The Mechanism of Carcinogenesis

A Study of the Significance of Cocarcinogenic Action and Related Phenomena

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In a previous communication (5), the author has shown that when the effective carcinogenic potency of 3,4-benzpyrene for the mouse's skin was reduced by application of the hydrocarbon in a dilution of 0.05 per cent, the low tumor yield (0 to 6 per cent at the 20th week) was increased to 80 per cent by application of croton resin concurrently with the benzpyrene.1

This augmentation of carcinogenesis, or "cocarcinogenic action," could not be explained as a summated effect of two weak carcinogens, since croton resin gave no decisive evidence of carcinogenicity when applied alone to the skin. Nor could the effect be attributed to a nonspecific irritative action on the part of croton resin, since other skin irritants, such as xylene or turpentine, failed to augment carcinogenesis under similar conditions. It was suggested, therefore, that the cocarcinogenic effect of croton resin constituted a specific reaction distinct from the carcinogenic process itself. Yet the mechanism of cocarcinogenic action remained unexplained.

The present communication is concerned with an extension of this work, certain problems having been chosen for special study as likely to throw light on the nature of cocarcinogenic action and on its relation to the normal process of carcinogenesis. The problems chosen for investigation were the following: 1. The effect of croton resin on carcinogenesis of the skin by concentrated solutions of weak carcinogens; 2. the effect of croton resin on carcinogenesis associated with injections of benzpyrene; 3. the effect of croton resin applied to the skin before or after a limited period of benzpyrene application; and 4. the effect of croton resin on the malignant transformation of skin tumors induced by benzpyrene.

The methods employed in these experiments were essentially the same as those previously used by the author, and need not, therefore, be described again in detail. The white mice and white rats both belonged to fairly homogeneous (though not genetically pure) strains, bred in this laboratory over a period of many years, without any admixture from other sources. All reagents for application to the skin were dissolved in acetone, application being made once a week to a small area of skin in the region of the shoulder blades, the hair being clipped short with scissors prior to each application. Other details of actual experiments can best be left for consideration under the separate headings.

THE EFFECT OF CROTON RESIN ON CARCINOGENESIS OF THE SKIN BY CONCENTRATED SOLUTIONS OF WEAK CARCINOGENS

In previous experiments (Series 1 to 5, Table I), it was found that the effect on the mouse's skin of the addition of croton oil or resin to benzpyrene treatment differed, depending on whether the benzpyrene was applied as a dilute solution (0.05 per cent) or in a concentrated form (1 per cent). With a dilute solution of benzpyrene, croton resin produced a striking augmentation of carcinogenesis, whereas with a concentrated solution, the effect was insignificant. This difference could be explained in one of two ways: Either the carcinogenic potency of 1 per cent benzpyrene in acetone is already maximal for the mouse's skin, so that further increase in potency was theoretically impossible, or else the mechanism of cocarcinogenic action of croton resin is such that the augmentation represents nothing more than an increase from the artificially lowered potency (produced by dilution) up to the potential limit for the particular carcinogen.

The problem was put to the test by determining the influence of croton resin on the carcinogenic action of weak carcinogens applied in concentrated solutions. If the first explanation is correct, croton resin should be able to augment the carcinogenic potency of a concentrated solution of a weak carcinogen as readily as...
of a dilute solution of a potent carcinogen; if the second is correct, croton resin should be ineffective when acting with a concentrated solution of any carcinogen, irrespective of whether its potency is high or low.

Four separate groups of mice received weekly applications to the skin of a saturated solution of 1,2,5,6-dibenzanthracene in acetone, of a similar solution together with 0.5 per cent croton oil, of a saturated solution of 1,2-benzanthracene, and of a similar solution together with 0.025 per cent croton resin.2

The results of this experiment are summarized in Table I, Series 6 to 9. No significant augmentation of carcinogenesis occurred either with 1,2,5,6-dibenzanthracene or 1,2-benzanthracene, so that, of the two possible explanations of cocarcinogenic action mentioned above, the second appears to be the correct one.

