Attempts to Produce Cancer in Rhesus Monkeys with Carcinogenic Hydrocarbons and Estrogens*

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By 1936 a great amount of work had been done on cancer in the lower mammals, the results of which were being used with increasing frequency in attempts to interpret human cancer, yet very little was known about cancer in other primates. The only experimental study in this regard was that of Bonne, Lodder and Streef (2) on the effects of cutaneous application of coal tar on the Java-nese monkey. It therefore seemed that a careful investigation should be made of the effects of carcinogenic hydrocarbons and estrogens on the Rhesus monkey. Despite the large numbers of Rhesus monkeys that have been under observation for long periods of time, including the latter half of the life span, only 2 cases of spontaneous cancer have been described in this monkey, an osteogenic fibroma (1) and a prostatic carcinoma (6). This paucity of reported cases would suggest a low susceptibility to cancer but cannot in itself be considered adequate proof that such is true. In the study to be reported here it soon became evident that cancer would not be induced by brief periods of treatment. Therefore, a relatively large number of monkeys was set up for chronic treatment to continue until the animals died. No cancers have been observed, and the present time was arbitrarily chosen to report the results of these experiments, since only 4 animals remain alive. Of these 4, 3 have been treated for 8 to 10 years and are still in good health.

MATERIAL AND METHODS

For this experiment, which has run from 1936 to the present time, 50 Rhesus monkeys were used. Three of these monkeys were mature males; the rest were females. Twenty-one of the females were castrated and 3 were hysterectomized. They were purchased from a commercial dealer. It is estimated that the monkeys were between 3 and 10 years of age when treatment was begun, although some may have been older. They usually weighed between 4 and 5 kilos. During the winter the animals were kept indoors. No attempt was made to keep the room at a constant temperature, the temperature probably fluctuated from 70° to 85° F. In the summer the monkeys were kept in outside enclosed paddocks open to the sun and air.

The type of food varied a great deal during the period of treatment but was controlled to the extent that the animals remained in good health and maintained or gained weight depending on the age of the individual animal. From 1936 to 1938 the diet was made up of a variety of fruits and vegetables, but thereafter a more standardized diet was used. This consisted of prepared dog meal and pellets which provided an adequate supply of all of the known vitamins except vitamin C. The latter was supplied by feeding canned tomatoes, oranges or fresh cabbage. In 1938 Eastern States Dog Food was fed during the winter as a mash supplemented with canned tomatoes. During the summer months this food was given in pellet form, supplemented with oranges. During 1939 Purina Fox Chow and later Purina Dog Chow were substituted for the Eastern States Dog Food, primarily because of taste preference. Since 1942 all of the prepared dog food has been fed in pellet form and vitamin C has been supplied almost exclusively by feeding fresh cabbage. During the entire period the monkeys were fed Chim Crackers (Kennel Food Supply Co.) once or twice a week for variety. Water was available at all times.

Nearly all the organs and tissues that frequently become cancerous in the human being were treated with carcinogenic hydrocarbons and estrogens. The specific treatment for each animal is given in Table I. Both systemic and local applications were used.
Estrogens and carcinogens were administered directly to the breasts. In several animals a specific carcinogen was injected directly into one or more of the 8 or 10 ducts opening into the nipple so that a particular sector was impregnated with the carcinogen.

Estrogens.——A variety of estrogens was used. At the beginning of the experiment estradiol benzoate (10,000 I.U. per cc.) and theelin (1,000 I.U. per cc.) in sesame oil were employed. In 1938 estradiol dipropionate (50,000 I.U. per cc.) was substituted for the theelin and estradiol benzoate. In the same year, when synthetic estrogenic substances became available, one animal was started on triphenylethenylene and one on diethylstilbestrol. At this time all estrogens were dissolved in oil and injected subcutaneously or intramuscularly into the region of the thigh except where injections were made directly into the breast. In general 5,000 I.U. were injected twice weekly but a considerable range of dosage was represented in the animals treated (Table I). Beginning in 1938 estradiol dipropionate in pellets was administered to one animal. Most of the estrogen-treated animals started after 1939 received either estradiol dipropionate or estrone in pellets placed subcutaneously in the breast and thorax. After 1942 all monkeys that were still on chronic estrogen treatment were implanted with pellets of 30 per cent stilbestrol in cholesterol and reimplemented as soon as the estrogen effects began to fade.

