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 high-boiling catalytically cracked oil and hereafter referred to as MH 101, elicited papillomas and carcinomas on the skin of mice, rabbits, and monkeys, but had no tumorigenic action on the skin of rats or guinea pigs. Details of our experiments upon rodents have been previously reported (9-11). The present paper describes our experience with monkeys and records, insofar as we are aware, the first instance of experimental induction of skin cancer in the Macacus rhesus.

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 8 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. As painting with the oil continued, the number and size of the papillomas increased on the ears, back, groin, and suprapubic region for the males; and on the ears, back, and breast region for the females.

Male monkey No. 1, had his first papilloma after 1 year and 168 days. This monkey had no tumors at first, but after 1 year and 117 days, the number of tumors increased (Fig. 2). Histological 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. 5). Histological examination of the bleeding tumors on the groin and suprapubic region revealed squamous-cell carcinoma (Fig. 5).

Female monkey No. 2, a male, was the most sensitive to the action of oil painting. The first tumor, a benign papilloma, appeared on the 822d day after the beginning of the painting. As painting with the oil continued, the number and the size of the papillomas increased on the ears, back, and breast region (Fig 1) but none on the groin and suprapubic 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.

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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°</th>
<th>8°</th>
<th>30°</th>
<th>4°</th>
<th>5°</th>
<th>6°</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>Several small pap. on ear, back</td>
<td>2 small paps. on ear</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 pap. on ear, back</td>
<td>Several small pap. on ear, back</td>
<td>0</td>
</tr>
<tr>
<td>1/10/47</td>
<td>0</td>
<td>1 large pap. on ear</td>
<td>Several small pap. on ear, back, breast</td>
<td>Several pap. on ear, back, breast</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2/10/47</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</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 pap. on ear, back, groin</td>
<td>Several pap. on ear, back</td>
<td>Several small pap. on ear, back</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 ear, back</td>
<td>Many paps. on ear, back, breast</td>
<td>A few paps. on ear, back, breast</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>1 bleeding tumor on back</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many paps. on ears, back, breast</td>
<td>A few paps. on ears, back, breast</td>
</tr>
<tr>
<td>7/20/49</td>
<td>Many paps. on ears, back, groin, sup., sq.† carcinoma on biopsy</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many paps. on ears, back, breast</td>
<td>Many paps. on ears, back, breast</td>
<td>Accidentally killed; had sq. carcinoma on ear, back</td>
<td></td>
</tr>
<tr>
<td>8/22/49</td>
<td></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>Many paps. on ears, back, groin, sup.; some bleeding tumors on back, sq. carcinomas</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many paps. on ears, back, groin, sup.</td>
<td>Many small paps. on ears, back, breast; one pap. on groin</td>
<td>Died; had paps.</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>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 small paps. on ears, back, breast; one pap. on groin</td>
<td>Died; had paps.</td>
</tr>
<tr>
<td>10/18/50</td>
<td>Many paps. on ears, back, groin, sup.; some tumors on back, groin, hemorrhagic</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 small paps. on ears, back, breast; one pap. on groin</td>
<td>Died; had paps.</td>
</tr>
<tr>
<td>12/24/50</td>
<td>Many paps. on ears, back, groin, sup.; scrotum; tumors on back, groin, hemorrhagic</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 small paps. on ears, back, breast; one pap. on groin</td>
<td>Died; had paps.</td>
</tr>
<tr>
<td>5/22/51</td>
<td>Many paps. on ears, back, groin, sup.; ulcerated area on groin, sq. carcinoma</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 small paps. on ears, back, breast; one pap. on groin</td>
<td>Died; had paps.</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>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 small paps. on ears, back, breast; one pap. on groin</td>
<td>Died; had paps.</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.
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 322 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 biopsy 3 years and 328 days old at the time of death. At autopsy, lymph nodes in the left groin region were greatly enlarged. There was a large ulcer, 10 × 8 cm. in the right groin, elevated and infiltrating into subcutaneous and muscular layers. The peritoneal cavity showed no free fluid or evidence of tumor invasion. There were multiple lung abscesses and marked pulmonary edema thought to be the cause of death of this monkey. The monkey had many small and large tumors on the ears, back, scrotum, groin, and suprapubic region, mostly papillomas. Histological examination of the ulcerated tumors on the skin of each of six rhesus monkeys revealed squamous-cell carcinoma (Fig. 6). There were no metastases.