Thus, in the former experiment, the two agents were allowed to act together on connective tissue instead of on skin; in the latter, the cocarcinogenic action of croton resin was tested on the skin once again, but with the benzpyrene brought to it in small concentrations through the blood stream, instead of as a dilute solution from the surface.

The effect of croton resin and of turpentine injected subcutaneously in rats together with varying concentrations of benzpyrene.—Twelve groups of rats, 6 in each group, were used for this experiment. The reagents for injection were dissolved in sesame oil, 0.5 cc. of the appropriate solution being injected subcutaneously into the left flank at the commencement of the experiment, after which no further treatment was given. The animals were kept under observation for 9 months, the time of appearance of a tumor being recorded and its size measured periodically. At the end of the experiment, the animals were killed, and any tumors present were examined histologically. The amounts of benzpyrene injected were 2.5 mgm., 0.05 mgm., and 0.001 mgm., respectively. These amounts were injected alone in some groups or together with 50 mgm. of turpentine or 0.005 mgm. of croton resin in others. Three control groups received sesame oil alone, turpentine in sesame oil without benzpyrene, and croton resin in oil without benzpyrene.

With the highest concentration of benzpyrene (2.5 mgm.), the tumor yield was 5/6 with the hydrocarbon alone, 6/6 with benzpyrene plus turpentine, and 6/6 with benzpyrene plus croton oil. The tumors were all spindle cell sarcomas, and their times of appearance and rates of growth did not differ significantly in the 3 groups. Not a single tumor appeared at the site of injection in any of the animals of the other series (i.e., those which received 0.05 mgm. and 0.001 mgm.

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**Table I: Results of Application of Croton Oil or Resin Concurrently with Different Carcinogens**

<table>
<thead>
<tr>
<th>Series</th>
<th>Reagent for application, dissolved in acetone</th>
<th>Number of mice used</th>
<th>Time of appearance of first tumor, in weeks</th>
<th>Time taken for 50 per cent of survivors to develop tumors, in weeks</th>
<th>Mice with tumors/survivors, 20th week</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1% 3,4-benzpyrene</td>
<td>36</td>
<td>12</td>
<td>19</td>
<td>22/36</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>1% 3,4-benzpyrene and 0.5% croton oil</td>
<td>36</td>
<td>19</td>
<td>17</td>
<td>26/33</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>0.05% 3,4-benzpyrene</td>
<td>36</td>
<td>21</td>
<td>1</td>
<td>2/34</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.05% 3,4-benzpyrene and 0.5% croton oil</td>
<td>36</td>
<td>11</td>
<td>24</td>
<td>12/32</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>0.05% 3,4-benzpyrene and 0.025% croton resin</td>
<td>40</td>
<td>9½</td>
<td>18</td>
<td>16/20</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>Saturated solution 1,2,5,6-dibenzanthracene</td>
<td>36</td>
<td>12</td>
<td>28</td>
<td>5/33</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>Saturated solution 1,2,5,6-dibenzanthracene</td>
<td>36</td>
<td>13</td>
<td>26</td>
<td>6/32</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>Saturated solution 1,2-benzanthracene</td>
<td>20</td>
<td>12</td>
<td>28</td>
<td>5/33</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>Saturated solution 1,2-benzanthracene and 0.025% croton resin</td>
<td>30</td>
<td>(5½)*</td>
<td>..</td>
<td>0/30</td>
<td>0</td>
</tr>
</tbody>
</table>

* A localized thickening of the skin in one mouse, recorded as a papilloma, proved to be a hyperkeratosis without any outgrowth of epithelium when examined histologically.

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**The Effect of Croton Resin on Carcinogenesis Associated with Injections of 3,4-Benzpyrene**

The object of this method of approach was to determine whether croton resin was cocarcinogenic under conditions other than those in which both the resin and the benzpyrene are applied to the skin.

