The Effect of Ultraviolet Irradiation on the Carcinogenic Potency of Certain Hydrocarbons*

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As the results of published investigations on concurrent application of carcinogenic agents and irradiation with ultraviolet, visible and infrared light (2, 6-8, 10-19) differ considerably, we have found it of interest to investigate the activity of different carcinogenic hydrocarbons after ultraviolet irradiation.

Cook and Martin (5), Dufraisse and Gerard (9) and Veluz (20) have shown that ultraviolet irradiation of carcinogenic hydrocarbons causes the formation of oxides that have the character of peroxides. Methylcholanthrene and 9, 10-dimethyl-1,2-benzanthracene are more readily photo-oxidized than 1, 2, 5, 6-dibenzanthracene and 3,4-benzyrene.

Cook and Martin (5) have shown that the photo-oxide of 9,10-dimethyl-1,2-benzanthracene is non-carcinogenic in mice. Boyland and Boyland (4) found that the alkali-soluble product of 1,2,5,6-dibenzanthracene after ultraviolet irradiation was non-carcinogenic in mice whereas Allsopp (1) found that the alkali-soluble product of 3,4-benzyrene after ultraviolet irradiation was carcinogenic in mice.

MATERIALS AND METHODS

In our investigation the hydrocarbons were dissolved in benzene and placed for 72 hours in open Petri dishes at a distance of 50 cm. from an air-cooled 350 watt mercury quartz lamp. After evaporation of the solvent the irradiation products were dissolved in ground nut oil for subcutaneous injection (dose 0.5 mgm. in 0.2 cc. of oil) and in benzene (0.5 per cent) for painting. Mice of the Street strain were used in all our experiments.

The irradiation products thus obtained are, from a chemical viewpoint, by no means pure compounds, but it can be supposed that these "crude mixtures" are in fact products of the same process (photo-oxidation?), as occurs on the skin of mice after concurrent application of ultraviolet light and carcinogenic hydrocarbons. Figs. 1 to 4 give the absorption curves of the compounds used.

1,2,5,6-Dibenzanthracene.—The absorption spectrum of this comparatively weak carcinogenic hydrocarbon is, as shown in Fig. 1, completely altered by irradiation since the irradiation products have lost their characteristic absorption band. Nonetheless, its carcinogenic power is reduced to only a quarter or even half that of the original hydrocarbon.

In the control group of animals painted with nonirradiated hydrocarbon, the first tumor appeared after 9 months. In the 14 mice surviving the 9 months, 7 instances of papillomas or carcinomas were found; whereas in the 20 mice painted with the irradiated hydrocarbon, only 5 tumors appeared, and strangely enough 2 of these were subcutaneous sarcomas at the site of application. One case of spontaneous mammary cancer appeared in both the experimental and control groups, the latter case being complicated by leukemia. In the last group there appeared also one case of gastric ulcer with initial cancer development.

Of the 26 control mice subcutaneously injected with 1 mgm. of hydrocarbon, 17 developed sarcomas (approximately 65 per cent); 1 of these was complicated by leukemia. Besides these, 1 case of mammary cancer occurred. Four cases of sarcoma (approximately 15 per cent) appeared in the group of 27 animals injected with the irradiation product. In this same group there arose 2 cases of mammary cancer (1 complicated by pulmonary adenoma), and 1 isolated case of pulmonary adenoma.

Boyland found that the alkali-soluble irradiation product of 1,2,5,6-dibenzanthracene was noncarcinogenic. It is not stated, however, how many mice are included, nor is the dose of the photo-oxide given. In our investigations the carcinogenic potency of...
the crude mixture of irradiation products showed the reduction to be only 25 to 50 per cent. This may be explained by assuming that the mixture contains unaltered hydrocarbon.

3,4-Benzpyrene.—From Fig. 2 it is seen that 3,4-benzpyrene loses its characteristic absorption band after irradiation, although the absorption intensity is not reduced to anything like the same degree as that of the irradiation product of 1,2,5,6-dibenzanthracene.

