SOME OBSERVATIONS ON THE NATURE, ORIGIN AND POSSIBLE FUNCTION OF THE SQUALENE AND OTHER HYDROCARBONS OF HUMAN SEBUM*

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In a study of the general composition of the surface skin fat or sebum from the human forearm (MacKenna, Wheatley and Wormall (1)) it was found that this material contains a high proportion of hydrocarbons constituting about half the non-saponifiable matter or about one-sixth of the whole sebum. Squalene was identified as one of the major constituents, accounting for about one-third of the total hydrocarbons. At about the same time Sobel (2) showed that this substance was a normal constituent of human hair fat, ear wax and smegma, and Čmelik, Petrak-Longhino and Mihelić (3) established its presence in vernix caseosa. The presence of squalene in these sebum and sebum-like materials presents an interesting biochemical problem as to its origin and possible functions in the skin. Its role is even more significant as it appears to be a possible intermediary in the metabolism of cholesterol (Langdon and Bloch (4)). We have also found that paraffins are present in the hydrocarbon fraction of human sebum (5, 6) and we now report some investigations which have been made in an endeavour to elucidate the role played by the sebum hydrocarbons.

EXPERIMENTAL METHODS AND MATERIALS

Collection of sebum

Sebum samples were obtained by the following three methods.

(*a*) From the forearm by extraction with acetone, either from single subjects or from groups of individuals as already described (1).

(*b*) By swabbing the back with fat-free cotton-wool moistened with carbon tetrachloride and extracting the sebum from these by reflux distillation with chloroform (Hodgson-Jones, MacKenna and Wheatley (7)).

(*c*) By a slight modification of the cup method previously described (Hodgson-Jones and Wheatley (8)). A larger cup was prepared from a polythene funnel, 6 cm. in diameter, which was cut across about 2.5 cm. from the top. The upper part of the funnel formed a cup 6 cm. in diameter with sloping sides. The greater flexibility of the plastic as compared with glass enabled the cup to mould itself to the curvature of the body when pressed against the skin and so eliminate the tendency to leaking found with cups of similar size made of glass. Two 5 ml. portions of carbon tetrachloride were used to extract the area of skin enclosed by this cup.

Analytical methods

Fractionation of bulked specimens followed previously described methods (1). The hydrocarbon content of single forearm specimens was determined by a chromatographic method and squalene estimations were performed by an iodometric method (Wheatley (9)).

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Squalene

A sample of pure squalene, kindly supplied to us by Dr. H. J. Channon of Unilever Ltd., was used as supplied in the experiments involving topical application and mixed with five times its weight of butter in the feeding experiments. A sample of the non-saponifiable matter from shark liver oil was also prepared and was found to contain 76% squalene; this preparation was used as a source of squalene in some of the clinical trials.

RESULTS

Nine batches of pooled sebum collected from the forearms of groups of 15–82 normal male medical students were found to contain 8.2–15.8% (average 11.9) hydrocarbons. This apparent wide variation was confirmed by determining the hydrocarbon content of single samples of sebum from the forearms of 10 normal male subjects. These gave results of 10.2–26.9% (average 15.2). Owing to losses during separation and analysis it is not possible to estimate accurately the squalene content of sebum collected in this way by the 'acetone method', but the hydrocarbon fractions of these single sebum samples were estimated to contain 25–65% squalene (average 47).

In an earlier investigation we obtained evidence (6) that human sebum contains a *normal* chain paraffin, and we have tried to confirm this by using the urea adduct method of Zimmerscheid, Dinerstein, Weitkamp and Matschner (10). A batch of sebum was collected from the forearms of 31 subjects and the hydrocarbon fraction isolated from the non-saponifiable matter by chromatography. Treatment of the whole hydrocarbon fraction (13.2% of the sebum), or this fraction after the squalene had been removed by treatment with HCl followed by chromatography, failed to give a urea adduct. Control tests showed that when *n*-paraffins (paraffin wax) were added to the sebum hydrocarbon fraction (freed from squalene) their recovery as urea adduct was good (93%). Solid hydrocarbons were then isolated by crystallising (from ethanol) the residue obtained after removal of squalene from the sebum hydrocarbon fraction, and an attempt to form a urea adduct was again made but without success. Finally an attempt was made to remove possible interfering substances by chromatography on silica, but the residue after chromatography still failed to yield a urea adduct. It has not been possible at this stage of the work to check these findings by means of infrared spectrometry but it appears that the *n*-paraffins previously isolated from human sebum (6) were contaminants and that only branched-chain paraffins are naturally present.