Carcinogenic hydrocarbons.——The carcinogens used were methylycholanthrene, benzpyrene and dibenzanthracene in crystalline form. For subcutaneous injection they were dissolved in sesame oil in such concentration that each cc. contained 10 mgm. of carcinogen. It was necessary to keep the dibenzanthracene in an incubator in order to keep it in solution. These three substances were also painted on the skin from a benzene solution (10 mgm. per cc.). In addition dibenzanthracene was painted from a methyl salicylate solution (10 mgm. per cc.). Injections of one mgm. of carcinogen in oil were usually made twice weekly. Cutaneous applications were made once or twice a week, but this method of application, together with the volatile nature of the solvent, made it difficult to determine the exact amount of carcinogen administered. It was estimated, however, to be about 1 mgm. per application. Usually more than one site of injection as well as painting was used at the same time, so the weekly dose of carcinogenic hydrocarbons varied from 2 to 6 mgm. Other methods of application were also used. Methylycholanthrene was implanted in paraffin pellets or in capillary tubes. Also crystals of methhylcholanthrene were dusted into the peritoneal cavity, sewed into an omental pocket or placed over the uterus and held in place by suturing the omentum to the uterus. Pellets of methylycholanthrene and benzpyrene were placed subcutaneously, into the myometrium, into the cervix, into the submucosa of the vagina, under the serosa of the stomach, or into the mammary glands. These were introduced by means of a number 13 gauge trocar. Methylcholanthrene was suspended in horse serum for intravenous injection and was given orally in large doses to one animal. The oral application was made either by stomach tube or fed in glucose solution. Beginning in 1943 the respective carcinogens were given in pellet form to all of the chronically treated animals because the areas where injections and paintings had been made were so scarred that it was doubtful that much of the carcinogen was retained following injection, or absorbed following painting.

 OBSERVATIONS
Since the animals received such varied treatment and each treated area gave a variety of responses, abbreviated protocols will be given for 2 monkeys which have been used rather extensively as material for illustrating this paper. These protocols will be given before the general description, since for brevity it is necessary to condense the observations under the headings of the various chemicals used.

ABBREVIATED PROTOCOL I
Lesions: Breast.—Within 2 months both nipples had doubled in size and were firm. A ligneous induration extended out radially from each nipple and the skin became firmly adherent to the underlying tissue. After 7 months small abscesses formed and ulcerated, first in the right breast and then in both breasts. This effect became so extreme as to necessitate intermittent treatment during the following months. When healing, the ulcerated areas

(Continued on Page 100)
<table>
<thead>
<tr>
<th>Number and sex</th>
<th>Methylenecholanthrene</th>
<th>Dosage*</th>
<th>Diameter/Total gm</th>
<th>Dosage*</th>
<th>Weekly Total gm</th>
<th>Dosage*</th>
<th>Period of Treat. gm</th>
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<td></td>
<td></td>
<td></td>
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<td>Thigh intramuscularly $</td>
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<td></td>
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<td>Thigh intramuscularly $</td>
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<td>0.072</td>
<td></td>
<td>Abdomen$</td>
<td>0.080</td>
<td>Thigh intramuscularly $</td>
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</table>

*Also received 75 mgms. of testosterone propionate.
§Castrated.
showed a tendency for keloid formation. Irregular pigmentation and fibrotic reactions persisted after healing.

During the second year there was little change in the breasts; both continued to show marked induration surrounding the nipples. During the third year the dibenzanthracene treatment in the left breast maintained it in a chronic state of inflammation at the injection site. The left breast continued to show much greater reaction to the carcinogen than did the right breast which received benzpyrene. However, a mass having the characteristics of a fibroma developed in the right breast. It was apparently non-tender, and was fixed to the overlying epidermis. During the fourth year the carcinogen caused one to form two distinct masses, one below the nipple and fixed to the overlying epithelium and the other in the superior lateral quadrant and fixed to the underlying tissue. Due to the marked induration and scar formation of the left breast, the left nipple became twice as large as that of the right and was slightly cyanotic and colder. These conditions (Fig. 1) were maintained until the animal came to autopsy.

Left ear.—Injections into the left ear were begun in January, 1937; the helix and antitragus of the pinna were the sites of injection. Fibrous tissue masses were induced rather rapidly, and ulcerations not infrequently produced. In August, 1939, the injection site was changed to the anterior portion of the pinna adjacent to the point of attachment of the head, and to the area just anterior to the attachment of the pinna. This area was anatomically able to tolerate the injections of the foreign material. A mass developed here and gradually increased in size until February, 1940, when it underwent internal necrosis with the discharge of thick gummosus material from several sinuses. The injection site was then moved slightly anterior to the tragus, where a cleanly circumscribed mass developed which was in part subcutaneous fibrous proliferation and in part epithelial proliferation. The surface of this mass desquamated, and, if rubbed, left small bleeding points beneath the easily detachable cornified layer. The old injection sites remained readily visible as scarred desquamated or proliferated areas (Fig. 4). This condition remained until autopsy.

