Lack of Extractable Carcinogens in the Skin of Patients With Multiple Precancerous Keratoses of Actinic Origin

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Although it is well known that ultraviolet light exerts a potent carcinogenic effect upon the skin, it is not known whether this effect is exerted directly upon a chromosome or a gene, or whether it is exerted indirectly through the formation of a carcinogenic compound. Indirect evidence that ultraviolet light does not exert its carcinogenic effect through the formation of a chemical resembling the carcinogenic hydrocarbons was provided by the observations of Rusch, Kline and Baumann (8) when they showed that in contrast to the additive effect between different carcinogenic hydrocarbons there was no additive effect between ultraviolet rays and carcinogenic hydrocarbons. Unpublished experiments by these same authors giving direct evidence of the lack of formation of carcinogenic chemicals by ultraviolet irradiation showed that benzene extracts of irradiated mouse skin or of irradiation tumors failed to induce neoplasms when injected subcutaneously in corn oil. Similarly, Passey (6) was unable to demonstrate carcinogens in extracts of the skins of rats irradiated for 1 year with ultraviolet rays. The observations of these workers were made with relatively small amounts of material so there was a possibility of insufficient dosage.

The possibility that the skin of patients with multiple precancerous keratoses of actinic origin might contain extractable carcinogenic chemicals was considered. The availability of relatively large areas of keratotic skin for extraction by washing with benzene offered the advantage of providing large amounts of material for exhaustive tests for possible carcinogenic activity. Furthermore, by repeatedly washing one side of the face with benzene and observing the incidence of carcinoma on that side as compared to the unwashed side it was reasoned that, if carcinogens were being formed, their presence would be reflected in a reduced incidence of cancerous change in the lesions on the washed side of the face.

MATERIALS AND METHODS

Selection of patients.—Twelve patients with multiple precancerous keratoses of the face were selected. All of these patients had previously developed at least one carcinoma and most had developed several carcinomas in their keratoses. Except for one 9 year old boy with xeroderma pigmentosum and one young woman with delayed xeroderma pigmentosum, the patients were elderly people with typical multiple actinic keratoses or the so-called "farmers' skin" or "sailors' skin".

The patients were given a supply of 2 X 2 inch gauze pads and a supply of benzene. They were instructed to wash one side of their faces with the benzene and to save the pads used for this purpose in a large bottle. The procedure was repeated daily for periods of from 20 to 706 days with an average period of 162 days.

In addition, small amounts of skin washings and excised keratoses from 25 other patients with multiple keratoses were pooled for extraction with benzene and assay for carcinogenic activity.

In most cases, a record was kept of the size and location of the keratoses on both sides of the face so that any change in size or development could be followed.

Preparation of the extract.—The pads containing the skin washings were continuously extracted with hot benzene in a Soxhlet extractor for a minimum of 8 hours. The benzene extract was then concentrated by heating over a water bath under vacuum. The final product was a thick yellow or brown oil which became semisolid in the refrigerator.

Tests for carcinogenicity.—The concentrated material made from the skin washings was tested for carcinogenicity in mice by the following means: (a) painting on the skin in benzene solution (b) painting on the skin in combination with croton oil (c) painting on the ear plus irradiation with ultraviolet light, and (d) subcutaneous injection in mice and rats; in addition whole keratoses were implanted subcutaneously.
RESULTS

Painting on the skin.—The material extracted from precancerous skin was painted on the skin of mice in two experiments.

In the first experiment, the material was dissolved in benzene so that 1 cc. of the benzene extract contained the amount of material washed from the skin in an average period of 1.7 days. Ten white mice derived from the Bar Harbor A.B.C. stock were used and the material was painted on the interscapular region after clipping the hair. Three strokes with a camel’s hair brush were made 3 times a week. This was continued until all the mice were dead. Four mice were still alive at the end of 1 year of painting and the last mouse died after 22 months. There were no developments suggestive of precancerous or cancerous changes in any of the painted mice. There was some scaliness and some epilation in a few of them but no more than would be expected from the benzene alone. No carcinomas were found elsewhere in the bodies of these mice.

The second experiment was carried out mainly for the purpose of determining if an increased dosage of the extract might result in the production of neoplasms. Therefore in this experiment, the dosage was increased so that 1 cc. of the benzene solution contained the material from 3 daily washings of the skin of the patients with precancerous lesions. For control purposes another group of mice were similarly painted with a benzene solution of 0.2 per cent of methylcholanthrene. Twenty mice were used in each group. After 6 months of painting 3 times a week, the mice painted with the skin extract showed no precancerous or cancerous changes whereas 100 per cent of the 14 remaining mice in the group which were treated with methylcholanthrene had developed carcinoma. The painting with the skin extract was continued and at the end of a year there still was no neoplastic change in the eight surviving mice. The last mouse died after two years of painting and in no instance did carcinoma develop. The only neoplasm to appear in the group was a lymphosarcoma of the spleen.

