Changes in Epidermal Hydroxyproline and Tyrosine Accompanying Induced Epidermal Carcinogenesis

I. GORDON FELS AND JOSEPH GRECO

(Radioisotope Service, Veterans Administration Hospital, Hines, Illinois)

SUMMARY

A marked and progressive decrease in epidermal hydroxyproline accompanied induced epidermal carcinoma by methylcholanthrene in C3H mice. A decrease was also noted in the benzene-treated controls; but this was reversible, whereas the effect produced by the carcinogen was not. A progressive increase in epidermal tyrosine was observed which was more marked in the carcinogen-treated animals than in the controls.

Acid hydrolysates of the carcinogen-treated skins produced a darker, humin-like coloration than did those of the controls. The possible origin of this is discussed. The results are interpreted in terms of the sensitivity of fibroblasts to methylcholanthrene.

Several authors have reported a marked decrease in collagen of rodent skin following tumor formation. This has been found to be true both for tumors induced with methylcholanthrene (13) as well as for transplanted tumors (5, 10). The degeneration of collagen fibers accompanying induced tumors had been observed much earlier by Orr (15), who also believed that the carcinogen prevented the occurrence of an adequate fibrous reaction (16).

These findings, in addition to being of considerable interest in themselves, point to a system whereby alterations in a well characterized protein may be followed with tumor induction. This is readily achieved chemically by the determination of hydroxyproline and an aromatic amino acid such as tyrosine. Both are characteristic of collagen, although in different ways. Hydroxyproline is found virtually nowhere else but in collagen, and tyrosine is almost completely absent in this protein. Epidermal changes in these two constituents are reported herein, accompanying induced tumor formation with methylcholanthrene.

MATERIALS AND METHODS

The painting technic of Cramer and Stowell (1) was adopted with male C3H mice weighing approximately 30 gm. Methylcholanthrene dissolved in benzene in a concentration of 0.6 per cent was applied 3 times a week to an area of skin extending from the nape of the neck to the rump. Each application deposited about 0.1 mg. methylcholanthrene to the area. This was continued for 90 days, after which time the applications were halted. Control animals received only benzene for the same period of time. At regular intervals three animals from each group were sacrificed, and the painted area, free of hair, was removed. This was then defatted with acetone-ether and dried in a desiccator. The specimens then were hydrolyzed in 6 N HCl in sealed vials, and neutralized aliquots of the hydrolysate were used for analysis. Tyrosine was determined spectrophotometrically (8), and hydroxyproline was assayed by the method of Neuman and Logan (14) with applied corrections for tyrosine content. The average values were expressed in terms of nitrogen, determined by the recent ninhydrin procedure (7). In general the determinations within a group of animals varied less than 10 per cent for hydroxyproline, 20 per cent for tyrosine, and 10 per cent for nitrogen. The variation of duplicate determinations on the same specimen, however, was approximately one-tenth of these percentages.

Indirectly related to these studies is the observation that the acid hydrolysates of the methylcholanthrene-treated skins were invariably darker than those of the controls. This was not because of the presence of the carcinogen, since normal skins

* Presented on September 15, 1960, at the 138th annual meeting of the American Chemical Society, New York, N.Y.

Received for publication July 5, 1960.
hydrolyzed in the presence of methylcholanthrene did not produce the dark hydrolysate. The optical densities of these hydrolysates were measured at 600 m\(\mu\) and were expressed in terms of the nitrogen content of the sample.

RESULTS

The morphologic and histologic changes accompanying induced cutaneous carcinoma have been described amply in the literature (1–3). The subject has been reviewed recently by Stewart (18) and will not be emphasized here. In general, the carcinogen-treated group developed papillomas some time after 2 weeks of painting. This was followed shortly by ulceration. Malignant tumors became evident only after 2–3 months.

The progressive changes in epidermal hydroxyproline in the experimental and control animals are given in Chart 1. Immediately apparent was the sharp drop in this constituent during the painting period. This was true for the benzene-treated controls as well as for the experimental animals, although the effect was more marked in the latter group. The distinctive differentiation between the two groups was demonstrated after the cessation of painting. The control animals, apparently still possessing the capacity for recovery, were capable of reconstituting the lost hydroxyproline. On the other hand, the methylcholanthrene-treated animals, after an abortive attempt at recovery, declined until the tissue was completely devoid of hydroxyproline.

The optical densities of the acid hydrolysates of the two groups are pictured in Chart 4. An emphatic separation of the two groups appeared to take place after 10 days. This became more marked with time, especially after the painting period.

DISCUSSION

The results of the present experiments are interpreted best in terms of a tissue reaction to injury. At present this is not possible at the molecular level. In the case of the control animals, the fibroblasts, which are believed to be concerned primarily with collagen synthesis, are capable of recovery...
after the toxic effects of benzene are removed. The effect of solvent and carcinogen, however, appears to be irreversible. The fact that the collagen values drop to zero would indicate that the advancing tumor is capable of destroying the pre-existing collagen. The possibility exists that the pre-existing collagen has been diminished on a percentage basis by the overgrowing tumor cells. However, the sensitivity of the chemical procedure, plus the negative results with undiluted aliquots of hydrolysate, make this possibility unlikely.

In the case of tyrosine, the elevated values of this amino acid in both groups of animals represents a new synthesis of tyrosine containing protein resulting from the chemical injury. The effect of carcinogen plus solvent is more marked than that of solvent alone. The cessation of painting, however, did not bring the control values back to normal, although this might have occurred in an experiment of longer duration.

One may visualize the effect of methylcholanthrene on skin as being multiple, this being so because of the heterogeneity of the cellular population. Of the several cell types present, the dermal fibroblasts have been shown to possess the greatest sensitivity to the toxic effects of the carcinogen (4, 6, 12), the effect depending upon the concentration.

Of particular interest is the observation that the acid hydrolysates of the carcinogen-treated tissues are darker in color than those of the benzene-treated controls. This was observed as early as the 10th day and became more marked with time. The dark coloration following acid hydrolysates of tissues is usually ascribed to humin formation, a condensation product of tryptophan and an aldehyde (9), in this case presumably of carbohydrate origin. Since no change in tryptophan content has been observed between methylcholanthrene tumors and normal, homologous tissue (17), one may presume that the difference in coloration is due to an enhancement of a carbohydrate constituent accompanying tumor formation.

Finally, it may be mentioned that Hamer and Marchant (11) found no changes in hydroxyproline or tyrosine after 12 weeks of painting of methylcholanthrene in acetone on outbred albino mice. Since no demonstrable tumors were produced, only "small warts," this negative result may be ascribed to a species difference. If one makes the acceptable assumption that the hydroxyproline content of skin reflects its collagen content, then the present results broadly verify those obtained by previous workers using other methods (5, 10, 13); namely, that the collagen content is decreased with tumor formation.

ACKNOWLEDGMENTS

The authors are grateful to Doctor Alice Dakin, Pathology Section, Laboratory Service, Veterans Administration Hospital, Hines, Illinois, for pathological appraisal of the experimental animals.

REFERENCES

3. ———. The Early Stages of Carcinogenesis by 20-Methylcholanthrene in the Skin of the Mouse. Ibid., pp. 379-402.


Changes in Epidermal Hydroxyproline and Tyrosine Accompanying Induced Epidermal Carcinogenesis

I. Gordon Fels and Joseph Greco


Updated version  Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/21/1/40](http://cancerres.aacrjournals.org/content/21/1/40)

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.