Right ear.—The right ear was also started on treatment in January, 1937, but with benzpyrene, the injection sites being the same as those for the left ear. There was little reaction during the early treatment, but pressure necrosis resulting from the instillation of the oily material into this site occurred at intervals. In August, 1939, when the injection site of the left ear was shifted, the injection site in the right ear was moved to the anterior portion of the helix adjacent to the point of attachment, and to the skin at, and anterior to, the tragus. A large, firm mass resulted at this point (Fig. 5). A well demarcated epithelial reaction over this mass was also observed. The skin thickened and desquamated but did not ulcerate. The reaction years this layer became densely adherent to the living skin, and its removal caused small bleeding points. In June, 1938, painting with methylcholanthrene in benzene was substituted for dibenzanthracene in benzene on the interscapular area, and methylsulicylate was substituted for benzene as a solvent for dibenzanthracene for painting the perianal region. During the early treatment there was no appreciable loss of hair, but gradually the hyperkeratinization of the hair follicles and sebaceous glands became quite extreme. Localized areas of extreme epidermal proliferation occurred late in the treatment and persisted until autopsy.


Photographs: All treated areas were photographed June 15, 1940.


Autopsy: Gross findings. After July, 1940, all injections were made in such a way as to not disturb the areas of reactions any more than necessary, with the result that at autopsy there were no ulcerated areas of necrosis. The hyperkeratinization and fibrotic masses described above were still present, although the skin appeared more normal in texture and the fibrotic masses had a more flexible feel. The animal had a heavy layer of subcutaneous fat and showed no evidence of emaciation. The digestive tract was typical of all other animals dying of dysentery. All other organ systems were negative as to pathology. The ovaries and uterus were quite small for an adult female. The cervix appeared to be greatly fibrosed.

Histological findings.—The skin in the treated areas was about 4 times as thick as normal skin. In addition to a piling up of the epithelium, there were nests of squamous cells with occasional pearl formation. This proliferation was orderly, however, and the basement membrane was intact throughout. The epithelium contained occasional mitotic figures. The fibrotic masses consisted of moderate

### Table II: Carcinogenic Treatment of Monkey No. 66

<table>
<thead>
<tr>
<th>Carcinogen*</th>
<th>How applied</th>
<th>Date started</th>
<th>Areas treated</th>
<th>Amount weekly</th>
<th>Total</th>
<th>gm.</th>
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<td>injected in oil</td>
<td>1/1/37</td>
<td>left ear</td>
<td>1</td>
<td>0.323</td>
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<tr>
<td>DBA</td>
<td>&quot; &quot; &quot;</td>
<td>&quot; &quot; right ear</td>
<td>1</td>
<td>0.430</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>&quot; &quot; &quot;</td>
<td>&quot; &quot; right breast</td>
<td>10</td>
<td>3.180</td>
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<td></td>
</tr>
<tr>
<td>MC</td>
<td>&quot; &quot; &quot;</td>
<td>&quot; &quot; perianal</td>
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<td>0.380</td>
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</tr>
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<td>DBA</td>
<td>painted in benzene</td>
<td>6/1/38</td>
<td>interscapular</td>
<td>10</td>
<td>1.820</td>
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* DBA, dibenzanthracene; BP, Benzyrene; MC, Methylcholanthrene.