Painting on the skin in combination with croton oil.—From the group of mice remaining after a year of painting with the skin extract in the preceding experiment, 5 were selected for painting with croton oil in combination with the skin extract. The croton oil was added to the benzene solution of the extract in a concentration of 0.5 per cent.

The purpose of the croton oil, a co-carcinogen described by Berenblum (1) was to produce an inflammatory reaction which might elicit frank carcinoma from a possible latent or preneoplastic stage. Continuation of the painting for a period of 1 year, when the last mouse died, resulted in the formation of no carcinomas although there was considerable epilation, thickening of the skin, and some ulceration and crust formation. After 5 months of this combined painting there were 5 mice living, and at 9 months 2 survived.

Painting on the ear plus irradiation with ultraviolet light.—To determine whether the material extracted from precancerous human skin might have an augmenting effect on carcinogenesis by ultraviolet light, the right ears of 24 A.B.C. mice were painted with the material before irradiation, while the left ears were left unpainted. Ultraviolet irradiation was carried out three times a week in wire holders according to the technic of Rusch and his associates (7). In addition 25 mice which were not irradiated were also painted three times a week on the right ear with a benzene solution, 1 cc. of which contained the material from 1.7 daily washings.

Inasmuch as the time of onset of the carcinomas was essentially the same in the painted and unpainted ears no detailed tabulation of the results is given. By the fourth month the ears became thickened and nodules began to appear; by the eighth month 10 mice were left in the irradiated group and all had cancers affecting both ears about equally; after 1 year all had died of cancer. The neoplasms were sarcomas and squamous cell carcinomas and the incidence of the two types was similar in both ears.

No cancers were produced in the non-irradiated mice though the right ears were painted thrice weekly till the last mouse died. Nineteen mice were alive at 8 months, 4 were alive at 16 months and the last mouse died after 20 months. In no instance was any abnormality noted on the ears other than the occasional hyperkeratosis due to the benzene.

Therefore, the results showed no augmenting effect of the skin extracts upon ultraviolet carcinogenesis and the skin extracts by themselves again failed to induce cancer.

Subcutaneous injection in mice and rats.—The sebum extracted from the precancerous skins was injected in single doses into 20 mice and into 10 rats. The material was also injected in multiple doses into 30 mice. One cubic centimeter of the sebum represented the amount of material obtained.
from the skins of patients by daily washing for periods of from 22 to 65 days.

In a preliminary experiment one mouse received an injection of 0.25 cc. of the thick yellow oil while the second mouse received 0.35 cc. It was observed that this dosage was too great inasmuch as it led to the development of sterile abscesses and ulcers. Accordingly, the dosage was reduced to 0.1 cc. in a group of 8 mice and 0.05 cc. in 0.05 cc. of corn oil in a group of 10 mice. In addition 10 rats each received an injection of 0.15 cc. of the undiluted extract.

These single injections resulted in the formation of nodules that persisted for many months but in no instance did a neoplasm develop at the injection site. After 1 year there were 16 mice and 4 rats living. After 2 years only 2 mice and 2 rats were still alive. Several subcutaneous nodules, which had persisted for periods varying from 14 to 26 months, were studied microscopically and were found to consist of masses of amorphous material surrounded by dense fibroblastic proliferation. In no instance was neoplasm present. Two adenocarcinomas arose in the mammary glands and one sarcoma appeared at some distance from the injection site. Most of the mice developed persistent subcutaneous lumps and a few developed ulcers, but in no instance did a malignant neoplasm develop at the site of injection. The mice were kept until they died when they were examined to determine the cause of death. After a year 18 of the 30 mice were still living. After 18 months there are 4 mice living and none of these have any evidence of neoplastic change in the injection site. Only 1 neoplasm was observed—a fibrosarcoma which arose in the mammary gland at some distance from the injection site.

Accordingly, it is concluded that the sebum extracted from the skin of patients with multiple precancerous lesions contained no carcinogenic substances which could be demonstrated by subcutaneous injections in mice and rats.

**Implantation of whole keratoses.**—Senile keratoses excised from 9 different patients were implanted in the subcutaneous tissues of 20 Sprague-Dawley rats. After one year all 20 of the mice were still alive and many of them had nodules, but in none did cancer develop. After 18 months no neoplasms had developed in the 8 rats remaining; therefore, they were discarded. It is concluded that subcutaneous implantation of keratoses does not elicit neoplasia.

**Effect of washing the precancerous skin with benzene on the incidence of cancer.**—The patients were requested to wash only one side of their face with the benzene and to leave the other side unwashed as a control. The keratoses on both sides of the face were measured with calipers at each visit. When carcinoma developed, the lesions were excised by the chemosurgical technic (5).