Among 21 animals painted with nonirradiated 3,4-benzpyrene, 20 tumors (9 papillomas and 11 carcinomas) were found. One of the carcinomas was complicated by leukemia. Of 22 animals painted with irradiated benzpyrene, however, only 11 developed tumors (3-papillomas and 8 carcinomas). The carcinogenic potency is thus reduced by nearly 50 per cent.

In 20 animals treated by subcutaneous injection of nonirradiated hydrocarbon, 7 cases of sarcoma were seen as compared with only 2 cases of sarcoma from 24 mice treated with irradiated benzpyrene.

One case of leukemia and 1 of pulmonary adenoma appeared in the control group, and among the animals treated with irradiated benzpyrene, 1 case of pulmonary cancer was observed.

Thus the difference in the effect is very pronounced. However, the irradiation product of this hydrocarbon, too, possesses no appreciable carcinogenic potency.

The reduction in the carcinogenic potency of 3,4-benzpyrene after irradiation is similar to that of 1,2,5,6-dibenzanthracene; namely, to about 50 per cent by painting and to 25 per cent by subcutaneous injection.

9,10-Dimethyl-1,2-benzanthracene.—By irradiation, the characteristic absorption band disappears and the absorption curve is depressed considerably (Fig. 3). From other investigations it is known that in mice of the Street strain 9,10-dimethyl-1,2-benzanthracene causes cancer in nearly 100 per cent of the animals. In the groups painted with the nonirradiated hydrocarbon, however, only a few tumors occurred because the very toxic effect of the vigorous treatment, the violent epilation, and emaciation caused the death of all animals within 5 months, and partly because the carcinogenic potency has been altered considerably. The change, however, seems to be of a somewhat different nature from that of the other hydrocarbons mentioned above. In the latter, (dibenanthracene and benzpyrene) irradiation effected a certain reduction of the carcinogenic power, but the tumors that appeared were of the same type as those produced by application of the original hydrocarbon. The effect upon the skin of the irradiated 9,10-dimethyl-1,2-benzanthracene is a development of minimal papillomas, which, in spite of continued painting, regressed in most cases or remained unchanged for months. Only 1 minimal, well defined, carcinoma occurred in a 12 months old mouse, and 2 subcutaneous sarcomas in mice 11 and 12 months old.

It seems as if the irradiation product of 9,10-dimethyl-1,2-benzanthracene has lost one component of its carcinogenic power but retained another. Bercnblum (3) supposes that “carcinogenesis is not a single process but consists of several component phases which may be dissociated.” Possibly this irradiation product is a compound which may bring about what Bercnblum calls proneoplastic hyperplasia, but is incapable of advancing the process further to manifest carcinoma as can the original hydrocarbon.

By oxidation of 9,10-dimethyl-1,2-benzanthracene with sodium bichromate (Na₂Cr₂O₇) the absorption spectrum is, as may be seen from C in Fig. 3, altered in quite another way than by irradiation. By painting with 9,10-dimethyl-1,2-benzanthracene ox-
dized with sodium bichromate no tumor development was seen among 30 animals 20 of which lived longer than 10 months. As mentioned above, in 1 animal a gastric ulcer was found. By oxidation with NaCrO₃ the carcinogenic potency has thus been completely eliminated.

By subcutaneous injection of unaltered, irradiated, and sodium bichromate oxidized 9,10-dimethyl-1,2-benzanthracene, the following results were obtained:

Of 28 mice injected with 1 mgm. of original hydrocarbon, 10 sarcomas and 1 squamous cell carcinoma appeared in animals from 6 to 11 months old. In 2 of these, generalized leukemia and a large lymphosarcoma in the groin were found. The group of animals that did not develop sarcomas, produced 3 additional cases of leukemia, and 4 other animals developed small papillomas at the site of injection.

By injecting irradiated 9,10-dimethyl-1,2-benzanthracene into 18 animals only 1 sarcoma in a 12 month old mouse was produced. In 30 animals injected with the sodium bichromate oxidized hydrocarbon no tumors appeared at the site of application. One mouse 17 months old died of leukemia. In 7 animals small cysts (3 to 10 mm.) were seen; they contained a transparent liquid and their walls consisted of apparently normal connective tissue. Five of these cysts, which were left untouched, regressed spontaneously after several months.