Cancer Research
proliferations of mature fibroblastic elements and a minimal infiltration of lymphocytes and plasma cells. The entire treated area showed a diffuse pattern of increased numbers of collagenous fibers with dispersed small areas of chronic inflammation. The older treated areas showed typical scar tissue. The painted areas showed the typical extreme infiltration of lymphocytes and plasma cells. The entire alveolar system was appreciably stimulated, especially one showing much more edema than the right one. The terminal ducts of the mammary glands were normal, and the glands of the uterus were moderately well developed. At 4 months both breasts showed developed gradually. At 4 months both breasts showed the formation of non-inflammatory fibrous masses at the site of injection, and, in contrast to the usual effect, the early response in the right breast to injected benzpyrene was of greater magnitude than that of the left breast receiving methylcholanthrene. By November, 1938, some of the fibrous tissue in the right breast had resolved into a small nodule which was firm and movable. It was thought possible that it might be composed of mammary tissue. The left breast had now begun to show more striking effects. There was a thick, horizontal band, presumably derived from the epidermis, across the mammary region at the level of the nipple. None of the tissue changes involved visible inflammatory reactions. From this period on, the reaction of the left breast to methylcholanthrene increased, while that of the right breast (benzpyrene treated) remained relatively unchanged for long periods or showed periods of slow regression followed by periods of slow progression. The tissue mass in the left breast became organized into a distinct nodule. It continued to grow and presumably represented a moderate proliferation of the basal layers of the skin with infiltration of dense connective tissue. By February, 1940, a firm irregular mass had formed around the left nipple. This was firmly affixed to the overlying skin. The reaction in the left breast continued to be quite marked. There was considerable thickening of the epithelium, desquamation and loss of hair, with a vertical extension of this reaction near the nipple. By May, 1940, this surface reaction had become more widespread, and the entire painting site about the left nipple presented a picture of multiple punctate areas of proliferation of the surface epithelium with a scaling of these irregular macules and papules. This condition is illustrated in the photograph (Fig. 3) which was taken in June, 1940.

Table III. Carcinogenic Treatment of Monkey No. 705

<table>
<thead>
<tr>
<th>Carcinogen*</th>
<th>How applied</th>
<th>Date started</th>
<th>Areas treated</th>
<th>Amount weekly</th>
<th>Total mgm.</th>
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<td>left ear</td>
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<td>182</td>
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<td>11/43</td>
<td>left breast</td>
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<td>364</td>
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<tr>
<td>BP</td>
<td>injected in benzene</td>
<td>5/5/47</td>
<td>right ear</td>
<td>1</td>
<td>182</td>
</tr>
<tr>
<td>MC</td>
<td>pellets</td>
<td>3/16/45</td>
<td>left breast</td>
<td>25</td>
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<td>pellets</td>
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<tr>
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<td>left breast</td>
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<tr>
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<td>pellets</td>
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<td>left breast</td>
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<td>5/15/47</td>
<td>right ear</td>
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Total: MC, 1.292 gm.; BP, 0.564 gm.; DBA, none; Estrogen, none.

*MC, methylcholanthrene; BP, benzpyrene; DBA, dibenzanthracene.

right mammary gland showed extreme metaplasia (Fig. 15). The ovaries were atrophic, the cervix was fibrosed, but the glands of the uterus were moderately well developed, despite the marked atrophy of the ovary.

ABBREVIATED PROTOCOL 2


Lesions: Breasts.—Reaction to the injected carcinogen developed gradually. At 4 months both breasts showed the formation of non-inflammatory fibrous masses at the site of injection, and, in contrast to the usual effect, the early response in the right breast to injected benzpyrene was of greater magnitude than that of the left breast receiving methylcholanthrene. By November, 1938, some of the fibrous tissue in the right breast had resolved into a small nodule which was firm and movable. It was thought possible that it might be composed of mammary tissue. The left breast had now begun to show more striking effects. There was a thick, horizontal band, presumably derived from the epidermis, across the mammary region at the level of the nipple. None of the tissue changes involved visible inflammatory reactions. From this period on, the reaction of the left breast to methylcholanthrene increased, while that of the right breast (benzpyrene treated) remained relatively unchanged for long periods or showed periods of slow regression followed by periods of slow progression. The tissue mass in the left breast became organized into a distinct nodule. It continued to grow and presumably represented a moderate proliferation of the basal layers of the skin with infiltration of dense connective tissue. By February, 1940, a firm irregular mass had formed around the left nipple. This was firmly affixed to the overlying skin. The reaction in the left breast continued to be quite marked. There was considerable thickening of the epithelium, desquamation and loss of hair, with a vertical extension of this reaction near the nipple. By May, 1940, this surface reaction had become more widespread, and the entire painting site about the left nipple presented a picture of multiple punctate areas of proliferation of the surface epithelium with a scaling of these irregular macules and papules. This condition is illustrated in the photograph (Fig. 3) which was taken in June, 1940.

The above conditions continued until the end of 1941. By this time there was considerable thickening of the skin. This hyperkeratinization plus a deposit of the painted carcinogen made it doubtful whether the new carcinogenic applications were being absorbed. It was, therefore, decided to implant pellets of the carcinogens. The hyperkeratinization then gradually cleared up, and although the papillae and nodules still persist to some extent, they have taken on much the same consistency as the normal tissues around them. A biopsy of one of the nodules in May, 1945, showed that most of the dense fibrous tissue of the nodule had been replaced by fat.

