DIBENZANTHRACENE TUMORS IN CONTROLLED STRAINS OF MICE

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At the annual meeting of the American Association for Cancer Research in Washington, three years ago, Dr. C. C. Little expressed the belief that all experimental cancer research on animals should be repeated with controlled strains of animals from genetically well established stock.

Having seen the material of Cook, Hieger and Kennaway (1, 2) in 1932, and being particularly impressed with the fact that 1:2:5:6-dibenzanthracene offered a stable and consistent carcinogenic agent, we believed that it was an ideal compound with which to work out this idea. We decided, therefore, to attack the problem from the angle of the response to painting with dibenzanthracene, of a strain of mice having a naturally high incidence of spontaneous tumors as compared with a strain having no spontaneous tumors. Painting was chosen primarily because of our limited facilities and because Burrows (3, 4), Peacock (5), Maisin (6), Lacassagne (7), and others had already well established the injection method. We also wished to ascertain whether or not the efficiency of this new carcinogenic agent might be in any way affected by reticulo-endothelial block.

It has since developed that Andervont (8) had much the same idea and began work simultaneously with us in August 1933. While his carefully controlled experiments were carried on along different lines and his results were not susceptible of exact correlation with ours, nevertheless he was led to essentially the same conclusions.

We obtained two groups of stock mice from the Roscoe B. Jackson Memorial Laboratory: Strain "A," an albino strain having a high incidence of spontaneous tumors, particularly in breeding females, and "C57," a black strain said to be practically immune to all neoplasia (9). These were well known stock mice whose genetic history had been carefully controlled through many generations.

Although most of the 1:2:5:6-dibenzanthracene used was the commercial product of the E. K. Co., our first lots came from Prof. A. A. Morton of the Massachusetts Institute of Technology, under whose guidance Mr. D. D. Clapp evolved the substance.

Our technic differed in no essential from that followed by other workers. For painting the depilated skin, usually between the shoulders or lower on the back, we used a 0.5 per cent solution of 1:2:5:6-

1 Read before the American Association for Cancer Research, New York, April 17, 1935. For discussion see page 193.
dibenzanthracene in chemically pure benzol, biweekly. For subcutaneous injections, which were done in some few instances, 1.0 per cent 1:2:5:6-dibenzanthracene in sesame oil was used, being injected beneath the loose skin of the back, once a week. If the animal appeared sick or the lump from the previous injection had not practically disappeared, a rest period of a week or two was allowed before injections were resumed. After studying types of solvents, the fatty injection media of other workers, and trying out several oils ourselves, it seemed to us that we could get larger doses of the compound into the mice with less general disturbance and with quicker absorption by using sesame oil.

The diet of the mice was that recommended by the Jackson laboratory and was kept constant. It consisted of a well balanced dog biscuit, water, and fresh vegetables of one kind or another. The living quarters were adequate, though somewhat drafty. Breeding went on uninterruptedly except in those mice with advanced tumors. Altogether we have bred between 2500 and 3000 mice, roughly representing five generations. No mice were used for experimental purposes under two months of age.

For some seemingly inexplicable reason, even in the A strain females who had several litters and received no treatment we had but seven spontaneous tumors of the breast. It now appears that no untreated or breeding females were allowed to live long enough to fall into the time period of eighteen to twenty-four months recommended by Dr. Little (10). The average length of life of our mice was slightly under one year, although it is only fair to state that this figure was materially lowered by the high incidence of pneumonia and toxic deaths. A graph of the time-age incidence of spontaneous tumors in the A strain mice, furnished by Dr. Little, shows the peak between nine and fifteen months. This being true, the low incidence of spontaneous tumors in both our treated and untreated breeding females must be attributed in part to death at an early age. This phase of our experiment is being repeated. Reinhard (11) presents evidence pointing to a reduction in the incidence of spontaneous tumors in a "high"-cancer strain after painting with tar.

The percentage of tumor production did not differ significantly in male and female mice, there being less than a 2 per cent variance between them, in spite of the fact that practically all females had been bred at least once. The control mice were uniformly negative throughout.

