Influence of Age on Total Epidermal Lipid During Carcinogenesis Induced by Methylcholanthrene in Mice*

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In our first paper (1) we reported that epidermal cancers induced by cutaneous applications of methylcholanthrene appeared more quickly and in a higher percentage of animals in young than in old New Buffalo mice. The obvious conclusion reached was that the epidermis of these young New Buffalo mice was less resistant to the carcinogenic action of methylcholanthrene than the epidermis of old ones—that an age factor operates.

But in similar experiments young CBA mice developed cancer at almost identically the same rate and in the same percentage of animals as did the older ones. This very conspicuous similarity in the response of these young and old animals indicated that the age factor, if present, was somehow submerged and did not operate. Since the outstanding difference between the New Buffalo and CBA mice is the hereditary difference between the two strains, the conclusion seemed logical that heredity is an important factor in the production of cancer under these particular experimental conditions.

Further comparison of the two series showed that the young New Buffalo mice developed cancer more quickly and in a higher percentage of individuals than did the young CBA mice; whereas the old mice of both strains responded by cancer production in much the same way. Consequently the strain or hereditary factor was strongest in the young, since the young mice of the two strains showed a signal difference in their response to tumor formation by methylcholanthrene whereas the response of the old mice of both strains was essentially the same.

The next step in the study of these age and hereditary factors evidently was to investigate the chemical composition of the reacting epidermises in the hope of finding out whether chemical composition of epidermis conditions the production of cancer. We started to work with calcium because in the major research project of Barnard Hospital (2), which is to integrate the changes that take place in epidermal carcinogenesis induced by methylcholanthrene, a decrease to approximately 50 per cent of normal was observed in epidermal calcium (3); and also for the reason that calcium is a kind of strategic element in physiological processes likely to be associated with basic phenomena such as those of aging.

In this second contribution (4) we first determined the average calcium content in mgm. per 100 mgm. of epidermis, which was as follows:

	New Buffalo	CBA
Young	0.043	0.040
Old	0.054	0.055

Apparently the degree of malignant epidermal response to methylcholanthrene does not depend on the calcium content of the reacting epidermis; for although young New Buffalo mice, with less epidermal calcium, responded more actively than old ones, with more epidermal calcium, the response was about equal in young and old CBA mice despite the difference in epidermal calcium. Again, in these analyses the hereditary factor appeared because the depression of calcium content in hyperplastic stages of epidermal carcinogenesis was greater in New Buffalo mice than in CBA. The decrease in epidermal calcium was about the same in young and old New Buffalo mice despite the differences in response by cancer formation. Moreover, the decrease was less in young than in old CBA mice although the epidermal response was practically the same, so that the concentration of calcium in epidermis does not condition either the speed or the percentage incidence of cancer development.

The experiments herein described relate to the lipid content of the reacting epidermis, and stem likewise from previous work on the major project, in which Wicks and Suntzeff (5) showed that the total lipid-protein nitrogen ratio of the reacting hyperplastic epidermis of young New Buffalo mice decreased 50 to 60 per cent. Moreover, we could think of many

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possible reasons why differences in lipid concentration of epidermis might prove a potent factor. The purpose of these experiments, therefore, was to discover whether any difference exists in the decrease in epidermal lipid in young and old mice of the two strains subjected to the same carcinogen.

EXPERIMENTAL PROCEDURE

Because Wicks and Suntzeff (5) employed the same New Buffalo strain of mice, 3 to 4 months old and treated in the same way with methylcholanthrene in benzene as in our own previous experiments, it did not seem necessary to repeat their work although they determined the total lipid-protein nitrogen ratio whereas we have measured the mgm. of total lipid per 100 mgm. of fat-free epidermis.

Their results are summarized on the left in Table II. It is a simple matter to calculate the percentage decrease in their ratio, as it is the percentage decrease in mgm. of total lipid per mgm. of protein nitrogen.

