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VARIATION IN SKIN SURFACE LIPID COMPOSITION AND SEBUM EXCRETION RATE WITH TIME

J. A. Cotterill, W. J. Cunliffe and B. Williamson

From the Department of Dermatology, The General Infirmary, Leeds, St. James's (University) Hospital, and The General Hospital, Harrogate, England

Abstract. Serial collections of sebum for the determination of sebum excretion rate and skin surface lipid composition were made during a 27 hour period. The sebum excretion rate varies with the time of collection and also with the order of collection. Both squalene and wax ester composition are related to the order of collection whereas the free fatty acids show a possible diurnal variation. The variation in triglycerides is more complex, showing a relation to order of collection and possibly also to the time of day.

The rate of sebum excretion in man has been shown to exhibit a circadian rhythm (2) and it therefore seemed possible that the composition of skin surface lipid might also vary with the time of day. We have investiagted this problem.

SUBJECTS AND METHODS

Nine male subjects aged 19 to 62 years were investigated. Three had moderate acne vulgaris, 3 were healthy normal medical students with minimal acne, 2 patients had leg ulcers and 1 minimal contact eczema. The eldest patient who had a leg ulcer also showed mild parkinsonian features. All subjects were admitted to hospital for the investigation and were asked to wash their hair on the evening before the investigation but to refrain from washing the face thereafter.

Collections of sebum were made from the forehead using absorbent papers and serial determinations of the sebum excretion rate were made by the modified method of Strauss & Pochi (3, 13). The overall design of the experiment was identical with that of Burton et al. (2). The investigations were performed on one of two occasions separated by an interval of several weeks. On the first occasion the collection began at 1500 hours and in the second group the collection began at 0900 hours. Biochemical analysis of skin surface lipid by thin-layer chromatography began immediately after the collection of samples whatever the time of day or night to minimise any possible biochemical changes due to storage (6).

RESULTS

The serial sebum excretion rates and biochemical analyses are shown in Figs. 1–9. Because of the wide scatter of results the data has been expressed as a percentage of the mean value for each individual subject. The results have been analysed with regard both to the order of collection and to the actual time of collection.

Fig. 1 shows that the amount of sebum collected varied with the order of collection. By use of Student's *t*-test, a significant difference was disclosed between the first and third collection ($p < 0.005$) and between the second and third collection ($p < 0.01$).

Fig. 2 shows significant variation of the sebum excretion rate with actual time of collection. Six out of 9 subjects showed the minimum excretion of sebum between 0300 and 0600 hours and this was significantly lower than the excretion rate of the preceding collection. There was also a significant difference between the low excretion rate at 0300 to 0600 hours and the subsequent collection ($p < 0.005$).

From Fig. 3 it is clear that there is an association between the squalene content and the order in which the sebum was collected. The initial collection contained the least squalene and the final collection most and statistical comparison of these two results showed a significant difference ($p < 0.001$). Analysis of squalene content with time of day (Fig. 4) indicates that the changes are related to the order of collection rather than to the time of day.

The wax esters behaved similarly to squalene (Fig. 5) there being significantly less excreted at the first collection in contrast to the peak ex-

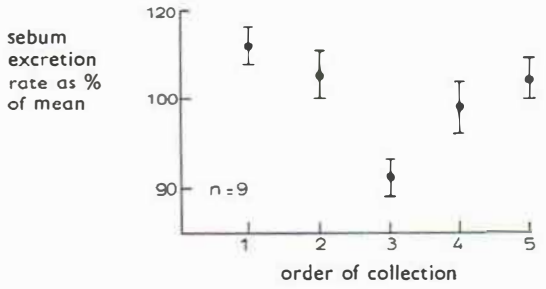

Fig. 1

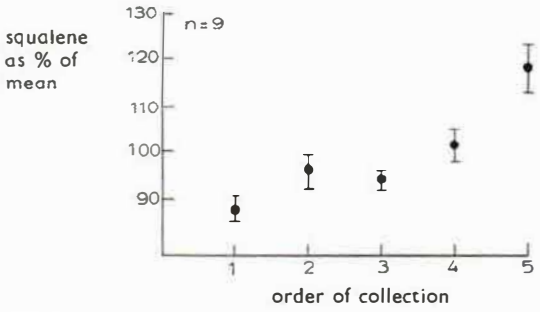

Fig. 3

cretion period which was also the final collection ($p < 0.01$). There was also a significant rise between the first and second collection ($p < 0.02$). There was no significant variation with time of day (Fig. 6).

