The Possible Role of Squalene as a Protective Agent in Sebum*

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Human sebum contains squalene, which is a polyunsaturated triterpenoid hydrocarbon (2, 7). This substance constitutes approximately 5 per cent of skin surface sebum of the adult (2). Oxygen is taken up avidly when squalene is exposed to air, with the rapid formation of peroxides. This fact suggested that it might function as a protective agent in sebum. It has been demonstrated that it is fungistatic *in vitro* against certain pathogenic dermatophytes (10). When dimethylbenzanthracene and methylcholanthrene are exposed to squalene in air for several weeks, they are altered chemically so that the hydrocarbons can no longer be detected by fluorimetric measurements (8). Benzpyrene, on the other hand, may be recovered in theoretical amounts, but it in turn prevents the uptake of oxygen by squalene. These investigations were undertaken to determine whether the product(s) of reaction between methylcholanthrene (MCA) and squalene had retained its carcinogenic activity.

MATERIALS AND METHODS

Squalene (Eastman) was freshly purified by passage through alumina before use. The mineral oil was pharmaceutical grade liquid petrolatum. Solutions of 3-methylcholanthrene (Eastman) in squalene and mineral oil were prepared by adding 300 mg. of the carcinogen in 10 ml. of chloroform to 100 ml. of the solvent hydrocarbon. Five ml. of these solutions was introduced into pint-sized wide-mouth mason jars. The chloroform was removed under a stream of nitrogen. Similar quantities of the hydrocarbons alone were also introduced into the mason jars. These preparations were made up once a week and were placed in a 37.5° C. oven for 4 weeks.

The following preparations were used for painting the skin of mice: (*a*) benzene containing 0.3 per cent MCA; (*b*) mineral oil; (*c*) mineral oil containing 0.3 per cent MCA exposed at 37.5° C. for 4 weeks; (*d*) freshly purified squalene; (*e*) freshly purified squalene containing 0.3 per cent MCA; (*f*) squalene exposed at 37° C. for 4 weeks; (*g*) squalene exposed at 37° C. for 4 weeks to which 0.3 per cent MCA was added; (*h*) squalene containing 0.3 per cent MCA exposed together for 4 weeks. It was necessary to warm the preparations containing exposed squalene to approximately 40° C. to reduce the viscosity sufficiently to permit satisfactory painting.

The mice were C57BL and C57BR. They were painted on the back 3 times a week for 14 weeks and were then examined several times a week for the presence of tumors.

RESULTS

Carcinogenic activity of MCA after exposure with squalene.—There was no hair loss in the mice painted with mineral oil and squalene. However, hair loss occurred following painting with exposed squalene; but the hair regrew when painting was discontinued. This is of interest, since it has been reported that squalene causes hair loss in the rabbit (1). In some instances the newly grown hair was gray.

Groups 1 and 2 were observed for 32 weeks following the first painting. Group 3 was observed for 28 weeks, at which time all the surviving mice died during a heat wave. The results are shown in Table 1. No tumors appeared in the groups painted with mineral oil, fresh squalene, or exposed squalene. Mice painted with MCA in benzene, mineral oil, fresh squalene, and squalene that had been previously exposed were all found to have the usual expansive necrotizing tumors originating from several foci. Although certain differences appeared in the time of development of the tumors following each treatment (Table 1), these differences were not especially noteworthy.

No tumors appeared in Groups 1 and 2 following painting with MCA exposed with squalene. In Group 3, three mice had solitary papillomas which were well localized. One papilloma appeared on the 16th week of the experiment, but it did not increase in size. On the 26th week it became detached from its base. The second papilloma ap-

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peared on the 23d week and the third on the 25th week. Both were well circumscribed. The histopathologic diagnosis was squamous-cell papilloma with foci of atypical changes. In the first and second tumor the dyskeratotic changes were insufficient to label them carcinoma *in situ*. In the third an area of carcinoma *in situ* was observed.