By subcutaneous injection the carcinogenic potency of the irradiated hydrocarbon was thus reduced to a minimum and the sodium bichromate oxidized hydrocarbon was, as by painting, quite noncarcinogenic.

Methylcholanthrene.—The characteristic absorption spectrum of methylcholanthrene is totally changed after irradiation (Fig. 4). The hydrocarbon loses almost completely its absorption band in the ultraviolet region. The curves bear besides (Figs. 1 to 4), a great resemblance to the curves of 1,2,5,6-dibenzanthracene and its irradiation product. Both irradiation products, however, differ completely from a biological point of view since the irradiated 1,2,5,6-dibenzanthracene is less carcinogenic than the original hydrocarbon but nevertheless still has a distinct tumor-producing power; whereas the irradiation product of methylcholanthrene has for practical purposes lost its carcinogenic potency, as will be seen from the following:

By painting with the original hydrocarbon 13 cases of papillomas and 13 of carcinomas were produced in 27 mice. The tumors appeared rather quickly and none of the animals lived longer than 8 months. Of the 26 animals affected, 5 cases of leukemia and 1 of subcutaneous sarcoma at the place of painting were seen.

By painting with irradiated methylcholanthrene no tumor development at the place of painting was seen in 25 animals, but there was one case of leukemia in a 6 months old mouse.

By subcutaneous injection of 1 mgm. of original methylcholanthrene 25 sarcomas and 1 carcinoma appeared among 38 animals. Of the affected animals there was 1 case of leukemia and another of pulmonary adenocarcinoma and large gastric ulcer with carcinoma in addition to a sarcoma.

Of the animals subcutaneously injected with irradiated methylcholanthrene only 1 sarcoma occurred in the group of 20.

The carcinogenic potency of irradiated methylcholanthrene when injected is thus reduced to a minimum, and when applied by painting, is completely lost.

A summary of these results is shown in Table I. It demonstrates clearly the decreased carcinogenic potency of the irradiated hydrocarbons. Furthermore, another quality of the reduced carcinogenic potency of the irradiated hydrocarbon is seen, namely, that the irradiation mixtures have no or only slight accelerating effect on the characteristic spontaneous tumors, especially the leukemias, of mice of the Street strain. Among the animals treated by painting and by subcutaneous injection with original hydrocarbons a considerable increase in the incidence of leukemia is seen, and many of the leukemias appear at an earlier age than usual in this strain. Of 85 animals treated by painting with original hydrocarbons, 10 cases of leukemia were found, of which 3 were in the 9,10-dimethyl-1,2-benzanthracene-treate group and 5 in the methylcholanthrene-treated group. From 112 animals treated by subcutaneous injection of original hydrocarbons, 8 cases of leukemia occurred, 5 of which were in the 9,10-dimethyl-1,2-benzanthracene-treated group. Altogether,

**DESCRIPTION OF FIGURES 1 THROUGH 4**

Fig. 1—A=Nonirradiated 1,2,5,6-dibenzanthracene. B=Irradiated 1,2,5,6-dibenzanthracene.

Fig. 2.—A=Nonirradiated 3,4-benzpyrene. B=Irradiated 3,4-benzpyrene.

Fig. 3.—A=Nonirradiated 9,10-dimethyl-1,2-benzanthracene. B=Irradiated 9,10-dimethyl-1,2-benzanthracene. C=Sodium bichromate oxidized 9,10-dimethyl-1,2-benzanthracene.

