

Influence of Age on Calcium in Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice*

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The major research project of Barnard Hospital is to integrate the changes that occur in experimental epidermal methylcholanthrene carcinogenesis in mice. A summarizing report of progress has recently been published (4). It is important to determine the influence of age on the kind of carcinogenesis. We have already found that cutaneous applications of 0.6 per cent methylcholanthrene produce cancers more quickly and in a higher percentage of young than of old New Buffalo mice, whereas the response of young and old CBA mice (a strain more resistant to this carcinogen) is approximately the same (3). It therefore appears that hereditary constitution can submerge the age influence. Since other experiments in the major project have demonstrated significant decreases in epidermal calcium and iron in New Buffalo mice treated with methylcholanthrene (1, 2, 6) the following experiments were made to ascertain the correlation, if any, between this difference in the response of young and old mice of these two strains and changes in epidermal calcium.

EXPERIMENTAL PROCEDURE

New Buffalo and CBA strains of mice of both sexes were used. For each strain there were two age groups: one 3 to 4 months old and the other 12 to 13 months old. The method of application of carcinogen already has been described (1). It has been somewhat modified from that used in our first experiments (3) in order to provide sufficient epidermis for analysis. The calcium content of completely ashed (at 450° C in a muffle furnace) samples was determined by the method (on a micro scale) of Lindner and Kirk (5). Previously we used nucleoprotein phosphorus as a basis of reference for the amount of tissue, but in this paper the results are expressed in mgm. of calcium per 100 mgm. of epidermis (wet weight). This change was made because experiments clearly demonstrated that quantitative alterations in calcium and other minerals were the same, whether wet weight or nucleoprotein

phosphorus was employed as the basis of reference, and wet weight is simpler.

RESULTS

Inspection of Table I shows that the epidermal calcium content of the old New Buffalo mice is about 18 per cent greater than that of young mice of the same strain. After 3 applications of the carcinogen the decrease in the calcium content of about 58 per cent was the same for both age groups. Furthermore, despite repeated applications for a period of 2 months, the diminution of calcium was maintained at about the same level for both groups.

The changes in the calcium content of epidermis from young and old CBA mice, brought about by similar treatment with methylcholanthrene, are shown in Table II. The old group had about 27 per cent more calcium than the young group to start with. The diminution in calcium content induced by 3 applications of the carcinogen in the young CBA was about 30 per cent, while the drop in the old group was some 44 per cent. Repeated treatments of the epidermis of the old mice for 6 weeks, 1 month, and 2 months produced a decrease in the calcium content by 35 per cent, 44 per cent, and 36 per cent respectively, while in the young group under identical conditions the drop in calcium amounted to 20 per cent, 20 per cent, and 18 per cent respectively, or about 50 per cent less than that of the old group. Treatment of the young CBA mice for 3 and 4 months did not cause a further drop in the calcium content.

The results of all the analyses are shown graphically in Fig. 1, in which the percentage of decrease in calcium is plotted against the time. Upon treatment of the epidermis of both age groups of the New Buffalo strain with the carcinogen, the diminution of the calcium was pronounced, and remained essentially the same up to 60 days, at which time the mice had received 24 applications. On the other hand the drop in the calcium content for both age groups of the CBA strain was distinctly less after 3 treatments with the carcinogen, a greater decrease occurring in the old group. The initial diminution produced by 3 treat-

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TABLE I: CALCIUM CONTENT OF YOUNG AND OLD NEW BUFFALO MICE

Young mice (3-4 months old*)				Old mice (12-13 months old)			
No. of mice	No. of paintings	Time after first painting to killing of mice, days	Ca per 100 mgm. tissue, mgm.	No. of mice	No. of paintings	Time after first painting to killing of mice, days	Ca per 100 mgm. tissue, mgm.
NORMAL, UNTREATED MICE							
7			0.035	10			0.044
8			0.042	8			0.047
11			0.050	10			0.066
11			0.040	10			0.057
10			0.040	9			0.061
12			0.052	8			0.050
59 (total)			Average 0.043	55 (total)			Average 0.054
METHYLCHOLANTHRENE-TREATED MICE							
7	3	10	0.019	6	3	10	0.022
4	3	10	0.019	6	3	10	0.023
5	3	10	0.017	7	3	10	0.023
4	3	10	0.019	—			—
20 (total)			Average 0.019	19 (total)			Average 0.023
5	6	20	0.016	5	6	20	0.020
5	6	20	0.011	5	6	20	0.023
7	6	20	0.023	—			—
7	6	20	0.013	10 (total)			Average 0.022
24 (total)			Average 0.016				
5	12	30	0.016	10	12	30	0.018
5	12	30	0.012	11	12	30	0.018
7	12	30	0.019	—			—
7	12	30	0.014	21 (total)			Average 0.018
24 (total)			Average 0.015				
5	24	60	0.022	8	24	60	0.025
6	24	60	0.021	7	24	60	0.019
5	24	60	0.023	—			—
16 (total)			Average 0.022	15 (total)			Average 0.022

* From previous publication (5).