Squalene content of sebum

The squalene content of fresh samples of sebum was estimated in the following manner. The samples were collected from the center portion of the back of the subject by the polythene cup method; three areas were extracted in the case of adults and four in the case of children in order to obtain sufficient sebum for the analysis. The total carbon tetrachloride extracts were filtered through a sintered-glass funnel, evaporated to small bulk *in vacuo* and then diluted to 15 ml. Duplicate 5 ml. portions of this solution were set aside for the squalene estimation while the weight of sebum contained in the solution was obtained by evaporating duplicate 2 ml. samples to dryness in weighed microflasks and reweighing the flasks. It was proposed to utilize the surface film method of Jones, Spencer and Sanchez

TABLE 1

The squalene content of the sebum from the back of normal subjects

Subjects	Number	Range	Average
		(%)	(%)
Children	10	2.5–9.8	5.8 ± 2.7
Adults, male	19	6.5–13.4	9.2 ± 2.4
Adults, female	20	7.0–13.0	10.0 ± 1.6

(11) for the latter estimation and the reliability of this method was investigated (cf. Hodgson-Jones and Wheatley (8)) by making determinations in triplicate, using the surface film method on a random selection of 50 weighed sebum samples. A dot diagram of the results showed that the method had an average error of ±16.1% as compared with the weighing method. This method was, therefore, not considered sufficiently accurate for the present investigations. A further objection is that hydrocarbons do not spread on the surface film, and are not measured by this method.

Normal subjects. Estimations were performed on normal subjects (10 children aged 1–12, 20 adult females aged 18–37 and 19 adult males aged 18–74) and the results are summarized in Table 1. In order to establish more clearly any correlation between squalene content and age a dot diagram was plotted (Fig. 1). These results show that the average squalene content of the sebum of children

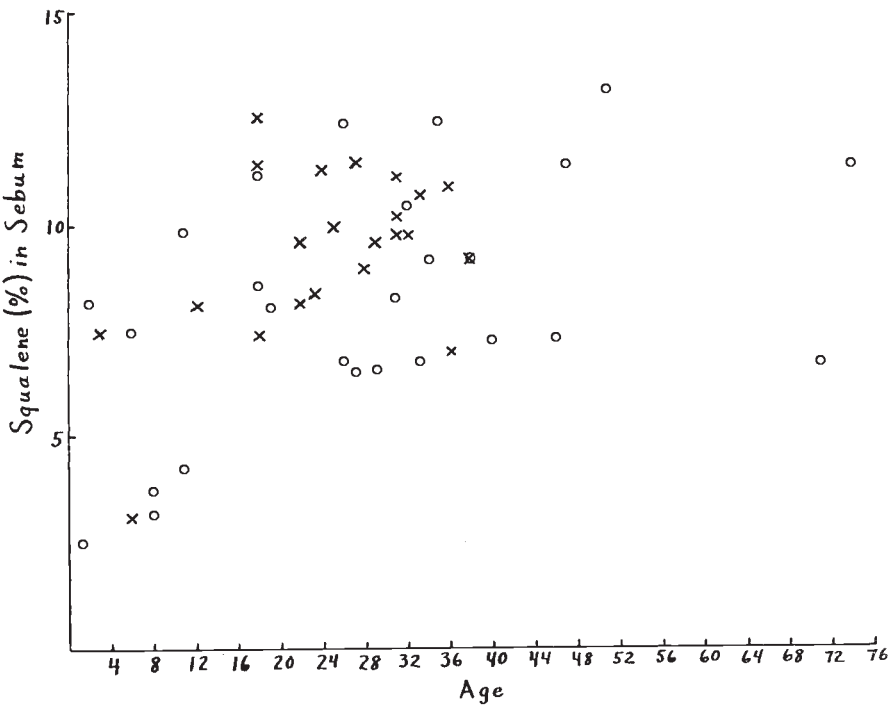

FIG. 1. Squalene content of human sebum from the back of subjects of various ages. ×—Females; ○—Males.

is much lower than that of adults. There is also a slight difference between the average values for adult males and females, but in view of the wide variations which occur in both sexes this difference is not statistically significant. One male subject was found to give a sebum with an unusually high squalene content (15.8%), and further it was found that the sebum "level" (8) was also high (324 μg./sq.cm.). This subject was not one included in the present series, and he is being further investigated.