Two widely different experiments were undertaken for this purpose, one of which, carried out on rats, was concerned with the effect of croton resin (and also of turpentine) injected subcutaneously, together with different concentrations of benzpyrene; the other, carried out on mice, dealt with the possibility of producing skin tumors at the site of application of croton resin when benzpyrene was injected at a distance.

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2 Croton resin is the active constituent of croton oil, and a solution of 0.025 per cent croton resin corresponds approximately in cocarcinogenic action to a solution of 0.5 per cent croton oil.
of benzpyrene with or without turpentine or croton resin, and the 3 controls). Thus the possibility of cocarcinogenic action on connective tissue is not supported by the present experiment.\(^3\)

The effect of croton resin and of turpentine on the skin of mice periodically receiving intraperitoneal injections of benzpyrene.—Two groups of mice, 20 in each group, received 5 intraperitoneal injections of 0.1 cc. of a 0.1 per cent solution of benzpyrene in sesame oil at monthly intervals, one group receiving, in addition, weekly applications to the skin of 0.025 per cent croton resin in acetone, the other receiving similar applications of 30 per cent turpentine in acetone. The experiment was continued for 25 weeks.

Though two animals in each group developed intraperitoneal tumors (i.e., at the site of injection of the benzpyrene), none showed tumors of the skin at the site of application of the croton resin or turpentine. This experiment was, therefore, also negative.

The Effect of Croton Resin Applied to the Skin Before or After a Limited Period of Benzpyrene Application

It has been shown (6) that when two different chemical carcinogens are allowed to act on the same area of skin for separate periods, the carcinogenic effects are summated, the skin being apparently incapable of distinguishing between one and the other (except quantitatively, of course, when the potencies are different), and responding, therefore, as if one carcinogen were acting all the time. It was thought interesting to investigate whether the same was also true when a carcinogen and a cocarcinogen were allowed to act under similar conditions. Such experiments would show (a) whether a cocarcinogen is capable of producing those biological changes in the skin which characterize the “latent period” of carcinogenesis preceding the actual development of a tumor, even if it cannot produce a tumor by itself, and (b) whether it is capable of “precipitating” the development of a tumor when such biological changes are already present as the result of previous action of a carcinogen.

Two experiments were, therefore, undertaken. In one, the croton resin applications preceded the benzpyrene treatment; in the other, they followed a limited period of benzpyrene treatment.

The effect of preliminary treatment of the mouse skin with croton resin on the subsequent response of the same area of skin to the carcinogenic action of benzpyrene.—The animals used for this experiment were 20 mice which had been receiving weekly applications of croton resin (0.025 per cent in acetone) to the skin for 26 weeks. The original purpose of this experiment was to determine whether croton resin was itself carcinogenic. When it was found that no tumors had appeared after 26 weeks and all the animals were still alive, the croton resin treatment was discontinued and application of 1 per cent benzpyrene in acetone was substituted.

The results of this experiment can briefly be summarized as follows: No tumors arose during the first 9 weeks from the commencement of benzpyrene treatment; from then onwards, warts began to appear at the site of application, and, after 16 weeks (42 weeks from the beginning of the original experiment), 10 out of 19 survivors bore tumors.

When compared with numerous control experiments from other series (i.e., in which 1 per cent benzpyrene in acetone was applied at weekly intervals to similar mice, without preliminary treatment of any sort), the above results fail to indicate any significant shortening of the latent period as the result of the preliminary applications of croton resin. The time taken for 50 per cent of the animals to develop tumors was admittedly a little shorter than in control groups (16 weeks as compared to 18 to 19½ weeks); on the other hand, the time taken for the first tumor to appear (9 weeks) was well within normal limits (5 to 13 weeks).

It may be concluded, therefore, that little or no effect is produced by prolonged application of croton resin, which might suggest the development of those biological changes which are characteristic of the latent period of carcinogenesis.