TABLE II: CALCIUM CONTENT OF YOUNG AND OLD CBA MICE

Young mice (3-4 months old*)				Old mice (12-13 months old)			
No. of mice	No. of paintings	Time after first painting to killing of mice, days	Ca per 100 mgm. tissue, mgm.	No. of mice	No. of paintings	Time after first painting to killing of mice, days	Ca per 100 mgm. tissue, mgm.
NORMAL, UNTREATED MICE							
10			0.045	10			0.054
8			0.046	8			0.052
11			0.036	9			0.054
13			0.035	8			0.059
12			0.039	—			—
12			0.037	35 (total)			Average 0.055
—			—				—
66 (total)			Average 0.040				
METHYLCHOLANTHRENE-TREATED MICE							
7	3	10	0.034	6	3	10	0.035
8	3	10	0.029	5	3	10	0.033
7	3	10	0.033	5	3	10	0.026
8	3	10	0.034	—			—
10	3	10	0.024	16 (total)			Average 0.031
10	3	10	0.021				
8	3	10	0.022				
—			—				—
58 (total)			Average 0.028				
7	6	20	0.030	7	6	20	0.039
9	6	20	0.036	7	6	20	0.033
8	6	20	0.033	—			—
7	6	20	0.029	14 (total)			Average 0.036
—			—				—
31 (total)			Average 0.032				
8	12	30	0.034	7	12	30	0.028
8	12	30	0.031	8	12	30	0.035
—			—				—
16 (total)			Average 0.032	15 (total)			Average 0.032
6	24	60	0.029	6	24	60	0.032
7	24	60	0.031	6	24	60	0.038
8	24	60	0.036	—			—
—			—	12 (total)			Average 0.035
21 (total)			Average 0.033				
8	36	90	0.031				
9	36	90	0.030				
—			—				—
17 (total)			Average 0.030				
17	48	120	0.030				

ments in both age groups was not further accentuated by painting the epidermis at regular intervals for 2 months.

These determinations of the epidermal calcium content of young and old mice of both strains do not afford an explanation of the observation described in a previous paper (3), that young New Buffalo mice

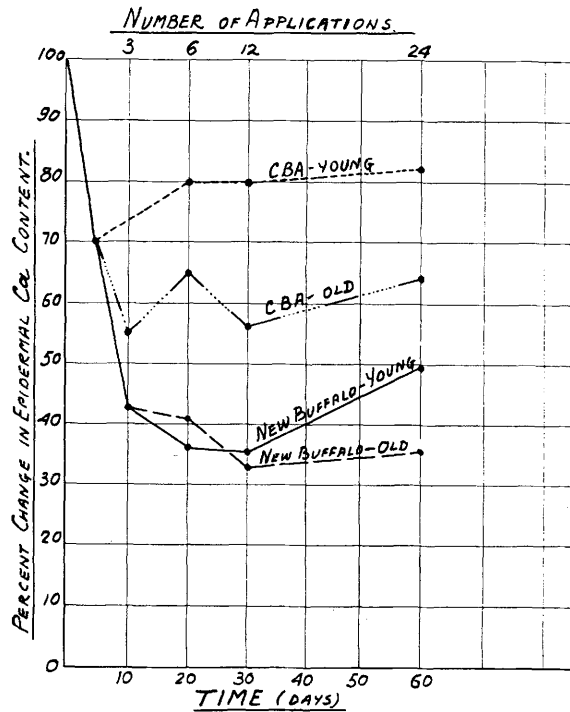


FIG. 1

develop tumors earlier in a higher percentage of individuals than do the old ones, for there is no significant difference in decrease in the calcium content between the young and old groups. But there is some correlation between tumor development and depression in epidermal calcium in the CBA strain, since the older mice develop tumors somewhat earlier and in a higher percentage than do the young, while at the same time there is a greater fall in the calcium content in the older than in the younger group.

Microscopical changes of the epidermis of both strains treated with the carcinogen were more or less similar, except that after 2 months the epidermis of the old CBA mice was much less hyperplastic than that of the old New Buffalo group. It is not only probable, but quite certain, that factors other than the calcium content operate in epidermal carcinogenesis and may explain the differences in response to carcinogen, not only between the young and old mice of each strain, but also between both strains.

SUMMARY

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

Old mice of both strains were found to contain more epidermal calcium than the young. The epidermis of both age groups of the New Buffalo strain responded similarly when treated with methylcholanthrene; that is, they underwent a nearly 50 per cent decrease in the calcium content. However, the diminution in the calcium content of the CBA strain was less than that of the New Buffalo, and the young group showed about 50 per cent less drop than did the old.

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