Skin cancer is known to have occurred in some individuals whose occupations have involved long-continued exposure to coal tar, shale oil, or certain petroleum derivatives. The etiological role of these materials has been supported by experiments in which the same substances have induced skin cancer after repeated application to the skin of mice or rabbits. Literature on this subject has been cited by Holt et al. (6) in a paper which suggests certain industrial hygiene measures to minimize or eliminate any possible damage to humans working with oils of the type studied in the present work.

In an effort to anticipate any possible tumor hazard that might be presented by a derivative of a relatively recent advance in petroleum technology, we tested this derivative for carcinogenic activity by repeated applications to the skins of animals. A sample of this derivative, known as the MH 101 used on the monkey is the same material on which the physical properties and analytical data are listed in the papers by Eby, Priestley, Rehner, and Hall (4) and by Diets, King, Priestley, and Rehner (5).

METHODS AND RESULTS

Six young Macacus rhesus monkeys (three males and three females, about 2 years old) were used. The animals were given food (bread, milk, banana, orange, lettuce, Purina Laboratory Chow) and water ad libitum. The basal diet was supplemented with a small amount of cod liver oil weekly. The oil MH 101 was applied 3 times a week to clipped areas of skin—ears, back, scrotum, groin, and suprapubic region for males; and ears, back, breast, vulva, vagina, groin, and suprapubic region for females. The painting was done so as to deposit about 0.2-1.0 gm. at each area at each application. Occasionally, tumors were biopsied and examined histologically. The results obtained from this study are summarized in Table 1. During the first 300 days there was no definite abnormality of the skin at the painted areas. All monkeys presented a healthy appearance comparable to that of untreated monkeys. All three female monkeys have had regular menstrual cycles. On the 892nd day, monkey No. 5, a male, showed two small papillomas on the ear. As painting with the oil continued, all the other monkeys also developed tumors. The number and size of the tumors increased on the ears, back, groin, and suprapubic region for the males; and on the ears, back, and breast region for the females.

Female monkey No. 4 died after 1 year and 118 days. The animal had several small tumors on the breast region. Histological examination of tumors showed only hyperkeratotic papillomas. After 3 years and 311 days, monkey No. 5, female, was accidentally killed. The animal had many tumors on the ears, back, and breast region (Fig. 1) but none on the groin and suprapubic region. Histological examination of large tumors on the ear and back revealed squamous-cell carcinomas in situ (Figs. 2 and 3). Visceral organs were normal.

Monkey No. 1, male, had his first papilloma after 1 year and 168 days of being painted, and squamous-cell carcinoma after 3 years and 978 days. This was proved by histologic examination of biopsied materials. The animal died after 4 years and 117 days. This monkey had not been active for 2 months before death, was emaciated, pale, and losing blood from the gum. At autopsy, the lungs were found to be filled with sootlike material, but there was no evidence of tuberculosis. Visceral organs and tissues appeared normal, but spinal bone was soft and spongy, the ribs swollen. Histological and x-ray examinations of the ribs revealed rickets. The monkey had many large and small skin tumors on the ears, back, groin, and suprapubic region (Fig. 4). Histological examination of the bleeding tumors of the groin and suprapubic region revealed squamous-cell carcinoma (Fig. 5).

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### Table 1

**Summary Showing Times of Appearance of Papillomas and Carcinomas in Monkeys**

(Painting with the oil MH 101 was started on October 15, 1945.)