Our major experiments were begun in August 1933, on 150 mice each of the A and C57 strains. Only those mice which lived three months or longer under treatment are included in our statistics. Of the albinos, 85 survived, 33 or 38.8 per cent showing malignant epitheliomata of the skin. These were painted an average of thirty weeks before tumor production, the extremes being fifty-three weeks and fifteen weeks.

To determine if the effects of this highly absorbable compound were
in any way altered by reticulo-endothelial block, two series of 75 mice were chosen from each strain, blocked with trypan blue, and then painted as in the preceding experiment. Of the albinos, 35 lived, presenting 10 tumors or 28.5 per cent, while the C57 strain had 40 survivors with 23 tumors or 57.5 per cent.

### Table I: Experimental Results

<table>
<thead>
<tr>
<th>Strain of mice</th>
<th>Total</th>
<th>Died</th>
<th>Exposed</th>
<th>Lesions</th>
<th>Per cent</th>
<th>Average weeks of painting</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, painted</td>
<td>150</td>
<td>65</td>
<td>85</td>
<td>33</td>
<td>38.8</td>
<td>34</td>
</tr>
<tr>
<td>A, blocked and painted</td>
<td>75</td>
<td>30</td>
<td>35</td>
<td>10</td>
<td>28.5</td>
<td>34</td>
</tr>
<tr>
<td>C57, painted</td>
<td>150</td>
<td>53</td>
<td>97</td>
<td>59</td>
<td>60.8</td>
<td>30</td>
</tr>
<tr>
<td>C57, blocked and painted</td>
<td>75</td>
<td>35</td>
<td>40</td>
<td>23</td>
<td>57.5</td>
<td>30</td>
</tr>
<tr>
<td>A, injected</td>
<td>75</td>
<td>42</td>
<td>33</td>
<td>12</td>
<td>36.3</td>
<td>14</td>
</tr>
<tr>
<td>C57, injected</td>
<td>75</td>
<td>61</td>
<td>14</td>
<td>5</td>
<td>35.7</td>
<td>14</td>
</tr>
</tbody>
</table>

For reasons stated above, the dibenzanthracene was applied chiefly by painting. We did, however, inject a small group of each strain. The number of albinos which lived three months or longer was 33, of which 12 or 36.3 per cent showed sarcomatous growths in the subcutaneous tissues. Of the C57 blacks there were but 14 survivors, 5 of which, or 35.7 per cent, presented sarcomata. These were injected, as described above, over a period of fourteen weeks.

Macroscopically, the depilated areas showed no disturbance of hair growth, as commonly occurs in animals painted with tar. This fact was noted, also, by Seelig (12). The skin became dry and scaly and at about the third month began to show small, warty papillomata. These increased in size, irrespective of whether or not painting was continued, tending to become flattened out, in the form of button-like lesions entirely confined to the skin. These expanded rapidly, showing a tendency to break down and ulcerate, covering extensive areas, and almost invariably presenting smooth, heaped up, crater-like edges. Even the largest of these tumors rarely presented any marked invasive characteristics. Frequently a lesion several centimeters in diameter would appear to stop short at a fascial plane, over which it could be easily moved about. Out of a total of 125 malignant epitheliomas, varying from the earliest to the latest stages, we had but one which showed any evidence of metastasis. In this instance the mouse had nodules in either lung. After obtaining the necessary early lesions for histological correlation, we made a practice of allowing the tumor to grow until the animal died.

Microscopically the lesions were typically epitheliomatous, showing the customary anaplasia, mitoses, and invasion of stroma. The stroma was rather uniformly scant, poor in vessels and lymphatics, and tended to show a high incidence of chronic inflammatory changes. The early lesions showed a microscopic picture quite characteristic of a Grade I
epithelioma, but as the lesions became more advanced they seemed to undergo a mutation, sections through the older tumors being more nearly characteristic of Grade III epithelioma. There were neither gross nor microscopic differences between the animals blocked with trypan blue and the others.