Fig. 4.—A=Nonirradiated methylcholanthrene. B=Irradiated methylcholanthrene.
### TABLE I: SUMMARY OF RESULTS OF TEST OF CARCINOGENIC POTENCY OF IRRADIATED HYDROCARBON

#### A. SKIN PAINTING TWICE WEEKLY FOR 24 WEEKS

<table>
<thead>
<tr>
<th>Hydrocarbon</th>
<th>Ratio Tumors to Mice</th>
<th>Kind of Tumors</th>
<th>Comments</th>
<th>Kind of Tumors</th>
<th>Ratio Tumors to Mice</th>
<th>Comments</th>
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<tbody>
<tr>
<td><strong>5/1,2,5,6-Dibenzanthracene</strong></td>
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<td></td>
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<tr>
<td>Original hydrocarbon</td>
<td>7/14+</td>
<td>6 carcinomas</td>
<td>+1 case of breast cancer complicated by leukemia and 1 case of gastric ulcer with cancer.</td>
<td>20+/21</td>
<td>11+ carcinomas</td>
<td>+1 case complicated by leukemia.</td>
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<tr>
<td>Irradiated hydrocarbon</td>
<td>5/20+</td>
<td>1 carcinoma</td>
<td>+1 case of breast cancer</td>
<td>10/22</td>
<td>8 carcinomas</td>
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<tr>
<td>Hydrocarbon oxidized</td>
<td>0/30+</td>
<td>0%</td>
<td>+1 case of gastric ulcer.</td>
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#### B. SUBCUTANEOUS INJECTION

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<th>Hydrocarbon</th>
<th>Ratio Tumors to Mice</th>
<th>Kind of Tumors</th>
<th>Comments</th>
<th>Kind of Tumors</th>
<th>Ratio Tumors to Mice</th>
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<tbody>
<tr>
<td><strong>5-/3,4-Benzpyrene</strong></td>
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<tr>
<td>Original hydrocarbon</td>
<td>7/26+</td>
<td>17 sarcomas</td>
<td>+1 case complicated by leukemia.</td>
<td>7/20+</td>
<td>7 sarcomas</td>
<td>+1 case of leukemia and 1 pulmonary adenoma.</td>
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<tr>
<td>Irradiated hydrocarbon</td>
<td>4/27+</td>
<td>4 sarcomas</td>
<td>+2 cases of breast cancer, 1 of which complicated by pulmonary adenoma, and 1 isolated case of case of pulmonary adenoma.</td>
<td>2/24+</td>
<td>2 sarcomas</td>
<td>+1 case of pulmonary carcinoma.</td>
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<tr>
<th>Hydrocarbon</th>
<th>Ratio Tumors to Mice</th>
<th>Kind of Tumors</th>
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<th>Kind of Tumors</th>
<th>Ratio Tumors to Mice</th>
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<tbody>
<tr>
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<tr>
<td>Original hydrocarbon</td>
<td>12/23+</td>
<td>2 carcinomas</td>
<td>+1 case complicated by leukemia.</td>
<td>26+/27</td>
<td>13 carcinomas</td>
<td>+5 cases of leukemia and 1 of subcutaneous sarcoma</td>
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<tr>
<td>Irradiated hydrocarbon</td>
<td>8+/22</td>
<td>1 carcinoma</td>
<td>+Minimal papillomas.</td>
<td>0/25+</td>
<td>0%</td>
<td>+1 case of leukemia</td>
</tr>
<tr>
<td>Hydrocarbon oxidized</td>
<td>0/30+</td>
<td>0%</td>
<td>+1 case of gastric ulcer.</td>
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<th>Comments</th>
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<th>Ratio Tumors to Mice</th>
<th>Comments</th>
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<td><strong>5/Methylcholanthrene</strong></td>
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<tr>
<td>Original hydrocarbon</td>
<td>17+/26+</td>
<td>17 sarcomas</td>
<td>+1 case complicated by leukemia.</td>
<td>7/20+</td>
<td>7 sarcomas</td>
<td>+1 case of leukemia and 1 pulmonary adenoma.</td>
</tr>
<tr>
<td>Irradiated hydrocarbon</td>
<td>4/27+</td>
<td>4 sarcomas</td>
<td>+2 cases of breast cancer, 1 of which complicated by pulmonary adenoma, and 1 isolated case of case of pulmonary adenoma.</td>
<td>2/24+</td>
<td>2 sarcomas</td>
<td>+1 case of pulmonary carcinoma.</td>
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<th>Ratio Tumors to Mice</th>
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<tr>
<td>Original hydrocarbon</td>
<td>11+/28+</td>
<td>10 sarcomas</td>
<td>+2 cases of leukemia and lymphosarcoma</td>
<td>24+/38</td>
<td>23 sarcomas</td>
<td>+1 case of leukemia, and 1 of pulmonary carcinoma and gastric ulcer with carcinoma</td>
</tr>
<tr>
<td>Irradiated hydrocarbon</td>
<td>1/18</td>
<td>1 carcinoma</td>
<td>+1 case of leukemia</td>
<td>1/20</td>
<td>1 sarcoma</td>
<td></td>
</tr>
<tr>
<td>Hydrocarbon oxidized</td>
<td>0/30+</td>
<td>0%</td>
<td>+1 case of leukemia</td>
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from 197 animals treated with irradiated hydrocarbons only 1 case of leukemia was seen. Of 60 animals treated with 9,10-dimethyl-1,2-benzanthracene oxidized with sodium bichromate only 1 case of leukemia was found. The incidence of spontaneous leukemia in this strain is, as mentioned earlier, 1 to 2 per cent.