Menstrual cycle. Daily determinations were performed on three female subjects to decide whether or not there were cyclic changes in the squalene content of sebum during the menstrual cycle. The results (Fig. 2) are a little conflicting; one subject (A) showed a definite drop in the squalene content on menstruation

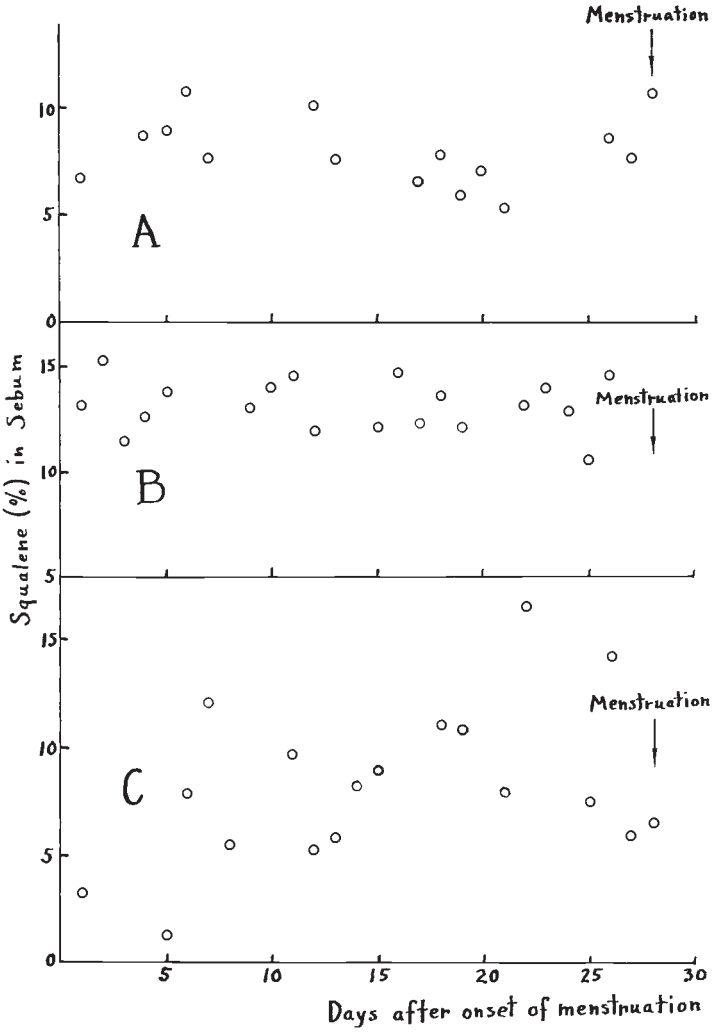

FIG. 2. Squalene content of human sebum from the back during the menstrual cycle. (Subjects A, B and C.)

TABLE 2

Squalene content of the sebum from various areas of the body

Subject	Sex	Area				
		Back	Chest	Abdomen	Arm	Leg
		(%)	(%)	(%)	(%)	(%)
H. B.	F	9.8	10.4	—	7.9	4.5
D. S.	M	6.5	10.1	12.0	8.5	8.9
J. H.	F	9.0	8.0	6.5	6.0	4.0

but this drop did not occur in the other two subjects. Marked daily variations were observed in all three subjects.

Variations over body surface. Sebum samples were taken from various areas of the body from three subjects and the squalene contents were determined. The polythene cup method was used for collection of the samples and either three or more adjacent areas of skin extracted according to the site and its