The method of painting with the carcinogen and the removal of the epidermis from the dermis was the same as that previously employed (1). The total lipid was extracted from the epidermis by refluxing it twice for 2 hours with 25 cc. of a solution containing 3 parts of 95 per cent alcohol and 1 part of reagent grade chloroform. The alcohol-chloroform mixture containing the lipid was then evaporated to dryness on a steam bath, the total lipid re-extracted with petroleum ether (b. p. 30 to 60° C.), and the latter evaporated to dryness on a steam bath in a light weight, 50 cc., glass stoppered, Pyrex Erlenmeyer flask. The total lipid was then weighed to the nearest milligram. As a basis of reference the fat free epidermis was dried at 105° C. to constant weight.

The effect of the benzene alone on epidermal lipid was not investigated, because Wicks and Suntzeff (5) found an average reduction in lipid-protein nitrogen ratio only from 5.25 to 5.17, which is not significant.

RESULTS

The results for old and young mice of the CBA strain are shown in Table I. The mgm. of total lipid per 100 mgm. of dry, fat-free epidermis for the young mice was 29.3, which dropped to 15.0, a decrease of 49 per cent, when the mice had received 3 applications of the carcinogen. The diminution of total lipid of 47 per cent of normal at 17 days after 6 treatments was about the same as that of the group that had received 3 treatments. These results are essentially in agreement with those of Wicks and Suntzeff (3), who found a 50 to 60 per cent decrease in the total lipid-protein nitrogen ratio for hyperplastic epidermis of young New Buffalo mice. In the old age group the amount of total lipid was 32.2 mgm. per 100 mgm. of dry, fat-free epidermis, which fell to 14.7, a decrease of 54 per cent of normal, after the epidermis had been treated 3 times with the carcinogen. However, the total lipid decreased to 10.7 mgm. per 100 mgm. at 17 days after 6 applications, a drop of 68 per cent of the normal. Here there is a greater drop in the total lipid in the old CBA mice than in the young.

The data for the New Buffalo mice are given in

TABLE I: TOTAL LIPID-FAT FREE DRY WEIGHT RATIO OF MOUSE EPIDERMIS, STRAIN CBA

Young mice (3 to 4 mos.)						Old mice (12 to 13 mos.)					
No. of mice	No. of paintings	Time after first treatment to killing of mice, days	Total lipid, mgm.	Fat-free, dry epidermis, mgm.	Mgm. of total lipid per 100 mgm. of fat-free epidermis, mgm.	No. of mice	No. of paintings	Time after first treatment to killing of mice, days	Total lipid, mgm.	Fat-free, dry epidermis, mgm.	Total lipid per 100 mgm. of fat-free epidermis, mgm.
NORMAL, UNTREATED MICE						NORMAL, UNTREATED MICE					
12			35	109	32.0	9			43	133	32.2
12			36	141	25.5	13			57	194	29.4
12			46	125	36.8	18			102	291	35.0
10			32	118	27.1	40	(total)			Average	32.2
11			36	143	25.2						
57	(total)			Average	29.3						
METHYLCHOLANTHRENE-TREATED MICE						METHYLCHOLANTHRENE-TREATED MICE					
10	3	10	26	186	14.0	13	3	10	59	397	15.0
10	3	10	25	185	13.3	10	3	10	43	300	14.3
12	3	10	61	365	19.5					Average	14.7
9	3	10	31	237	13.0						
41	(total)			Average	15.0						
11	6	17	25	164	15.2	8	6	17	22	224	9.8
11	6	17	28	173	16.2	8	6	17	26	219	11.8
22	(total)			Average	15.6	10	6	17	26	249	10.4
						26	(total)			Average	10.7