Since it has been shown that the triglycerides and free fatty acids content of sebum are inversely related (5, 11) an observation confirmed in the present investigation (Fig. 7)), then these two groups have been considered together with respect to both order of collection and time of day. Fig. 8 relates the free fatty acid and triglyceride content of sebum to the order of collection. Most triglyceride was collected in the first period and this was considerably more than in both the third ($p < 0.025$) and the final period ($p < 0.005$). The free fatty acids, however, showed no significant relationship to the order of collection.

Eight of the 9 patients investigated (88%) had a peak free fatty acid excretion between 0300 and 1200 hours and during the same period 6 patients (66%) had the least triglyceride excretion. Conversely, in 8 patients the peak triglyceride content occurred between 1500 to 2400 hours and in 7 patients (77%) this was associated with the lowest levels of free fatty acids (Fig. 9). The triglyceride excretion in the first 1500–1800 hour period was significantly greater than the 0300 to 0600 hour collection ($p < 0.02$) but not so when compared with the 2100 to 2400 hour collection ($p < 0.1$).

The shape of the curve for free fatty acid with two distinct peaks and the less marked reciprocal changes in triglyceride was suggestive of a circadian rhythm.

There was no correlation between the sebum excretion rate and composition of surface lipid, with the possible exception of triglycerides and free fatty acids. The triglyceride values were highest when sebum excretion was also high and during the 0300 to 0600 hour period when the sebum excretion rate was at its lowest the sebum free fatty acid rose to one of the two peaks observed during the period of the study (Fig. 7).

DISCUSSION

In any experiment concerning circadian rhythms there are many variables, and future experiments should be designed so that order of collection and

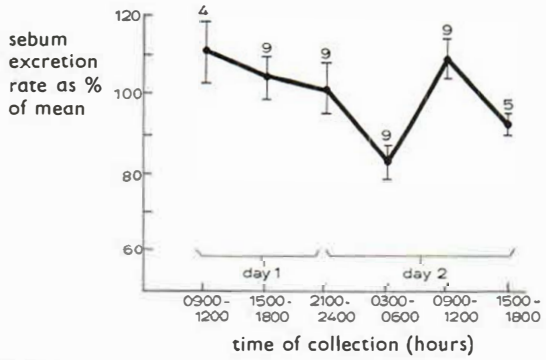

Fig. 2

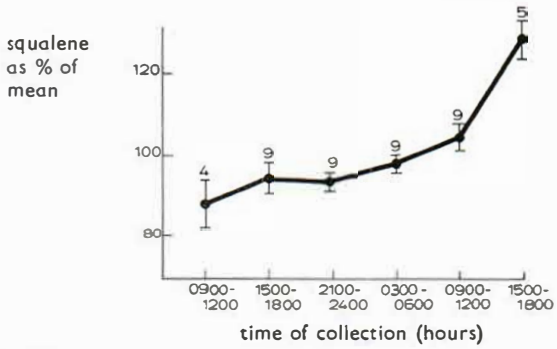

Fig. 4

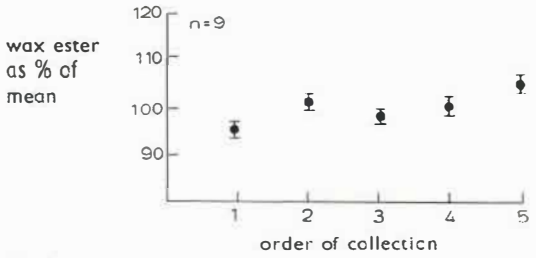

Fig. 5

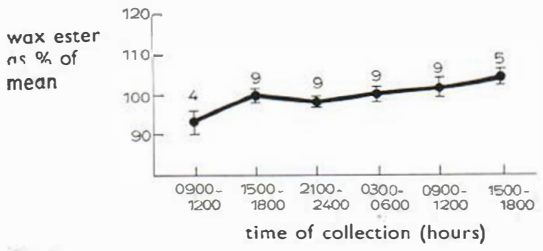

Fig. 6

time of collection are not confounded. For practical reasons such experiments are not easy to perform and thus we have used the same basic experimental methods of earlier workers (2).

With these reservations this experiment has demonstrated changes in the sebum excretion rate with the order of collection. The first 3 hour collection of sebum was significantly greater than subsequent collections and its is known that sebum collected in such a period is correlated with the excretion rate of collections made consecutively over a period of 12 hours (3). Sebum excretion rate was also shown to vary not only with order of collection but with time of day, thus confirming the earlier observations of Burton et al. (2) that there is a circadian rhythm, and the possible mechanisms have been discussed in detail by these authors.