Left ear.—From June, 1938, until August, 1939, methylcholanthrene was administered to the helix and antitragus of the pinna by injection and painting. The injections...
were not well tolerated here for anatomical reasons. Inflammation was evident within a few months, and ulceration soon began. Ulceration and sloughing were so frequent that the injection site was changed (August, 1939) to the anterior portion of the helix and adjacent epithelium. Although better able, anatomically, to tolerate the injection, the new site showed pronounced reactions by October, 1939. Firm non-inflammatory fibrotic masses formed, accompanied by thickening, pitting and scaling of the epithelium. By June, 1940, this condition had progressed to the state seen in Fig. 7. The general fibrosis continued to the extent that it became almost impossible to have the injected carcinogen retained. Therefore, treatment by implantation of pellets was begun in December, 1941.

Right ear.—Benzpyrene injections into the helix and antitragus of the pinna were begun in June, 1938. The injections were poorly tolerated, but otherwise the reactions were not very marked. In August, 1938, the injection site was changed to the anterior portion of the pinna and adjacent pre-pinnal epithelium. A fibrous tissue mass formed within a month with an epithelial reaction similar to that for the right ear. By June, 1940, this had progressed to the condition seen in Fig. 6. In this ear, also, pellets replaced the injection treatment in December, 1941.

At the present time, despite the number of pellets that have been implanted, the surface reaction of both ears has completely cleared and the fibrous masses and nodules have regained almost the same consistency as the surrounding tissue. This animal is still alive, in good health, and is being continued on treatment with implanted pellets of carcinogens.

Estrogens.—At the start of treatment there was a marked increase in the thickness of the sex skin, the edema in many cases extending over the back of the hind legs, dorsally over the crest of the ilium and forward over the forehead and face. The sex skin areas and the forehead showed a marked increase in color which usually reached a maximum within a month. After a few months of treatment both the swelling and color decreased rather slowly to a state about midway between the maximal and minimal conditions and remained at about this stage as long as estrogen was given.

Long-continued estrogen stimulation at or near the toxic level caused a loss of the normal glossiness of the hair, alopecia, loss of muscle substance, and partial atrophy of the hamstring muscles with resulting contracture of the lower limb at the thigh. The marked emaciation was followed by a cachectic condition which would result in death if sufficient rest periods were not allowed. However, there was, as could be expected, considerable variation in individual tolerance to estrogens, some animals being able to tolerate one or more milligrams weekly without the toxic effects becoming severe enough to force discontinuation of treatment.

With continued administration of smaller doses of estrogen, as has already been shown (14), menstruation occurred with quite definite periodicity even when the monkey was castrated. With reasonably large doses, the endometrium became refractory after a time and estrogen-withdrawal menstruation did not occur. One animal that received small doses of estrogen (0.05 mgm. weekly) for 3½ years, menstruated 18 days after estrogen withdrawal instead of the usual 7 to 9 days. Other animals treated for 6 months with 1.5 to 6.0 mgm. of estradiol dipropionate weekly had not menstruated by a month after estrogen withdrawal, at which time estrogen treatment was resumed. This endometrial exhaustion followed all types of estrogen treatment if the dose was high enough and the period of treatment sufficiently long.

Blood studies were made on several animals receiving fairly large doses of estrogen, but alteration of the blood picture was detected only when other toxic effects were visible. A dose which gave only a slightly toxic reaction was seen in one animal to cause a slight anemia without any change in the white cell count. Doses which became extremely toxic caused a striking alteration in the blood picture. Complete studies were made on only two animals of this type, but casual observations indicated that other animals receiving toxic doses followed the same course. There was a gradual development of anemia, accompanied by a rather gradual but distinct leukocytosis during most of the period of treatment. However, toward the end of treatment the leukocyte count fell, and with the extreme cachectic condition a sudden leukopenia occurred (1,000 WBC).