The figures in the individual groups are too small to estimate whether the irradiation products of the different hydrocarbons vary as regards the accelerating effect on the spontaneous development of leukemia of the strain. It is remarkable that most of the cases of leukemia appeared among the animals treated with 9,10-dimethyl-1,2-benzanthracene, in spite of the fact that the life span of the animals in the painted group is so short.

It is remarkable also that another of the spontaneous tumors of the strain, mammary carcinoma, appeared only in the 1,2,5,6-dibenzanthracene group in 5 out of 70 females, while there have been no examples of the tumor in the other groups. This tumor appears spontaneously in the Street strain at the rate of about 7 per cent in non-breeding females. No increase in pulmonary adenoma or carcinoma has been seen. Only 5 cases from all the animals have been found, but these tumors as a rule appear late in the life of the animals.

It has been mentioned that especially among the animals painted with irradiated 9,10-dimethyl-1,2-benzanthracene some cases of gastric ulcer were found and that these ulcers in some instances have been complicated by adenocarcinoma. Probably these lesions are not caused by the treatment since they have been found in untreated animals too. Street strain mice seems to develop gastric ulcers at an increasing rate.

**CONCLUSIONS**

The carcinogenic potency of the irradiation products of the carcinogenic hydrocarbon 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, 9,10-dimethyl-1,2-benzanthracene and methylcholanthrene, and of the product of oxidizing 9,10-dimethyl-1,2-benzanthracene with sodium bichromate (Na₂Cr₂O₇) has been investigated by means of painting and subcutaneous injection. The absorption spectra of the compounds used have been given. Irradiation effects a reduction in
in the carcinogenic potency of these hydrocarbons but it has been impossible to find a correlation between the depression in the absorption intensity and the carcinogenic potency.

The irradiation products of 1,2,5,6-dibenzanthracene and 3,4-benzpyrene, which show different absorption curves, are both less carcinogenic than the original hydrocarbons, but the reduction of carcinogenic power is the same in the two hydrocarbons, namely, to approximately 25 per cent of the original hydrocarbon by subcutaneous injection and to approximately 50 per cent by painting. The irradiation product of methylcholanthrene, the absorption spectrum of which strikingly resembles that of irradiated 1,2,5,6-dibenzanthracene, has almost completely lost its carcinogenic potency.

9,10-Dimethyl-1,2-benzanthracene oxidized with NaCrO₄ is quite noncarcinogenic, whereas the irradiation mixtures show greatly reduced carcinogenic potency. By painting with the irradiation product of 9,10-dimethyl-1,2-benzanthracene, many preneoplastic changes were seen, which in spite of continuous painting, in most cases remained at the same stage, or even regressed spontaneously.

The accelerating effect of the carcinogenic hydrocarbons on the spontaneous tumors in the strain used is reduced, although specific details cannot be deduced from the experiments.

It is probable that the results justify the suggestion that when an inhibitory effect has been noticed after concurrent application of ultraviolet light and carcinogenic hydrocarbons on the skin of mice this effect is due for the most part to photo-oxidation of the hydrocarbons.

REFERENCES

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