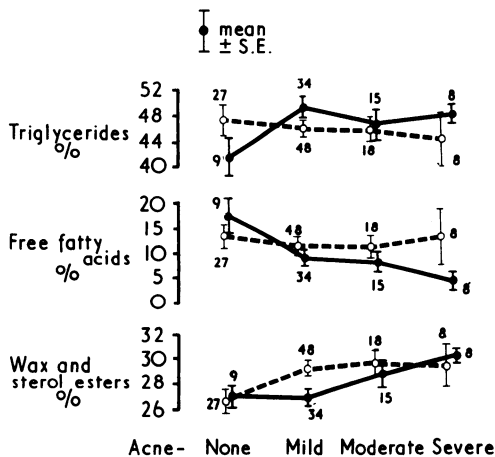


FIG. 2—Composition of skin surface lipid in relation to severity of acne in males and females aged 16-25 years. Solid line = Males. Broken line = Females.

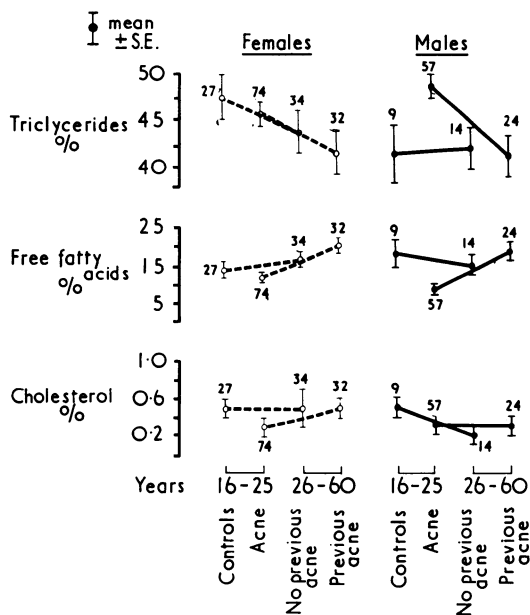


FIG. 3—Triglycerides, free fatty acids, and cholesterol in skin surface lipid.

We are grateful to the Wellcome Trust, the Leeds Regional Hospital Board, the Board of Governors of the United Leeds Hospitals, and the Leeds and West Riding Medical Research Trust for financial support. We also thank Mr. R. A. Forster, chief technician, Miss J. Day, technician, and Mrs. L. Lahe for secretarial help.

References

- Beveridge, G. W., and Powell, E. (1969). *British Journal of Dermatology*, **81**, 525.
- Burton, J. L., Cunliffe, W. J., and Shuster, S. (1970). *British Journal of Dermatology*, **82**, 497.
- Burton, J. L., Cunliffe, W. J., Stafford, I., and Shuster, S. (1971). *British Journal of Dermatology*, **85**, 119.
- Cotterill, J. A. (1972). M.D. thesis submitted to the University of Newcastle upon Tyne.
- Cotterill, J. A., Cunliffe, W. J., and Williamson, B. (1971a). *British Journal of Dermatology*, **85**, 130.
- Cotterill, J. A., Cunliffe, W. J., Williamson, B., and Forster, R. A. (1971b). *British Journal of Dermatology*, **85**, 35.
- Cunliffe, W. J., and Shuster, S. (1969a). *Lancet*, **1**, 685.
- Cunliffe, W. J., and Shuster, S. (1969b). *British Journal of Dermatology*, **81**, 697.
- Cunliffe, W. J., Cotterill, J. A., and Williamson, B. (1971). *British Journal of Dermatology*, **85**, 40.
- Cunliffe, W. J., Cotterill, J. A., and Williamson, B. (1972). *British Journal of Dermatology*, **86**, 311.
- Downing, D. T. (1970). *Journal of Investigative Dermatology*, **54**, 395.
- Freinkel, R. K., and Shen, Y. (1969). *Journal of Investigative Dermatology*, **53**, 422.
- Freinkel, R. K., Strauss, J. S., Shing, Y. Y., and Pochi, P. E. (1965). *New England Journal of Medicine*, **273**, 850.
- Greene, R. S., Downing, D. T., Pochi, P. E., and Strauss, J. S. (1970). *Journal of Investigative Dermatology*, **54**, 240.
- Kligman, A. M., Wheatley, V. R., and Mills, O. H. (1970). *Archives of Dermatology*, **102**, 267.
- Nicolaides, N., Fu, H. C., and Rice, G. R. (1968). *Journal of Investigative Dermatology*, **51**, 83.
- Nicolaides, N., Amsari, M. N. A., Hwei, C., Fu, H. C., and Lindsay, D. E. (1970). *Journal of Investigative Dermatology*, **65**, 487.
- Nikkari, I. (1965). *Journal of Clinical Laboratory Investigations*, Suppl. No. 17, 85, 1.
- Ramasastri, P., Downing, D. T., Pochi, P. E., and Strauss, J. S. (1970). *Journal of Investigative Dermatology*, **54**, 139.
- Strauss, J. S., and Pochi, P. E. (1961). *Journal of Investigative Dermatology*, **36**, 293.
- Strauss, J. S., and Pochi, P. E. (1965). *Archives of Dermatology*, **92**, 443.
- Van Scott, E. J., and MacCardle, R. C. (1956). *Journal of Investigative Dermatology*, **27**, 405.