<table>
<thead>
<tr>
<th>Date</th>
<th>10&lt;sup&gt;5&lt;/sup&gt;</th>
<th>20&lt;sup&gt;5&lt;/sup&gt;</th>
<th>30&lt;sup&gt;5&lt;/sup&gt;</th>
<th>40&lt;sup&gt;5&lt;/sup&gt;</th>
<th>50&lt;sup&gt;5&lt;/sup&gt;</th>
<th>60&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/28/46</td>
<td>0</td>
<td>2 small paps.* on ears</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10/18/46</td>
<td>0</td>
<td>2 paps. on ears</td>
<td>0</td>
<td>1 small pap. on ear, back</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11/22/46</td>
<td>0</td>
<td>1 large pap. on ear</td>
<td>1 small pap. on ear, back</td>
<td>Several small paps. on ear, back</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1/10/47</td>
<td>0</td>
<td>1 large pap. on ear</td>
<td>Several small paps. on ear, back</td>
<td>Several small paps. on ear, back</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2/10/47</td>
<td>0</td>
<td>2 small paps. on ears</td>
<td>2 paps. on ears, onear, breast</td>
<td>Died; paps. on ears, back, breast</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4/1/47</td>
<td>1 small pap. on ear</td>
<td>1 pap. on ear, groin</td>
<td>Several small paps. on ear, back, groin</td>
<td>Several small paps. on ear, back, breast</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1/15/48</td>
<td>Small paps. on ear, groin</td>
<td>Large and small paps. on ears, groin, sup.</td>
<td>1 large pap. on ear; many small paps. on back, groin, sup.</td>
<td>Many paps. on ears, back, breast</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3/18/49</td>
<td>Many paps. on ear, back, groin, sup.</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many paps. on ears, back, breast</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7/20/49</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many paps. on ears, back, breast</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8/22/49</td>
<td>Accidentally killed; had sq. carcinoma on biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/9/50</td>
<td>Died; had paps., sq. carcinomas on groin, sup.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/27/50</td>
<td>Many paps. on ears, back, groin, sup.; some bleeding tumors on back, sq. carcinomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/18/50</td>
<td>Many paps. on ears, back, groin, sup.; some tumors on back, groin, hemorrhagic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/24/50</td>
<td>Many paps. on ears, back, groin, sup.; scrotum; tumors on back, groin, hemorrhagic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/22/51</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/10/52</td>
<td>Died; had paps.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/8/52</td>
<td>Many paps. on ears, back, groin, sup.; ulcerated area on groin, sq. carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/27/54</td>
<td>Many paps. on ears, back, groin, sup.; very large tumor on groin, ulcerated, bleeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/8/54</td>
<td>Died; paps., sq. carcinomas; no metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* paps. = papillomas.  
† sup. = suprapubic.  
‡ sq. = squamous.
DISCUSSION

The findings just described show that application of 0.2–1 gm. of this oil 3 times a week elicited papillomas on the skin of each of six rhesus monkeys. The first papilloma appeared 32 days after the start of the experiment. Three of the animals were males, and three were females. In two of the males and one of the females, epidermoid carcinomas developed through secondary change in papillomas following continued application of the oil. The first cancer was diagnosed by biopsy 3 years and 9 months after the start.

In previous work (9, 10) with mice painted 3 times a week with approximately 15 mg. of this oil, the first papilloma was detected only 29 days after the start, and tumors were present in half of a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day. In a group of 100 mice on the 73d day. The first cancer was diagnosed by the 70th day.
expressing the effect of a drug acting at a distance. Since it is not known whether any remote actions are involved in the development of skin tumors, it seems more pertinent to consider the local concentration of the dose in relation to the size of the areas painted in the several species. This latter is expressed in Table 2. The local concentration of oil can be seen to have been less in the rapidly responding mice than in the more slowly responding rabbits or monkeys. The size of the painted areas was not greatly different in the different species, but in the mice it formed a much larger proportion of the total body surface. If one assumes that the individual cells are not greatly different in size in these species, it is apparent that a larger proportion of the total epidermal cells of the mice were exposed to the carcinogen. This may have been an important factor if some cells are more responsive than others to the action of carcinogens.

Bonne and his co-workers (2) obtained skin tumors relatively quickly on the small Javanese monkey, Macacus cynomolgus, after it was painted with gas works tar. Some of these animals were said to have developed carcinomas, but this diagnosis may be questioned, since the lesions regressed and disappeared. Among ten monkeys which lived for more than 6 months, eight had papillomas, and six of them later showed malignant transformation. The first papillomas appeared in a monkey after 50 skin paintings, in about 6 months, and the earliest time of appearance of carcinoma was about 9 months. The cynomolgus monkey has a surface area comparable to that of a rabbit. We do not have information on its life span.

In an extensive series of experiments carried out upon 50 rhesus monkeys by Pfeiffer and Allen (7), the pure polycyclic hydrocarbon carcinogens, methylcholanthrene, benzpyrene, and dibenzanthracene, were injected into mammary and subcutaneous tissues or various internal organs. No tumors resulted. Application of the carcinogens to the skin elicited hyperkeratoses, warts, and papillomatous masses, but no cancers.