Long-continued oil painting of the skin of the vulva and vagina failed to induce tumors.

During the course of study one female monkey was similarly subjected to skin painting with 0.8 per cent acetone solution of methylcholanthrene. It is interesting to note that at the end of 8 years (April 30, 1956) this monkey had failed to develop either papillomas or carcinomas at various painted areas (ears, back, breast, vulva, vagina, groin, and suprapubic region).

**DISCUSSION**

The time elapsed between the first application of this and other oils have been observed data seem pertinent. The pathology and rate of appearance of tumors in mice following application of this and other oils have been described in an earlier paper (11). In human beings, the “latent” period elapsing between the first exposure to a carcinogen and eventual appearance of cancer may be a substantial part of the total life span; for example, among pitch and tar workers the average time between beginning of employment and development of skin tumors has been given as 20–24 years, and among workers in petroleum oils as 50–54 years (5). In reference to data summarized in preceding paragraphs, the nearly 4 years that elapsed from the beginning of the present experiments until the first appearance of cancer in a monkey is approximately a quarter of the life expectancy reported for this species under laboratory conditions (7). The 2-year latent period that we observed in rabbits is about two-fifths of the life span of that species, while the 70-day latent period observed in mice is about one-tenth of their life span. The relatively long latent period usually described in cases of occupational skin cancer in men may possibly be due to hygienic precautions, such as washing, for the rate of development of tumors in mice painted with the oil used in the present tests can be greatly slowed by washing with soap and water after each application, and further delayed or completely obviated by treating the skin with certain barrier creams prior to each application of oil, provided that washing is done within 3 hours after the oil is applied (8).

It is striking that cancers arose so quickly in mice, since the dose applied to them was much smaller than the dose applied to rabbits or monkeys. If the doses used upon the several species are calculated on the basis of dose per kilogram of body weight, then a different picture is obtained and the speed of cancer formation is found to be, very roughly, proportional to the dose.

This latter calculation is more appropriate in
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>
<thead>
<tr>
<th>SPECIES</th>
<th>TIME TO FIRST APPEARANCE OF PAPILLOMAS</th>
<th>Dose applied</th>
<th>APPROX. WEIGHT OF ANIMAL</th>
<th>AREA TREATED</th>
<th>APPROX. LIFE SPAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>22 (days) 70 (days)</td>
<td>0.015 (gm.) 25 (gm.)</td>
<td>0.6 (gm.)</td>
<td>1 sq. cm.</td>
<td>0.015 (gm.) 2 years</td>
</tr>
<tr>
<td>Rat</td>
<td>0 (days) 0 (days)</td>
<td>0.1 (gm.) 200 (gm.)</td>
<td>0.5 (gm.)</td>
<td>4 sq. cm.</td>
<td>0.025 (gm.) 3 years</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>0 (days) 0 (days)</td>
<td>0.1 (gm.) 400 (gm.)</td>
<td>0.25 (gm.)</td>
<td>4 sq. cm.</td>
<td>0.025 (gm.) 6 years</td>
</tr>
<tr>
<td>Rabbit</td>
<td>26 (days) 411 (days)</td>
<td>0.5 (gm.) 3000 (gm.)</td>
<td>0.17 (gm.)</td>
<td>10 sq. cm.</td>
<td>0.051 (gm.) 5 years</td>
</tr>
<tr>
<td>Monkey</td>
<td>322 (days) 1573 (days)</td>
<td>0.9-1.0 (gm.) 7000 (gm.)</td>
<td>0.05-0.14 (gm.)</td>
<td>4-55 sq. cm.</td>
<td>0.06-0.06 (gm.) 16 years</td>
</tr>
</tbody>
</table>

* Refers to a single painted site.
Fig. 5.—Photomicrograph of tumor tissue (Fig. 4) from biopsy revealing squamous-cell carcinoma with pearl formation. ×200.

Fig. 6.—Monkey No. 8, showing a papillomatous growth on the ear, 1 year and 168 days.

Fig. 7.—Monkey No. 3, 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

The authors wish to express their appreciation to Dr. J. P. Holt, formerly with the Standard Oil Company (N.J.), Dr. R. E. Eckhardt of the Esso Research and Engineering Company (N.J.), and Dr. C. P. Rhoads for their interest and valuable advice; and to Mr. Albert Mitchell and Mr. William Du Bois for the arduous monkey handling.

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