The effect of croton resin on mouse skin previously treated with benzpyrene for a limited period.—One hundred mice received 8 weekly applications to the skin of a 1 per cent solution of benzpyrene in acetone, by which time one animal had already developed a tumor at the site of application. This animal was discarded. In the remaining mice, the benzpyrene treatment was discontinued, and the animals were divided into 3 groups. In one, the previously treated skin was painted with 0.025 per cent croton resin in acetone at weekly intervals; in the second, the same area of skin was painted with 30 per cent turpentine in acetone; while in the third, acetone alone was applied as control. The painting was continued for 22 weeks (i.e., 30 weeks from the commencement of

\(^3\) In analogous experiments Sall and Shear found that when appropriate dosages and conditions were employed the basic fraction of creosote oil promoted the development of tumors following the subcutaneous injection of 0.1 mgm. of benzpyrene in mice. These positive results support the conception of cocarcinogenic action. See: Sall, R. D., and M. J. Shear. Studies in Carcinogenesis. XII. Effect of the Basic Fraction of Creosote Oil on the Production of Tumors in Mice by Chemical Carcinogens. J. Nat. Cancer Inst., I:45-55. 1940.
the experiment), after which the animals were left untreated for a further 6 weeks and then killed for histological examination of the tumors.

The results of this experiment (Table II) may be summarized as follows: In the acetone control series, 5 of the animals (representing 18 per cent of survivors) developed tumors at the site of application; in the turpentine series, the number of animals with tumors was 11 (representing 44 per cent); while in the croton resin series, as many as 25 animals bore tumors at the site of application (representing 86 per cent of survivors). In the control and croton resin series, the tumors were small pedunculated papillomas which grew slowly and only a small proportion of these became malignant, whereas in the turpentine series most of the tumors were sessile from the start, grew rapidly, and tended to become malignant early.

The two last experiments show, therefore, that whereas application of croton resin prior to benzpyrene treatment has little or no demonstrable effect, application of croton resin following a limited period of benzpyrene treatment leads to a pronounced increase in the development of tumors. The results with turpentine are much less striking, while the evidence about malignancy appears rather anomalous.

### Table II: The Effects of Croton Resin, Turpentine, and Acetone on Mouse Skin Previously Treated with 3,4-Benzpyrene for 8 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Croton resin</th>
<th>Turpentine</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Number of mice used</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>B. Number of survivors at end of the experiment</td>
<td>29</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>C. Number of mice which developed tumors</td>
<td>25</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>D. Percentage (C:B)</td>
<td>86%</td>
<td>44%</td>
<td>18%</td>
</tr>
<tr>
<td>E. Times of appearance of tumors (in weeks)</td>
<td>2, 3, 5, 6, 8, 8, 1, 5, 6, 8, 12, 1, 3, 3, 20, 27, 9, 10, 10, 11, 15, 16, 17, 19, 11, 11, 11, 13, 22, 25, 14, 14, 15, 15, 18, 16, 17, 18, 22.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F. Analysis of tumors:

- Number of animals in which the tumors have
  1. Regressed
  2. Remained small
  3. Grown progressively without becoming malignant
  4. Become malignant

<table>
<thead>
<tr>
<th></th>
<th>Croton resin</th>
<th>Turpentine</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Regressed</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2. Remained small</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3. Grown progressively without becoming malignant</td>
<td>8</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>4. Become malignant</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

The experimental demonstration of such an effect presents certain practical difficulties, owing to the great individual variability in the rate of appearance of benign tumors of the skin in mice following applications of a chemical carcinogen, and the even greater variability in the time taken for such tumors to become malignant. The following experiment was designed to overcome, to some extent, these inherent practical difficulties.