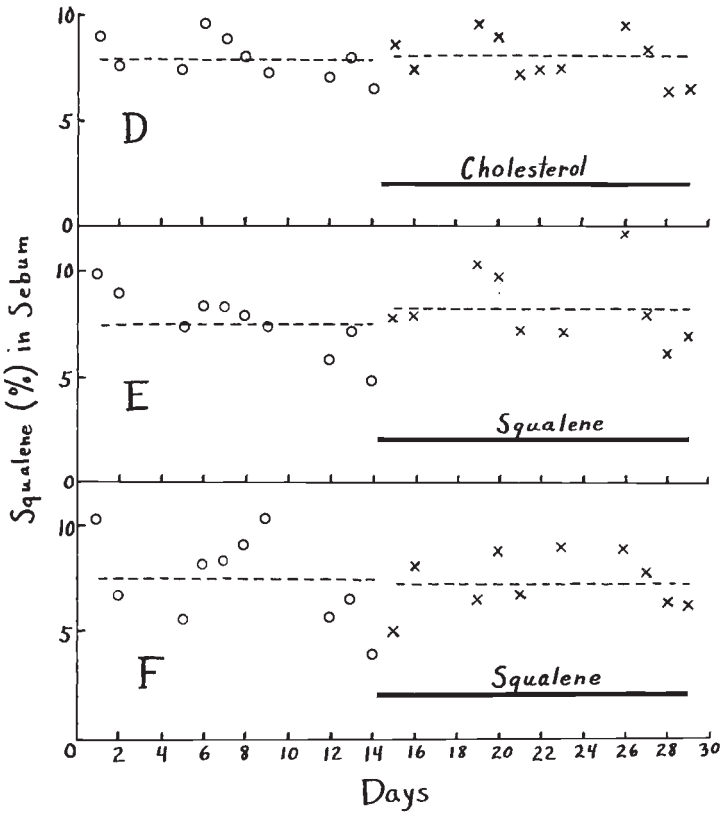

FIG. 3. Squalene content of sebum from the back of 3 male subjects, before and during the administration of cholesterol (D) or squalene (E and F). The dotted lines show the mean values for each period of the test.

TABLE 3

Squalene content of the sebum from normal subjects and from subjects suffering certain skin diseases

Skin Disease	Number	Range	Average
		(%)	(%)
(Normal subject)	20	5.4–11.7	8.5 ± 1.7*
Seborrhoeic dermatitis	20	4.0–11.4	7.2 ± 2.3*
Psoriasis	20	3.1–12.1	7.4 ± 2.1*

* Standard deviation.

known sebum richness. The results (Table 2) show that the squalene content of sebum is not constant but varies over the body surface.

Effect of feeding squalene and cholesterol. Daily sebum squalene determinations were made on three normal males for a period of 14 days. Two of the subjects were then fed with a dose of 1 g. squalene per day for 14 days, while the other had similar doses of 1 g. cholesterol. The test substance was mixed with five times its weight of butter, the mixture spread on a slice of bread and eaten. Sebum samples were then collected for a further period. The results (Fig. 3) show that there was no significant change in the squalene content of sebum following the ingestion of either squalene or cholesterol. The mean values (with S.D.) for the % of squalene in sebum were 7.8 ± 0.9 and 8.0 ± 1.1 respectively for the periods before and during the administration of cholesterol, and the corresponding values for the subjects taking squalene were: subject E, 7.4 ± 1.5 before and 8.1 ± 1.6 during squalene administration. Subject F, 7.4 ± 1.9 and 7.2 ± 1.4. In both subjects receiving squalene there was a marked fall in the squalene content of sebum just before the first test dose, but this may have been fortuitous.

Skin diseases. Sebum samples were collected from three groups of subjects; 20 normal, 20 with seborrhoeic dermititis and 20 with psoriasis. The samples were collected by swabbing the whole of the back of the subject with cotton-wool moistened with carbon tetrachloride, and the squalene content of the sebum recovered from the cotton-wool was then estimated. The results shown in Table 3 indicate average values in both skin diseases slightly lower than those for normal subjects but the differences are not statistically significant.

Effect of applying squalene to the skin

Patch tests on man were carried out with pure squalene and with the non-saponifiable matter from shark liver oil, these substances being left in contact with the normal skin for 72 hr. There was no detectable effect upon the skin or hairs; no change in the degree of pigmentation occurred, nor was any effect on keratinization observed. Squalene was injected into the skin itself in quantities of 0.2 ml. and other than a short-lived inflammatory response no alteration was noted. The effect of rubbing squalene on the unbroken skin and then applying ultra-violet irradiation from a mercury vapour lamp was also studied and no

TABLE 4

Summary of cases treated by the topical application of squalene and shark liver oil non-saponifiable matter

Subject	Diagnosis	Treatment with	Observations
B. B.	Flexural eczema	Shark liver oil n.s.*	No improvement
J. R.	Eczema	Squalene	No improvement
B. D.	Psoriasis	Shark liver oil n.s.	No improvement
P. D.	Psoriasis	Shark liver oil n.s.	No improvement
N. W.	Psoriasis	Shark liver oil n.s.	No improvement
A. F.	Psoriasis	Squalene	No improvement
P. M.	Psoriasis	Shark liver oil n.s.	No improvement
P. M.	Psoriasis	Squalene and ultra-violet irradiation	No improvement
F. T.	Pustular psoriasis	Shark liver oil n.s.	No improvement
P. J.	Vitiligo	Shark liver oil n.s.	No pigmentation
P. W.	Vitiligo	Shark liver oil n.s. and ultra-violet irradiation	No pigmentation
J. S.	Ichthyosis	Squalene	No improvement
T. R.	Hirsutism	Shark liver oil n.s. and squalene	No hair loss