The sarcomata grossly were subcutaneous nodules, irregular and semi-encapsulated, showing a distinct tendency to break down and infiltrate surrounding tissue. They grew very rapidly, forming large, rather firmly fixed masses which in a few instances actually exceeded the size of the host. Histologically they seemed to be rapidly growing, highly undifferentiated fibrosarcomata with a great many atypical mitoses, pronounced anaplasia, and a large number of giant tumor cells. They were supported by a small amount of moderately vascular stroma, which on the whole showed much less inflammatory change than that observed in the epitheliomas. These tumors require further study with differential stains to ascertain their exact nature.

An adequate explanation for the paradoxical difference in the incidence of malignant tumors in the A and C57 strains following repeated painting with 1:2:5:6-dibenzanthracene is difficult to arrive at, although in his first communication Little remarks that Korteweg of Amsterdam also found that strain C57 when painted with tar gave a higher incidence of tumors than did a naturally high-tumor strain (dba) similarly treated.

If the results of Reinhard, Korteweg, Andervont, and ourselves will bear close inspection, it would seem that some extrinsic factor other than the chromosome constitution must be responsible for the high incidence of spontaneous tumors in certain strains of female mice. Or do the extrinsic factors suppress and alter the chromosome characteristics?

It is interesting to note that in the animals blocked with trypan blue the ratio of carcinogenesis was practically the same as in the non-blocked group: A animals painted, 38.8 per cent; A animals blocked and painted, 28.5 per cent; C57 painted, 60.8 per cent; C57 blocked and painted, 57.5 per cent. I do not believe that the variation in the figures, statistically amounting to as much as 26 per cent in the first instance, is of any practical significance.

A further point of interest, and the source of considerable conjecture, was observed. Dibenzanthracene is distinctly fluorescent and can easily be identified in microscopic sections with the aid of ultraviolet light. The tumors produced by it do not show any great retention of the compound. It is excreted in the urine and feces; its presence in the gastro-intestinal tract and the kidneys in appreciable amounts is easily demonstrable. The mice painted with the substance always lick a considerable amount of the solution from their sides as soon as they are returned to their cages. Thus a large amount of the compound passes either through or over the epithelium of the gastro-intestinal and genito-urinary systems. We are dealing with a polyphasic, highly carcinogenic agent capable of creating malignancy of the epi-
dermis and of connective tissue. Yet of all our mice autopsied not one showed any tumors of either the gastro-intestinal or genito-urinary system. Further, we can find no such tumors recorded in the literature.

Many times we have elicited the primary papilloma, stopped the painting, and had the tumor progress to a lethal termination. In other words, once certain fundamental cell changes have been initiated, they continue to be governed by the laws of all neoplasia, and, as others have shown, can be continued indefinitely through transplants.

Another interesting phenomenon in the light of the enormous masses of tumor tissue present in a given host was the uniform lack of metastases. At least a dozen mice died with new growths as large or larger than themselves, yet with not a single metastasis, as determined both macroscopically and microscopically. This perhaps is not strange considering the fact that the tumors were primarily dermal, tending to grow expansively and possessing little stroma and few vessels or lymphatics.

**Summary**

1. When 1:2:5:6-dibenzanthracene is painted on the depilated skins of mice of high- and low-cancer strains, almost twice as many tumors develop in the skins of the low-cancer strain as in the other.
2. No such disproportion was observed in the few injected animals which developed sarcomata.
3. In an otherwise normally high-tumor strain of mice painting with 1:2:5:6-dibenzanthracene seem materially to reduce the incidence of spontaneous tumors in breeding females, but untreated breeding controls of the same strain also showed a very low incidence of spontaneous tumors.
4. Blocking with trypan blue had no appreciable effect on the carcinogenic activity of 1:2:5:6-dibenzanthracene.
5. In spite of the positive identification of 1:2:5:6-dibenzanthracene in the gastro-intestinal and genito-urinary tracts of treated mice, no instance of a new growth in these systems was encountered.

**Bibliography**

10. Little, C. C.: Personal communication.