Weekly applications of a 1 per cent solution of benzpyrene in acetone were made to the skin of 100 mice. As soon as any animal developed a wart, it was segregated and given an identification mark. The first animal with a tumor was placed in group A, the second in group B, the third in group C, the fourth in group A again, and so on, in rotation, until all the animals were separated in one or other of the 3 groups. By this means, each group contained the same proportion of animals with early, intermediate, and late developments of warts. Once an animal was segregated, the benzpyrene treatment was stopped, and acetone (in the case of group A), croton resin (in the case of group B), and turpentine (in the case of group C) were substituted for application to the same area of skin. Records were kept of the dimensions of the tumors at regular intervals, and 10 weeks after the appearance of a tumor, the animal in question was killed for histological examination of that tumor. In this way, it was possible to determine, in spite of the individual variability in response to carcinogenic action of the preliminary benzpyrene treatment, the average tendency towards malignant transformation in the 3 groups.

The Effect of Croton Resin on the Malignant Transformation of Skin Tumors Induced by Benzpyrene

In view of the above results, it was considered important to investigate more carefully whether croton resin or turpentine had any effect on the transformation of a benign into a malignant skin tumor.
The results of this experiment were as follows (Table III): In the acetone control series, malignant tumors were present in 7 out of 17 survivors (41 per cent), in the turpentine series, in 6 out of 15 survivors (40 per cent), and in the croton resin series, in 11 out of 18 survivors (61 per cent). When the rapidly growing tumors, already bordering on malignancy, are also taken into account, the results appear more decisive. The numbers of mice in which the tumors regressed or remained as small warts or grew slowly, compared to those in which the tumors grew rapidly and either bordered on or actually reached malignancy, were 9 and 8 in the acetone control series, 6 and 9 in the turpentine series, and 4 and 14 in the croton resin series; i.e., a ratio of 1:0.9, 1:1.5, and 1:3.5, respectively.

**Table III: Effects of Croton Resin, Turpentine, and Acetone on Warts Previously Induced by Applications of 3,4-Benzpyrene**

<table>
<thead>
<tr>
<th>Number</th>
<th>Croton Resin</th>
<th>Turpentine</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Number of mice used</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>2. Number of survivors at end of the experiment</td>
<td>18</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>3. Number of mice in which tumors regressed</td>
<td>1</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>4. Number of mice in which tumors grew but remained benign</td>
<td>4</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>5. Number of mice in which tumors reached early or probable malignancy</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>6. Number of mice in which tumors became definitely malignant</td>
<td>14</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>7. Ratio of $3 + 4$ to $5 + 6$</td>
<td>1:3.5</td>
<td>1:1.5</td>
<td>1:2.9</td>
</tr>
</tbody>
</table>

* Histological evidence of downward growth, without invasion of muscle.
† Histological evidence of invasion of muscle.

It is unfortunate that, in view of the complexity of the experiment, larger numbers of animals could not have been used. The results, nevertheless, suggest (though they cannot be considered as conclusive) that croton resin facilitates the conversion of warts from the benign to the malignant state. The results concerning the action of turpentine are inconclusive.

**Discussion**

In view of recent developments in our knowledge of the mechanism of carcinogenesis, and the confusion which must inevitably arise from endless repetition of such phrases as "the production of those biological changes which represent the latent period of carcinogenesis" or "the precipitation of a tumor at a site previously rendered preneoplastic," it has become necessary to adopt a special terminology. It is hoped that the use of the following terms will facilitate clarity of expression in discussion on the subject:

- **Anticarcinogenic action:** The inhibition of the process of carcinogenesis. The term was introduced by the author (2, 3, 4) to describe the action of dichlorehysulfide, cantharidin, etc.
- **Cocarcinogenic action:** The augmentation of carcinogenesis by a noncarcinogenic agent. This occurs when the appropriate agent is applied concurrently with a carcinogen which is acting under suboptimal conditions. The term was first introduced by Shear (12) for a certain noncarcinogenic fraction of tar and has been adopted by the present author for the action of croton resin (5).
- **Precarcinogenic action:** The production of a preneoplastic condition. Such an effect would be demonstrable by a shortening of the latent period of carcinogenesis in subsequent treatment with a carcinogen, or by preparing the ground for the subsequent action of an epicarcinogenic agent (see below).
- **Epicarcinogenic action:** The production of tumors in a tissue previously rendered preneoplastic.
- **Metacarcinogenic action:** The conversion of a benign into a malignant tumor.