* n.s. = non-saponifiable matter.

effect on pigmentation was seen. Finally to make sure that the squalene was reaching the dermis three areas of skin were blistered with cantharidin and the tops of the blisters removed. Pure squalene was applied to one raw area, shark liver oil to the second and the third area was untreated. All these areas were then irradiated equally with a mercury vapour lamp; subsequently all areas pigmented equally and healed in the same length of time. Squalene apparently had no inhibitory or stimulatory effect on either pigmentation or on healing.

A number of patients with skin diseases were treated by application of squalene or shark liver oil non-saponifiable matter to the affected areas and details of these tests are given in Table 4. No improvement in the skin condition of the subjects suffering from psoriasis (7 cases) or eczema (2 cases) occurred as a result of the application of squalene or the shark liver oil preparation. Hirsutism could not be reduced by the application of squalene nor could pigmentation be induced in vitiliginous areas with or without the use of ultraviolet irradiation. From the results of these limited tests on man it would appear that squalene has no significant action on human skin, and that it is without therapeutic value in the conditions tried.

DISCUSSION

Squalene is probably the most important hydrocarbon present in sebum. Considerable interest in the biochemical actions of squalene has recently been aroused, and the known actions of this unsaturated hydrocarbon may be summarized in the following manner.

1. Squalene is involved as an intermediary in the synthesis of cholesterol from acetate in the rat (Langdon and Bloch (4)). It is not the immediate pre-

cursor of cholesterol but may provide through some other intermediate isoprenoid units for sterol formation as indicated in the following scheme (Popják (12)):

$$\text{Acetate} \rightarrow \text{Isoprenoid unit} \rightarrow \text{X} \rightarrow \text{Cholesterol}$$
$$\uparrow\downarrow$$
$$\text{Squalene}$$

The suggestion that squalene may be converted into cholesterol was put forward as long ago as 1926 by Channon (13) and he showed that when this hydrocarbon is fed to rats there is an increase in the amount of unsaponifiable matter, including cholesterol, in the liver. Modern isotopic tracer studies by Bloch (4), Chaikoff (14), Popják (12) and their various collaborators have thrown much light on the possible changes involved.

2. *In vitro*, squalene inactivates free sulphydryl groups and inhibits succinic dehydrogenase (Flesch (15)).

3. When applied to the skin of certain animals (namely the rabbit and guinea pig) squalene has a depilatory action which is reversible (Flesch (15)).

4. Squalene is fungistatic *in vitro* against certain dermatophytes (Sobel, Marmoston and Arzangoolian (16)).

The metabolism of squalene in man is still obscure, but it seems probable that in man, as in other animals, there is a close metabolic relationship between squalene, cholesterol and possibly certain other steroids. The synthesis of cholesterol from acetate has been demonstrated in man by Hellman, Rosenfeld and Gallagher (17), and Eidinoff, Rosenfeld, Knoll, Marano and Hellman (18) also demonstrated radioactivity in sebum squalene after administration of C^{14}-labelled acetate. These results suggest that cholesterol synthesis in man probably proceeds by the same mechanism as in other animals and also that sebum squalene is most probably of endogenous origin.

The possible role of squalene in sebum has yet to be elucidated. The present experiments have shown that when applied to the skin it does not cause hair loss in man, neither will it inhibit skin pigmentation *in vivo*. Furthermore it appears to have no effect on keratinization. The differences between the amounts of squalene in the sebum of men and women are not sufficient to explain the cause of male baldness, and in the male subjects selected there appeared to be little difference between those with normal hair growth and those showing a tendency to baldness. On the other hand there were wider variations in the men than in the women. It has recently been observed by Nicolaides and Rothman (19) that the hair fats from a group of female subjects contained rather more squalene than did those from a similar group of male subjects, and also that the squalene content of the hair fat from children is much lower than that from adults. In the investigations described here similar differences have been found with sebum samples collected from the skin on the back.