It is obvious from the data that a complete or nearly complete loss of carcinogenic activity occurs following exposure of MCA with squalene.

C^{14}-labeled carcinogen and squalene.—To investigate the fate of the carcinogenic agents when exposed to squalene, C^{14}-labeled methylcholanthrene and C^{14}-labeled dimethylbenzanthracene were incorporated into the incubation mixtures as previously described (8).

Samples (100 mg.) of freshly purified squalene containing 500 μg. of carcinogen and .04 μc. of the C^{14}-labeled homolog were exposed to air at 37° C. for 4 weeks in 35-ml. round-bottomed centrifuge bottles. For control purposes, similar preparations were made with the squalene replaced by mineral oil. At the end of the incubation period, 10 ml. of methanol was added, followed by 3 ml. of cold 1 N sodium hydroxide. The mixture was extracted 6 times with 5-ml. portions of petroleum ether. The petroleum ether extract obtained under these conditions (Fraction 1) had previously been shown to contain all the unchanged carcinogen (8). Ten ml. of water was added to the aqueous phase, and the mixture was extracted 6 times with 5-ml. portions of ether (Fraction 2). The aqueous phase was then treated with 0.5 ml. of 10 N sulfuric acid and again extracted 6 times with ether (Fraction 3).

The recovery of radioactivity is shown in Table 2. When mineral oil was used as the solvent, a nearly quantitative recovery of radioactivity was found in Fraction 1. To determine if the radioactivity in this fraction was associated with the presence of unaltered carcinogen, 100 μg. of the corresponding fresh carcinogen was added to Fraction 1. The mixture was now subjected to chromatography on alumina. Upon fractional elution with 20 per cent benzene in petroleum ether, the radioactive substance was found to behave in a fashion identical to the unaltered car-

TABLE 1

SUMMARY OF RESULTS OF PAINTING MICE WITH EXPERIMENTAL SOLUTIONS

Treatment	Experimental group*	No. mice	Strain†	First tumor (weeks)	50 per cent with tumors (weeks)	100 per cent with tumors (weeks)
Benzene+MCA‡	1	17	BL	8	10	14
Mineral oil	1	16	BL			
Mineral oil+MCA	1	17	BL	13	17	19
	2	16	BR	11	14	20
	3	16	BR	9	14	17
Fresh squalene	1	14	BL			
	2	15	BR			
	3	18	BR			
Fresh squalene+MCA	1	17	BL	9	12	15
	2	8	BR	8	9	11
	3	12	BR	7	9	12
Exposed squalene	1	14	BL			
	2	12	BR			
	3	12	BR			
Exposed squalene+MCA	2	10	BR	10	13	16
	3	13	BR	5	14	18
(Squalene+MCA) exposed	1	18	BL			
	2	14	BR			
	3	12	BR	16§		

* All of the animals in Group 1 were run simultaneously; this was also true for Groups 2 and 3. Groups 1 and 2 were observed for 32 weeks; Group 3 for 28 weeks.

† BL refers to C57BL; BR to C57BR.

‡ MCA concentration, 0.3 per cent.

§ See text for details.

TABLE 2

RECOVERY OF C^{14} AFTER INCUBATION OF CARCINOGEN WITH SQUALENE (SQ) AND MINERAL OIL (M)

Fraction		DMBA Sq	DMBA M	MCA Sq	MCA M
		(per cent)			
1	Neutral pet. ether extract	22	97	10	96
2	Neutral ether extract	60	2	34	2
3	Acidic ether extract	18	1	56	2

cinogen, which was followed by its fluorescence. This indicated that when mineral oil was used almost no chemical alteration had taken place in the carcinogen, as was demonstrated previously (8). The squalene-containing preparations with DMBA and MCA yielded, respectively, 22 per cent and 10 per cent of their total radioactivity in Fraction 1. However, when subjected to the procedure indicated above, it was found that in both instances the radioactivity resided in a considerably more polar fraction and the recovered fluorescent fractions were free of radioactivity. Thus, in conformity with previous observations (8), DMBA and MCA were completely altered chemically.

