OBSERVATIONS ON THE INTRAVENOUS ADMINISTRATION OF COLLOIDAL SOLUTIONS OF CARCINOGENIC HYDROCARBONS IN RABBITS

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In 1933 Lacassagne (1) described in two rabbits a curious hyperplasia of the “broncho-alveolar” epithelium tending to metaplasia, but not truly malignant, subsequent to the intravenous injection of 1:2:5:6-dibenzanthracene in lard. Simonds and Curtis (2) in 1935 gave tar residue known to be carcinogenic to rabbits by intravenous injection and described lesions similar to those mentioned by Lacassagne. In this paper they referred to a Japanese, Nakano, who had reported carcinomatous changes in the lungs of 9 per cent of a series of rabbits treated with tar in lanolin intravenously. In the same year Boyland and Burrows (3) administered an aqueous emulsion of 1:2:5:6-dibenzanthracene with lecithin intravenously to four rabbits. Another group of four were injected with the hydrocarbon emulsified in olive oil. Some of these animals survived nineteen weekly injections and after 160 and 185 weeks still had no evidence of tumors. These workers also injected their colloidal solution subcutaneously without results. That 1:2:5:6-dibenzanthracene is capable of inducing a malignant growth in rabbits was shown by Haagensen and Krehbiel (4), who injected the hydrocarbon in paraffin oil subcutaneously into two rabbits. Sarcomas were discovered at the site of injection 308 and 379 days later. Lacassagne (1) produced a sarcoma in one rabbit’s testis with 1:2:5:6-dibenzanthracene in lard.

Colloidal solutions of 3:4-benzpyrene (5) were injected, presumably subcutaneously, into nine rabbits by Pourbaix and Denisoff (5) without any result. Schürch and Winterstein (6) painted rabbits’ ears with 3:4-benzpyrene twice a week. After 400 days one of the animals developed a typical squamous-cell epithelioma. Ten of the original twelve had papillomas after 362 days of painting. Peacock (7) injected colloidal 3:4-benzpyrene solutions into rabbits by vein, but the animals were killed shortly afterward and no effort to observe tumor formation was made.

When the study to be reported was begun we were not aware of the negative results of some workers who had given carcinogens intravenously. It was our intention to inject as much of the material as was feasible, and to observe the animals for prolonged periods. Hoping that the colloidal suspension would be engulfed by the macrophage system and perhaps irritate it, we followed the blood picture carefully. Healthy rabbits were used and several complete blood counts were made for control observations before the injections were started. Standard hematologic methods and materials were used, and all the differential counts were done by two of us (E. V. K. and G. V. L.).

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2 Douglas Smith Foundation Fellow.
3 Originally designated 1:2-benzpyrene.
The carcinogenic hydrocarbons used were 1:2:5:6-dibenzanthracene, 3:4-benzpyrene, and methylcholanthrene. The drugs were used as they came from the manufacturer without any purification. They were all found to be carcinogenic for mice by one of us (A. B.). The solutions were made after the method of Boyland (8): 0.3 or 0.5 gm. of the hydrocarbon was dissolved in 100 c.c. of pure acetone. One volume of this was poured into ten volumes of warm (45° C.) 0.5 per cent gelatine in distilled water. The addition was made slowly with constant, vigorous stirring. The acetone was removed in vacuo. The resulting solution was examined under the microscope, and if there were crystals in large aggregates present it was discarded. The solutions used were made so that the concentration was:

- 1:2:5:6-dibenzanthracene: 0.3 mg./c.c.
- 3:4-benzpyrene: 0.5 mg./c.c.
- Methylcholanthrene: 0.5 mg./c.c.

These suspensions are not isotonic, but with injection of only 5.0 c.c. doses, no difficulty was encountered. An occasional animal died shortly after the injection into the marginal ear vein, but most of them tolerated it quite well. The rabbits were injected at intervals varying from one to three weeks. Blood counts were made just before the injection was given. After from 20
Table II: Analysis of Blood Counts

<table>
<thead>
<tr>
<th>Hydrocarbon</th>
<th>Hemoglobin, Grams</th>
<th>Erythrocytes, Millions</th>
<th>Leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Maxim.</td>
<td>Minim.</td>
</tr>
<tr>
<td>1 : 2 : 5 : 6-dibenzanthracene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control:</td>
<td>13.51</td>
<td>16.9</td>
<td>11.0</td>
</tr>
<tr>
<td>Injected:</td>
<td>11.96</td>
<td>14.5</td>
<td>7.6</td>
</tr>
<tr>
<td>3 : 4-benzpyrene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control:</td>
<td>12.26</td>
<td>16.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Injected:</td>
<td>12.25</td>
<td>16.1</td>
<td>8.2</td>
</tr>
<tr>
<td>Methylcholanthrene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control:</td>
<td>12.62</td>
<td>15.2</td>
<td>9.8</td>
</tr>
<tr>
<td>Injected:</td>
<td>11.27</td>
<td>15.3</td>
<td>9.3</td>
</tr>
</tbody>
</table>

to 30 mg. of any one of the hydrocarbons had been given, the injections were discontinued and the animal observed until the study was ended.

Table I shows the duration of the period of observation for the rabbits receiving the different hydrocarbons. Table II indicates the numbers of rabbits receiving differing total doses of the carcinogens.

In none of the animals was any tumor discovered. When a rabbit died it was carefully autopsied and suggestive material was sectioned and studied histologically. At the end of the experiment all the survivors were killed and autopsied.

Table III summarizes the blood counts. Only the animals that survived longer than twenty-six weeks are included in the calculations. There was no significant change in either the hemoglobin value, the erythrocyte count, or the leukocyte count. In view of the hemolysis that must have attended the injection of hypotonic solutions, the slight decrease of the hemoglobin is interesting. Examination of the stained blood films during the course of the experiment disclosed an occasional nucleated erythrocyte and some polychromasias; but these are seen in normal rabbits. No abnormally juvenile white cells were seen, and the few times that a leukocytosis was discovered the animal was found subsequently to have some infection. Nothing that could be interpreted as an irritation or stimulation of the macrophage system was seen.

Much of the material removed at autopsy was extracted and studied spectrographically.

Summary

(1) Fifty-three rabbits were injected intravenously with carcinogenic hydrocarbons in colloidal solution.

(2) Of these, 31 survived for periods of from twenty-six to eighty-one weeks.

(3) All but 7 animals received more than 10 mg. of the carcinogenic agent, and 6 received from 25 to 30 mg.

(4) No tumors were found in any rabbit.
Conclusion

Intravenous injections of colloidal suspensions of carcinogenic hydrocarbons are well tolerated by rabbits, and in our experience induced no malignant changes.

Bibliography