Biochemical Response of Late Rickets and Osteomalacia to a Chupatty-free Diet

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British Medical Journal, 1972, **3**, 446-447

Summary

Eight Pakistani children with late rickets and two Pakistani women with osteomalacia were given a chupatty-free diet for seven weeks, substituting leavened bread of lower extraction. On this diet serum calcium levels rose to normal or near normal, levels of serum inorganic phosphorus rose slightly but significantly, and serum alkaline phosphatase levels showed a definite rise indicative of healing bone disease. It is concluded that the high phytate content of unleavened bread is the major cause of late rickets and osteomalacia in Pakistani and Indian communities in the United Kingdom. The simplest prophylactic measure seems to be the additional fortification with calcium carbonate of the high extraction flour used in preparing unleavened bread.

Introduction

Late rickets and osteomalacia are common among Indian and Pakistani immigrants to the United Kingdom (Dunnigan *et al.*, 1962; Felton and Stone, 1966; Swan and Cooke, 1971; Ford *et al.*, 1972). Women and children at the age of puberty are most commonly affected. Nutritional deficiency of vitamin D does not appear to be an aetiological factor, and other factors such as skin pigmentation, social custom, and atmospheric pollution, which may reduce the endogenous synthesis of cholecalciferol, seem less plausible than when first considered (Ford *et al.*, 1972).

Reinhold (1971, 1972) suggested that unleavened bread, with its high phytate content, may produce rickets or osteomalacia

when ingested over a prolonged period as a main source of cereal. Wills *et al.* (1972) reported the prompt healing of late rickets in an adolescent Indian boy treated in hospital with a low phytate diet without alteration in his vitamin D intake. The present study was undertaken to evaluate the rachitogenic properties of unleavened bread in the Pakistani and Indian dietary by substituting leavened bread of lower extraction without alteration in other dietary or environmental factors.

Subjects and Methods

Two Pakistani women aged 21 and 45 years and eight children (four boys and four girls) aged 9-16 years participated in the study. All had biochemical evidence of late rickets or osteomalacia. After biochemical confirmation of the diagnosis the participants were asked to stop eating chupatties and to substitute white bread *ad libitum*. No other dietary restrictions were imposed and it was emphasized that the dietary pattern should remain in other respects unaltered. No other treatment was given during the period of the trial. The participants were visited weekly in their homes, when non-fasting blood samples were taken for the estimation of serum calcium, inorganic phosphorus, and alkaline phosphatase. Sera were separated and deep frozen within one hour of withdrawal. Biochemical estimations were performed on an AutoAnalyzer. The statistical significance of the results was assessed by applying Student's *t* test to paired differences between levels of serum calcium, inorganic phosphorus, and alkaline phosphatase before and after beginning the chupatty-free diet.

Results

A dietary history showed that the individual consumption of chupatties by the participants ranged from two to four daily, each weighing 40-60 g. The consumption of unleavened bread thus ranged from about 80 to 240g daily. The flour from which

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