TABLE 3

SQUALENE IN FOREHEAD FAT OF NORMAL INDIVIDUALS AND OF INDIVIDUALS WITH CANCER OF THE FACE OR SCALP

(mg/sq cm of skin)

	No.	Range	Mean	"t"
Controls	8	1.7– 9.7	5.5±2.9*	
Basal-cell carcinoma	8	6.3–13.7	9.5±2.9	1.36
Squamous-cell carcinoma	5	1.8–3.9	2.4±1.3	1.26

* Standard deviation.

Squalene in surface fat of patients with cancer of the face and scalp.—Since MCA lost its ability to cause cancer on exposure to squalene, an investigation was undertaken to determine whether individuals with skin cancer produce less squalene in their sebum. In preliminary studies, the squalene content of the surface fat of the forehead was determined in individuals with cancer of other parts of the face or scalp.

The patient was instructed not to wash or touch the face on rising in the morning. In the late afternoon an area of approximately 60 square cm. was delineated on the forehead by means of a plastic sheet from which a suitable section was cut out. The exposed area of the skin was washed 4 times in a reproducible fashion with folded alcohol-soaked filter paper held with a forceps. The filter paper was covered with 5 ml. of ethyl alcohol and 2 ml. of 6 N potassium hydroxide. After standing overnight, the mixture was extracted 4 times with 4-ml. portions of petroleum ether. The extract was centrifuged to remove the water, and an aliquot was taken for analysis for squalene by the procedure previously described (7).

The results are shown in Table 3. Patients with basal-cell carcinoma had, on the average, more squalene; those with squamous-cell carcinoma, less squalene than the individuals of a similar age group who were free of skin lesions. However, the differences were not statistically significant.

DISCUSSION

It has long been held that the lipid surface film of the skin serves in some protective capacity. Its fungistatic and bacteriostatic action has been ascribed to the presence of fatty acids (5, 6). Squalene may serve as a potent agent in these respects (9).

There is certain evidence that sebum may condition the response to exposure of the skin to carcinogenic hydrocarbons. For example, when lanolin is used as a solvent for MCA, its carcinogenic activity is markedly inhibited. Such inhibition was not observed when human sebum obtained from ovarian dermoid cysts was used as a solvent (4).

In the experiments in which human sebum was used as a solvent for MCA (4) the sebum was freshly obtained, and the MCA-containing solutions were maintained so that contact with air was avoided. From the data presented previously (8) and the investigations presented herein, it is obvious that, when methylcholanthrene is exposed along with squalene, it becomes chemically altered and loses its ability to cause cancer, but it is necessary for the squalene to become peroxidized.

The results obtained with the C^{14}-labeled carcinogens suggest that they have in turn been oxidized. Coupled peroxidation of unsaturated fatty acids with carcinogens has been previously reported (3). It is too soon to speculate on the nature of the reaction products of MCA, but an acidic or phenolic fraction has been produced as well as two neutral fractions which have a considerably greater polarity than MCA.

Although the findings are of a preliminary nature, it is of interest that individuals with squamous-cell carcinoma appear to have less squalene in the surface fat than is normal. This form of cancer is usually produced by MCA. It remains to be determined whether this is related to a diminished squalene content of the sebum or to a diminished sebum production. The latter is probably the case, since it has been established clinically that this form of cancer is associated with "dry skin."

SUMMARY

When a solution of MCA in squalene is exposed to air for several weeks, its carcinogenic activity is lost or considerably diminished. When C^{14}-labeled MCA and DMBA are treated in the same fashion,

no unchanged carcinogen can be detected, and a number of more highly polar substances are formed. Alcohol washings from the foreheads of normal individuals and individuals with cancer of the face and scalp were analyzed for squalene.

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