Table II. The epidermis of old New Buffalo mice contained 32.2 mgm. of total lipid per 100 mgm. of dry, fat-free epidermis, the same as that of the old CBA. Three applications of methylcholanthrene reduced this value to 10.8, a drop of 66 per cent of normal, and 6 treatments to 9.6, a decrease of 70 per cent. The decrease in the total lipid of the old mice of the CBA and New Buffalo strains was almost the same at 17 days, that is, about 70 per cent of normal. On the other hand, the diminution of total lipid in young CBA mice was about 48 per cent of normal. Although our results are not strictly comparable to those of Wicks and Suntzeff, since the latter used protein nitrogen as a basis of reference, the decrease (50 to 60 per cent) that they found for young New Buffalo mice agrees well with our values for young CBA. The effect of benzene alone was not studied, since it has already been shown that the solvent for the hydrocarbon has no appreciable effect on the total lipid (3).

In our second paper (4) we reported that depression in epidermal calcium in both young and old New Buffalo mice was greater than that of both young and old CBA mice, indicating a definite difference in the response as far as calcium is concerned, but this was not linked with a difference in response as measured by tumor production.

The data presented in this, the third contribution, show that the depression in epidermal lipid in both young and old New Buffalo mice was about the same as that in both young and old CBA mice respectively. These observations reveal that the decrease in the total lipid was not paralleled by a difference in epidermal responsiveness to tumor production by the carcinogen.

SUMMARY

The role of total lipid as a factor in the age differences to the response of epidermis in old and young mice of the New Buffalo and CBA strains to methylcholanthrene has been investigated.

The decrease in the total lipid on the basis of dry, fat-free epidermis in the hyperplastic epidermis of old CBA mice was about 70 per cent of normal, while the drop in the young was nearly 50 per cent. In the New Buffalo groups under identical treatment the diminution in total lipid of the hyperplastic epidermis of the old group was 70 per cent of normal, while in the young group the decrease in the total lipid-protein nitrogen ratio was about 60 per cent of normal. No relationship was found between the decrease in total lipid content and the susceptibility to tumor production.

TABLE II

LIPID-PROTEIN NITROGEN RATIO OF MOUSE EPIDERMIS *

No. of mice	No. of paintings	Time after first treatment to killing of mice, days	Total lipid, mgm.	Protein N	Lipid-Protein N ratio
YOUNG MICE (3 TO 4 MOS.)					
NORMAL, UNTREATED MICE					
8			26.8	5.3	5.06
9			55.8	11.2	4.98
14			61.4	12.0	5.10
12			49.3	10.4	4.74
6			34.9	5.2	6.71
8			50.7	10.3	4.92
57	(total)			Average	5.25
METHYLCHOLANTHRENE-TREATED MICE					
8	1	10	43.8	20.6	2.13
8	1	10	49.0	15.1	3.24
10	1	10	43.7	19.8	2.21
26	(total)			Average	2.53
5	2	10	40.8	16.2	2.52
8	2	10	44.4	31.1	1.43
7	3	10	49.5	37.1	1.33
9	3	10	45.6	17.6	2.59
5	4	10	43.4	21.5	2.02
11	6	14	54.0	22.9	2.36
4	7	15	40.0	16.2	2.47
49	(total)			Average	2.10

* Data from Wicks and Suntzeff (3).

TOTAL LIPID-FAT FREE DRY WEIGHT RATIO OF MOUSE EPIDERMIS

No. of mice	No. of paintings	Time after first treatment to killing of mice, days	Total lipid, mgm.	Fat-free, dry epidermis, mgm.	Total lipid per 100 mgm. of fat-free epidermis, mgm.
OLD MICE (12 TO 13 MOS.)					
NORMAL, UNTREATED MICE					
11			46	158	29.0
11			49	163	30.0
15			52	185	33.5
15			57	158	36.1
52	(total)			Average	32.2
METHYLCHOLANTHRENE-TREATED MICE					
9	3	10	35	423	8.2
8	3	10	58	491	11.8
12	3	10	83	721	11.5
29	(total)			Average	10.8
5	6	17	21	268	8.0
9	6	17	23	206	11.1
14	(total)			Average	9.6

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