When a chemical carcinogen is applied repeatedly to the skin of a susceptible animal, a series of biological changes develops in an orderly sequence (the time relationship varying, however, from animal to animal). At least three well-recognized stages are involved: (a) the preneoplastic stage (or latent period of carcinogenesis), (b) the wart or papilloma stage, and (c) the stage of malignancy. In the first stage, no tumors are yet detectable, but the histological appearances of the skin (epithelial hyperplasia, inflammatory changes in the corium, etc.), though resembling in many respects those obtained with noncarcinogenic irritants, seem to possess certain specific features (8, 10). The essential characteristic of the second stage is that the warts which develop are usually multiple and arise as minute foci, suggesting that their origin is, in each case, from a single cell. The third stage (malignancy) develops, in the majority of cases, from one or other of the existing warts, the remainder of the painted area of skin being rapidly included in the malignant mass by invasion, and probably not by malignant transformation of the surrounding tissue.

Precarcinogenic action may, therefore, be considered as the conversion of normal skin into that of stage (a); epicarcinogenic action as the conversion from stage (a) into stage (b), and metacarcinogenic action as the conversion from stage (b) into stage (c), while anticarcinogenic action represents interference with epicarcinogenic action only, since it has been shown (3) that, when applied in subulcerative concentrations, neither the preneoplastic state nor the subsequent

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4 The phrase "by a noncarcinogenic agent" has been added by the Editors.
growth of warts, once these have been established, is inhibited by the anticarcinogens so far studied.

Since any one carcinogenic agent is capable of producing all three effects (pre-, epi-, and metacarcinogenic actions), it is generally assumed that these represent three inseparable stages of one single carcinogenic process. This unitarian conception of carcinogenesis is not supported, however, by the results described in the present communication.

Croton resin is not carcinogenic by itself, nor is it capable of producing, to any demonstrable extent, those prepreneoplastic changes which characterize the latent period of carcinogenesis. Yet, when applied to skin which has already been rendered prepreneoplastic by other means, it is capable of precipitating the development of warts in a high proportion of cases, and furthermore, when allowed to act on warts already established, it seems able to facilitate their conversion into malignant tumors.

These results can be taken as evidence of a dissociation of the process of carcinogenesis into several component parts, the whole process constituting, as it were, a chain reaction of essentially independent processes. That this interpretation is valid when applied to the normal process of carcinogenesis is evident from a consideration of recent results by Rous and his associates (7, 11).

These authors have drawn attention to the differences in the response of the skin of the mouse and rabbit respectively to the carcinogenic action of tar. While papillomas develop fairly readily in both species, those in the mouse tend to grow progressively, even after discontinuing the tarring, whereas those arising in the rabbit usually regress under similar conditions. Moreover, whereas the mouse papilloma has a strong bias towards malignancy, that of a rabbit becomes malignant only with difficulty, even under optimal conditions. These authors demonstrated, however, that in spite of the strong tendency to regression, tar papillomas in the rabbit possess all the valid criteria of neoplasia, since, after complete regression, they could be made to reappear by a variety of influences (renewed tarring, wound healing, and turpentine).

Thus, even in the normal process of carcinogenesis, the separate component parts (pre-, epi-, and metacarcinogenic actions) develop to different relative degrees in the two species. In the mouse, all 3 processes progress more or less rapidly, the impetus towards final malignancy being fairly pronounced even at an early stage, whereas in the rabbit, the precarcinogenic effect is extremely pronounced (the latent period being sometimes as short as 2 weeks). The epicarcinogenic effect is also pronounced, though continued encouragement is required for the warts to continue to grow, while the metacarcinogenic effect is relatively feeble.