It is possible that the excretion of squalene in human sebum represents the excretion of a metabolically useless substance or the elimination of the excess which may be formed in connection with the biosynthesis of cholesterol. Feeding of squalene might, therefore, be expected to increase the amount of squalene

in the sebum, but in our experiments this treatment has no significant effect on the concentration of squalene in sebum, nor is the total loss of squalene through the skin increased. The daily dosage given (1 g.) may have been too low to cause a detectable increase, though it was at least five times the estimated normal loss through the skin. There is also as yet no direct evidence of a relation between sterol metabolism in general and the metabolism of squalene and its excretion in sebum; since there are no consistent changes during the menstrual cycle (when rapid changes in the output of estrogens and similar steroid hormones occur) nor after feeding cholesterol. However, the marked difference between children and adults, in relation to the excretion of squalene in sebum, is consistent with such a relationship, and Rothman (20) suggests that this difference is due to the ability of children to carry out the biosynthesis of cholesterol more efficiently than adults. Lower overall synthesis of sterols may equally well explain this difference.

Amongst the significant positive findings which have come from the present studies are (*a*) wide daily variations occur in the squalene content of sebum, and (*b*) marked variations occur in the squalene content of sebum from different areas of the same subject. Similar daily variations in the cholesterol content and the iodine number of sebum from the forearm were observed by Washburn and Liese (21). This indicates that the composition of the surface film of fat on the skin is not constant but is continually changing. There has perhaps been a tendency to regard the 'fat mantle' as a rather static defense of the body against its environment. We have tacitly made this assumption when comparisons were made between normal subjects and those suffering with skin diseases. Such an approach may be incorrect and a study of the dynamics of sebaceous gland activity may be necessary to elucidate any role which sebum may play in the causation and prevention of skin diseases.

The bulk of the work described in this paper deals with the origin and function of squalene in sebum, and little further work has been done on the other hydrocarbon constituents. In view of the failure of repeated attempts to obtain a urea adduct from forearm sebum we believe that the solid hydrocarbon previously isolated by us from human sebum was a branched and not a straight chain paraffin, in spite of the evidence obtained by infrared spectrometry. This problem is being further investigated, but the present findings seem to indicate that the *n*-paraffins contained in sebum and human hair fat (Nicolaides and Rothman (22)) might also be of extraneous origin, and this would agree with later observations of Rothman and his colleagues (20).

Earlier findings indicated (MacKenna, Wheatley and Wormall (6)) the presence of possible cyclization products of squalene, and in this connection it is interesting to note the recent observation by Hougen (23) of the presence of polycyclic hydrocarbons in human hair fat. These last-named compounds are probably not metabolic products but arise from atmospheric soot; nevertheless it is interesting to speculate on their significance should they prove to be natural constituents of sebum. Also in this connection mention may be made of the observations by Sobel and Marmorston (24) that carcinogenic hydrocarbons are

destroyed in the presence of squalene, and that squalene is fungistatic *in vitro* against certain dermatophytes (16). These properties appear to be related to rapid peroxide formation which occurs when squalene is exposed to air. In earlier work we have also stressed (6) this rapid oxidation of squalene and it may yet prove to be the principle function of squalene in sebum. If this is correct, estimation of the squalene content of the surface film of fat would not reflect true differences in sebaceous gland activity. To study such differences it would be necessary to collect very fresh samples of sebum before any squalene was utilized in fulfilling its natural function on the skin. This may prove to be a more correct approach to the study of pathological sebaceous gland activity.

SUMMARY

1. Wide variations in the hydrocarbon content of sebum collected both from single subjects and from groups of individuals have been shown to exist.

2. The straight-chain paraffins previously reported to be present in human sebum are now believed to be external contaminants.

3. The squalene content of sebum of normal subjects of various ages and of individuals with certain skin diseases has been estimated. The results obtained with children were significantly lower than those for adults and there was a slight but not significant difference between those from men and those from women. No significant differences were observed between subjects with certain skin diseases and normal individuals.

4. No correlation between the menstrual cycle and the amount of squalene in sebum could be found, while the feeding of squalene or cholesterol to human subjects caused no alteration in the squalene content of the sebum.

5. Wide daily variations occur in the squalene content of sebum, and differences are observed in sebum from different areas of the same subject.

6. When applied to the skin of normal subjects and to that of subjects suffering with certain skin diseases squalene appears to have no effect on human skin and to be without therapeutic value in the conditions tried.

7. These observations are discussed and their possible significance evaluated.

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