Washing had but little effect on the keratoses; most of the lesions continued to enlarge, others regressed somewhat, but the same thing occurred on the unwashed side. One senile keratosis was observed to regress completely after vigorous washing with benzene. The patient stated that he felt stinging sensation in the lesion and it seems likely that the tissues were so defatted by the benzene that some of them were killed with resultant eradication of the keratosis.

The incidence of cancer in the 12 patients with multiple precancerous lesions was the same on both the washed and unwashed sides of the face. On the washed side there developed 3 squamous cell and 4 basal cell carcinomas while on the unwashed side there developed 2 squamous cell and 5 basal cell carcinomas. Therefore, washing the precancerous skin with benzene has essentially no effect either on the growth of the keratoses or on their tendency to become cancerous.

**DISCUSSION**

Although the foregoing experiments show a complete lack of carcinogenicity in the material washed with benzene from the skins of patients with multiple precancerous lesions, there remains the possibility that carcinogens might be extracted by other means. Benzene was chosen as the solvent because practically all of the substances known to be carcinogenic for the skin are soluble in it. However, although it seems improbable, it is conceivable that carcinogenesis by ultraviolet rays may be accomplished by the production of a water-soluble carcinogen.

It is also conceivable that the sought-for carcinogen might be so firmly bound to some cell constituent, possibly a protein, that it would not be obtained by simply washing the skin with benzene. Even if the carcinogen were not so bound, it is possible that the hypothetical carcinogen might be formed and retained in the basal cell layer from
which it might not be extractable by washing the surface of the skin. However, since sebum and desquamating horny scales are derived from basal cells this seems unlikely.

The possibility that the sebum itself might mask or inhibit the action of some carcinogenic substance is suggested by the findings of Simpson, Carruthers, and Cramer (10) who reported an inhibitory effect of lanolin upon methylcholanthrene carcinogenesis. However, Kline (7) has shown that the inhibitory effect of lanolin is lost when the methylcholanthrene and lanolin were dissolved in benzene and painted on the skin of mice. Therefore, since the material from precancerous skin was applied to the mice in benzene solution the sebum probably had no inhibitory effect on any carcinogenesis that might have taken place. Furthermore, Kline (4) has observed that carcinogenesis by benzpyrene is not inhibited by the use of lanolin as a vehicle for subcutaneous injection. Therefore, it seems unlikely that the sebum in the human skin extract would inhibit any carcinogen that might be present in the injected material. Further experiments to elucidate this point are in progress, however.

The lack of augmentation of ultraviolet carcinogenesis by the application just before irradiation of a benzene solution of the material from precancerous skin is evidence that this human sebum contained no special substance that was not already present, in the mouse at least upon which the rays might act to produce carcinogens.

One reason for persistently trying to demonstrate carcinogens in material washed from human skin was the well-known relation between non-circumcision and cancer of the penis which apparently is caused by the action of the retained smegma. A similar situation is the occasional origin of cancer in the lining of sebaceous cysts; in this case the sebaceous material appears to be the carcinogenic substance. If the materials thrown off from the skin and its appendages can act as carcinogens under such circumstances, why do the washings from precancerous skin fail to exhibit carcinogenic properties? One possible answer is that the rancidity which characterizes retained smegma and the contents of sebaceous cysts may be essential to the carcinogenicity of these materials.

Some success in the search for endogenous carcinogens has been attained by Schabad (9), Steiner (11), Hieger (3) and des Ligneris (2) but these workers usually used liver as the source of tissue. Steiner extracted burn and x-ray scars and found no carcinogenic activity, but he did observe 1 sarcoma in the region injected with material extracted from an epidermoid or sebaceous cyst (12). In the absence of demonstrable carcinogens the material extracted from precancerous skin was not subjected to spectroscopic or other analysis for chemicals similar to the carcinogenic hydrocarbons. However, the material was shown to have an intense, bluish fluorescence in ultraviolet light not unlike the fluorescence of keratoses.

The failure of washing with benzene to reduce the incidence of carcinoma in the skin of patients with multiple precancerous lesions is consistent with the failure to find carcinogens in the material washed from that type of skin.

SUMMARY AND CONCLUSIONS

Material washed with benzene from human skin affected with multiple precancerous keratoses contained no carcinogenic substances that could be demonstrated either by painting on the skin of mice or by injection into the subcutaneous tissue of mice or rats. Furthermore the material failed to augment ultraviolet carcinogenesis in mice when painted on the skin just before irradiation and it failed to produce precancerous changes from which carcinoma could be elicited with croton oil. Implantation of senile keratoses into rats also failed to demonstrate carcinogenic substances in these lesions.

Repeated washing of precancerous skin with benzene to remove hypothetical carcinogens did not reduce the incidence of cancerous change in the keratoses.

These findings are believed to be evidence in favor of the hypothesis that the carcinogenic influence of ultraviolet rays is exerted by a direct effect upon the cell constituent responsible for neoplastic change rather than by an indirect effect through the formation of a carcinogenic chemical similar to the carcinogenic hydrocarbons.

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