Evidence in favor of a dissociation of the process of carcinogenesis is, therefore, of two kinds: 1. The existence of certain substances which are capable of producing part, but not all, of the changes leading to neoplasia, and 2. the fact that the individual stages of carcinogenesis develop with relatively different degrees of impetus in different species. As a basis for further research, it may be helpful to present this new conception of carcinogenesis in a more concrete form:

1. Precarcinogenic, epicarcinogenic, and metacarcinogenic actions represent independent processes in carcinogenesis.

2. True carcinogens are capable of producing all three actions, though to different degrees in different species.

3. Croton resin differs from true carcinogens in lacking the power of precarcinogenic action, but resembles them in possessing both epi- and metacarcinogenic action.

4. It is possible that other kinds of incomplete carcinogens may exist, possessing, for instance, metacarcinogenic but not pre- or epicarcinogenic action.

5. The varying response to carcinogenesis of different tissues may possibly be attributable to a deficiency in one part of the chain reaction, rather than to a greater or lesser responsiveness to carcinogenesis as a whole.

But what then does cocarcinogenic action represent in this scheme of the mechanism of carcinogenesis?

When the cocarcinogenic action of croton resin was first described (5), some consideration was given to the possibility that the effect was part of a normal carcinogenic process; i.e., that the action was due to a summated effect on the part of two weak carcinogens, dilute benzpyrene and croton resin. This view was rejected because no evidence of carcinogenic action on the part of croton resin was obtained when this substance was applied alone to the skin, while the possibility that croton resin was too weak a carcinogen to produce tumors by itself, yet strong enough to act in conjunction with another weak carcinogen in the above manner, was shown to be untenable on theoretical grounds. Final refutation of this view concerning the mechanism of cocarcinogenic action is now available. If it were correct, one would have expected croton resin to augment the carcinogenic action of a saturated solution of a weak carcinogen as readily as of a dilute solution of a potent carcinogen. Yet this was found not to be the case.

This negative conclusion does not lead one very far, however, and the other results, described in the present communication, present equally negative evidence, and cannot do more, therefore, than merely indicate vaguely the probable nature of cocarcinogenic action.
The negative results obtained when benzpyrene was injected at a distance from the site of application of the croton resin are probably not very significant, since the concentration of benzpyrene actually reaching the painted area of skin may well have been too low for the cocarcinogenic effect of the croton resin to manifest itself. It is interesting to note in this connection that, according to Beck (1), intraperitoneal injections of small amounts of benzpyrene in mice failed also to augment the neoplastic response of the skin to cautery, x-rays, and ultraviolet irradiation, though, from earlier experience, tar appeared to be effective. In recent investigations which have not yet been published, by Dr. Schontal and the author, it was found possible to detect and measure the small amounts of benzpyrene present in the circulating blood of the mouse following subcutaneous and intraperitoneal injection of the hydrocarbon. When more data from these investigations are available, it may become possible to establish the conditions of injection required for an adequate amount of the benzpyrene to reach the skin through the blood stream.

The failure to demonstrate any cocarcinogenic action on the part of croton resin when injected subcutaneously into rats in conjunction with benzpyrene, though more convincing than the above experiment, must also be interpreted with caution. It is known (9) that tumors induced subcutaneously develop at some little distance from the actual site of injection of the carcinogen, and since in the present experiment the croton resin and the benzpyrene were injected together as one solution, it is possible that the resin may not have been able to diffuse out as far as the benzpyrene and may, therefore, have failed to reach the actual zone of potential carcinogenesis. The conditions of the experiment differed also from those in which cocarcinogenic action was demonstrated on the skin since, in the latter, a low concentration of benzpyrene was applied repeatedly at short intervals, so that the tissue was always under the influence of small amounts of the carcinogen, whereas in the former only one injection was given, and, in the case where a small amount of benzpyrene was injected, probably none was present at the time when the cocarcinogenic action of the croton resin could theoretically have become manifest.

The most significant experiment was that in which the croton resin was applied to the skin in conjunction with 1,2,5,6-dibenzanthracene and 1,2-benzanthracene, respectively, and, apart from the conclusions which have already been reached from this experiment, the results also help to throw a little light on the mechanism of cocarcinogenesis.