Squalene, wax esters and triglycerides appear to

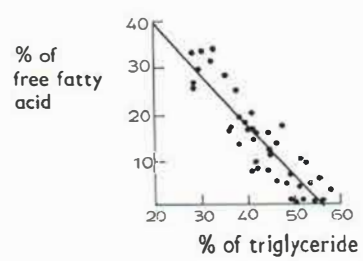

Fig. 7

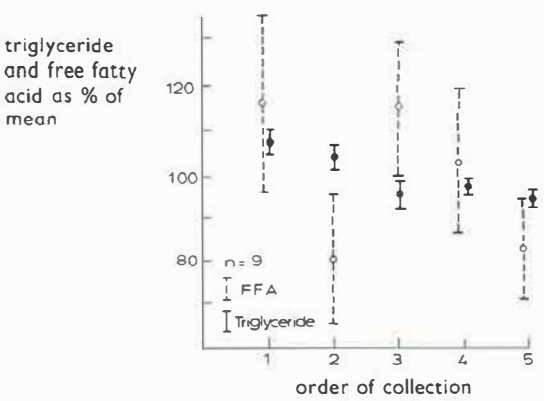

Fig. 8

be related to the order of collection and not to the time of day. Although there is a striking similarity in the excretion pattern of wax esters and squalene, the significant increase in the wax ester content at the second collection compared with the first collection is not understood. The increase in the percentages of wax esters and squalene in the samples as the experiment progresses suggests that towards the end of the 27 hour period the surface lipid has a greater sebaceous gland contribution since wax esters and squalene derive from the sebaceous gland rather than primarily from epidermis (8, 12).

Triglycerides and free fatty acids both show definite relationships to the time of collection and the shape of the curve for free fatty acids in relationship to time is strongly suggestive of a diurnal rhythm. The inverse relationship between triglycerides and free fatty acids which we have found confirms earlier observations (5, 11); the free fatty acids are derived from triglycerides by lipolytic enzymes present either in the sebace-

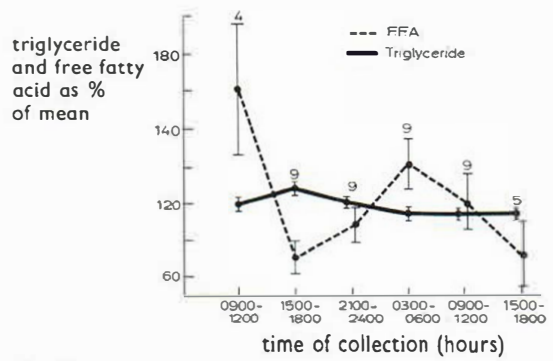

Fig. 9

ous duct cells or cutaneous micro-organisms (11). Although the first peak in free fatty acids may be due to collection of old skin surface lipid, rich in free fatty acids derived by hydrolysis of triglycerides, this seems unlikely as most triglycerides are also collected in the first sample in most patients. The second peak in free fatty acids occurs during a time of low sebum excretion rate and this may be due to increased lipolysis of triglycerides because of greater contact time with the necessary lipolytic enzymes.

The factors which control the degree of lipolysis of the triglycerides in vivo are not known. There is evidence suggesting that corynebacteria are more important than staphylococci in the production of the free fatty acids (10). In vitro studies on the ability of corynebacteria to produce lipolysis of triglycerides has shown that only certain strains can perform this enzymatic process (9) and this reaction is pH-dependent (7). Since data on skin pH is limited (1), we are not able to relate our findings to changes in cutaneous hydrogen ion concentration and this obviously merits further investigation. Temperature also might play a role in modifying surface lipid biochemistry. Increased skin temperature is known to increase the sebum excretion rate in the absence of sweating, although local changes in skin temperature do not explain the diurnal variations in sebum excretion rate (4). It is of interest in the present study that at the time of the lowest skin temperature (in the early morning period) the sebum excretion rate is also at its lowest and this is when the free fatty acids show one of their two observed peaks. The effect of such variables as skin temperature and skin pH on skin surface lipid composition are now underway and this may shed further light on the pathogenesis of acne vulgaris.

We conclude that both order and time of collection are important factors modifying both sebum excretion rate and the composition of skin surface lipid and these factors must be taken into account during serial investigations if meaningful comparison of data is to be made.

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J. A. Cotterill, M.D.
Department of Dermatology
The General Infirmary at Leeds
Great George Street
Leeds 1, Yorkshire
England