From the data published by these authors, it would appear that only seven of their animals were treated by being painted with carcinogens upon the skin, in each instance a 1 per cent solution of methylcholanthrene in benzene being applied once or twice a week, supplemented in one animal by benzpyrene and in another by dibenzanthracene. In the tabulation of their data, the duration of treatment of these seven monkeys was listed, respectively, as 6 months, 10½ months, 3 years and 3 months, 4 years and 10 months, 9 years, 10 years and 6 months, and 10 years and 9 months. The text, however, states that the treated areas became scarred, painting was replaced by injection of carcinogens in pellet form, and that this latter practice was followed for all animals from the 7th year of the program.

Application of carcinogens by painting could not have continued, therefore, for more than 7 years in the three animals treated longest. In the tests described in the present paper, we found no tumor in a female rhesus monkey painted 3 times

**TABLE 2**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>TIME TO FIRST APPEARANCE OF PAPILLOMA (DAYS)</th>
<th>TIME TO FIRST APPEARANCE OF CANCER (DAYS)</th>
<th>Dose Applied (GM.)</th>
<th>Area Treated (SQ. CM.)</th>
<th>Duration of Treatment (YEARS)</th>
<th>Approx. Life Span (Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>22</td>
<td>70</td>
<td>0.015</td>
<td>25</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>Rat</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>200</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>400</td>
<td>0.25</td>
<td>4</td>
</tr>
<tr>
<td>Rabbit</td>
<td>26</td>
<td>411</td>
<td>0.5</td>
<td>3000</td>
<td>0.17</td>
<td>16</td>
</tr>
<tr>
<td>Monkey</td>
<td>322</td>
<td>1573</td>
<td>0.2-1.0</td>
<td>7000</td>
<td>0.05-0.14</td>
<td>4-35</td>
</tr>
</tbody>
</table>

* Refers to a single painted site.

**FIG. 1.**—Monkey No. 5, showing several large and small tumors on the back, 3 years and 811 days.
**FIG. 2.**—Section of the tumor (Fig. 1) revealing squamous-cell carcinoma. X200.
**FIG. 3.**—Section of a tumor on the ear (monkey No. 5) revealing squamous-cell carcinoma in situ. X150.
**FIG. 4.**—Monkey No. 1, showing many large and small tumors on the groin and suprapubic region, 3 years and 278 days.
FIG. 5.—Photomicrograph of tumor tissue (Fig. 4) from biopsy revealing squamous-cell carcinoma with pearl formation. ×200.

FIG. 6.—Monkey No. 3, showing a papillomatous growth on the ear, 1 year and 168 days.

FIG. 7.—Monkey No. 4, showing a large ulcerated tumor in the right groin, elevated and infiltrating into subcutaneous and muscular layers, 6 years and 329 days.

FIG. 8.—Section of the tumor (Fig. 7) revealing squamous-cell carcinoma. ×200.
a week for 8 years with a 0.3 per cent solution of methylcholanthrene in acetone. Since precancerous skin lesions were observed by Pfeiffer and Allen, cancers might have been expected following treatment. Nevertheless, the substantial periods of time involved suggest that the tumors obtained in rhesus monkeys painted with the catalytically cracked oil resulted from the action of a compound or compounds other than methylcholanthrene or that co-carcinogenic substances in this oil may have speeded results.

It is noteworthy that tumors failed to develop in the vulva or vagina of our monkeys after painting of those areas with the oil that produced tumors on their skin. This finding illustrates limitations in utilizing results obtained upon the skin for prediction of effects upon mucosal surfaces.

A final point of some interest is that the scrotum has been a classical site for the occurrence of occupational skin cancer in men (5). In our experiments upon monkeys, we did not find the skin of the scrotum to be unusually susceptible to the carcinogenic action of the oil we studied; only one of three male monkeys developed a tumor in that site, and the single tumor observed there was merely a papilloma rather than a carcinoma. It would seem that the frequency of occupational skin tumors in this site in men may be related more to hygienic factors, such as less frequent washing of the scrotum, than to tissue susceptibility.

SUMMARY

Repeated application of a high-boiling catalytically cracked oil elicited papillomas on the skin of each of six rhesus monkeys.

Continued application of this oil was followed by malignant change in papillomas on three of these monkeys.

The response of the rhesus monkeys thus corresponded to that of mice and rabbits, both of which species developed papillomas and cancers after being painted with this oil, whereas rats and guinea pigs did not.

ACKNOWLEDGMENTS

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Experimental Production of Carcinoma in Rhesus Monkeys

Kanematsu Sugiura, William E. Smith and Douglas A. Sunderland


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