The simplest conception of cocarcinogenic action would be that it was merely a variant of epicarcinogenic action, on the supposition that the dilute benzpyrene produced the preneoplastic state, while the epicarcinogenic effect was carried out jointly by the dilute benzpyrene and the croton resin. If this were the case, one would have to assume, first, that the precarcinogenic action of benzpyrene is less influenced by dilution than its epicarcinogenic action and, second, that the low carcinogenic potency of dibenzanthracene and the still lower potency of benzanthracene are due primarily to deficiencies in precarcinogenic action. Until these assumptions are confirmed experimentally it is not possible to say whether this simple explanation of cocarcinogenic action is correct or not.

An entirely different explanation of the mode of action of cocarcinogenesis would be to suppose that croton resin merely facilitated the entry of the carcinogen into the cell so that a small number of molecules of the hydrocarbon, applied to the surface, would still have a reasonable chance of acting on the cell. This would account for the failure of croton resin to augment carcinogenesis in the case of concentrated solutions of carcinogens, irrespective of whether their potencies are high, medium, or low. This interpretation implies, however, that cocarcinogenic action is an entirely different process from pre-, epic-, and metacarcinogenic action. No decision can be made at the present stage as to the likelihood of this explanation being the right one.

In conclusion, it is necessary to stress the practical implications of the present results in relation to clinical and preventive medicine. The elucidation of the specific chemical nature of carcinogenic hydrocarbons and the demonstration that most of the ordinary skin irritants are not in themselves carcinogenic, have tended to distract clinical attention from the possibility that irritation might play a part in the development of tumors in man. However, the fact that certain noncarcinogenic irritants are capable of producing cocarcinogenic, epicarcinogenic, and metacarcinogenic effects introduces new conceptions of possible extraneous factors of a noncarcinogenic nature influencing tumor development in man.

**Summary**

The effect of croton resin on carcinogenesis was studied under varying conditions, in order to determine the nature of cocarcinogenic action (the augmentation of carcinogenesis which occurs when croton resin is applied to the skin concurrently with a dilute solution of 3,4-benzpyrene) and its relation to the normal process of carcinogenesis.

No cocarcinogenic effect was observed when the croton resin was applied to the skin and the benzpyrene was injected at a distance (intraperitoneally);

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5 See footnote 3.
nor was it possible to augment the carcinogenic effect of benzpyrene on subcutaneous tissues, by injection of croton resin together with the benzpyrene.

While augmentation of carcinogenesis was very pronounced when croton resin was applied to the skin concurrently with a dilute solution of a potent carcinogen (3,4-benzpyrene), none was observed with concentrated solutions of different carcinogens, irrespective of whether their potency were high (3,4-benzpyrene), moderate (1,2,5,6-dibenzanthracene), or very low (1,2-benzanthracene).

Preliminary treatment with croton resin for a period of 26 weeks failed to influence significantly the response of the mouse’s skin to subsequent applications of benzpyrene. On the other hand, croton resin applied to the skin subsequent to a limited period of benzpyrene treatment led to a striking increase in the development of tumors.

Croton resin applied to papillomas already established appeared to facilitate their conversion to malignancy.

From consideration of these results, the suggestion is put forward that the three phases of carcinogenesis—(a) the development of the preneoplastic phase (latent period), or precarcinogenic action, (b) the conversion of this into the wart stage, or epicarcinogenic action, and (c) the malignant transformation of these warts, or metacarcinogenic action—are probably not simply stages of one single carcinogenic process, but independent processes. The carcinogenic hydrocarbons possess all three actions; croton resin possesses only the second and third, and cannot, therefore, produce tumors by itself.

No precise knowledge is yet available as to the nature of cocarcinogenic action, but two possible modes of action are discussed.

Attention is also drawn to the clinical implications of the existence of cocarcinogenic, epicarcinogenic, and metacarcinogenic actions on the part of noncarcinogenic agents in man.

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