The fact that estrogen treatment readily causes cervical metaplasia in the monkey (13, 5), and cervical cancer in mice (7) suggested the female genital tract as the most probable site of malignancy in the chronically treated monkeys. However, no malignant growths were observed. Marked metaplasia of the epithelium of the cervical glands occurred (Figs. 25 to 35), and sometimes the glands showed a strongly cystic condition (Figs. 27 and 36). The metaplastic epithelium began to develop in localized areas (Figs. 25 and 26) at the base of the normal columnar epithelium and pushed up to displace it with a stratified epithelium (Figs. 29, 30 and 34). This metaplasia usually involved only the tips of the glands (Figs. 31 and 32), but occasionally became more extensive (Figs. 28 and 33). The cervical glands sometimes became so hyperplastic that the condition might almost be termed adenomatous (Fig. 35). However, nothing resembling cervical carcinoma developed even though the cervixes of 4 monkeys were subjected to repeated trauma (cauterization, biopsies and suturing with a large surgical needle) during 6 months to a year and a half (Fig. 37). After prolonged...
treatment the myometrium became quite fibrotic and often contained numerous hyalinized areas, resembling to a great extent the old age changes which occur in the uterus. The endometrial glands at first showed the typical estrogen response, but after the exhaustion phase of the uterus had set in (following prolonged treatment) the glands were few in number and were long and straight with marked hyalinization around the deep portions of the glands. The vagina was usually quite hyperplastic, and epithelial bulbs were frequently found along the basal layer (Fig. 38).

Although several animals (Table I) received very high doses of estrogen, over extended periods of time, there was no evidence of pathological change in the mammary glands. This observation held true even though 3 animals had the estrogen injected directly into the gland in an attempt to increase the local concentration of the hormone. As has been shown before (8), full development of the mammary glands can be obtained in the monkey with estrogen alone. This requires 5 to 6 months of treatment. In the chronically treated monkeys secretion was present in the glands regardless of whether the animal's ovaries were present or not. Occasionally cystic dilation of the ducts was seen. The metaplasia of the lactiferous ducts which occurred when carcinogenic hydrocarbons were given in conjunction with estrogen will be discussed under the effects of the carcinogens since these effects also occurred when the carcinogenic hydrocarbons were given alone.

**Methylcholanthrene.**—Since the most profound tissue changes were obtained with methylcholanthrene, the lesions caused by this substance will be discussed first. As was described in the section on material and methods, the carcinogenic agent was applied as directly to the tissue as possible. There were apparently no toxic effects of the methylcholanthrene as such, even when massive doses were given orally or intravenously. When the agent was painted on the skin, hyperkeratinization always occurred, the early tissue changes being detectable within a week. At first there was a gradual thickening of the epithelium which was due principally to an increase in the thickness of the cornified layers, particularly the sloughing stratum disjunctum. There was a gradual atresia of the sebaceous glands and a thinning of hair with the eventual formation of cystic sebaceous glands and hair follicles filled with sloughed cornified epithelium (Figs. 13, 19 and 22). The basal layers of the epithelium proliferated very rapidly and frequently formed broad pegs of epithelium (as shown in Fig. 20 after benzpyrene treatment) or malignant-looking branching down-growths (Fig. 21). The latter occurred following the extreme hyperkeratinization and ulceration. The surface epithelium at other times appeared to be extremely disorganized. These changes have never been observed to go further than is illustrated in Fig. 21, and they clear up after the application site is moved. There was an increase in fibrous connective tissue in the subcutaneous layer which became the primary tissue reaction when the carcinogen was injected subcutaneously in oil (Fig. 18; compare with Fig. 17).

The painting of areas with carcinogen in either benzene or methyl salicylate usually caused an extensive dermatitis. Painting with benzene alone produced only a mild superficial dermatitis. It was almost impossible to keep these areas aseptic and some of the extreme inflammation seen must have been partially due to infection. It is impossible, however, to separate the two effects. Inflammatory lesions were commonly produced and were followed by the formation of granulation tissue and scabbing, which, combined with the sloughed epithelium and dried carcinogen, formed warty excrescences (Figs. 11 and 12). Removal of the latter left a very hyperemic, easily bleeding, pitted surface which usually continued to ooze a serous exudate. The whole area eventually became walled off by coarse collagenous connective tissue fibers, and the thickened, scarred surface became relatively avascular. This, together with the hyperkeratinization, probably made the area relatively impervious to the carcinogen.

It was impossible to separate the effects of infection from the effects of the carcinogen when the carcinogens were injected in oil, particularly when ulcerations occurred since here again the treating agent was not aseptic. The general effect of the injection of methylcholanthrene in oil was to produce a mild granulation at the site of injection so that often a definite mass could be palpated within 7 days after the first injection of the carcinogen. These masses continued to enlarge gradually and after a few months often became more than 3 cm. in diameter. The latter were usually simply granulomatous masses (Figs. 39 to 43), but frequently, with continued injections, they became inflamed and often broke down and ulcerated, making it necessary to change slightly the injection site. Usually, however, there was a more diffuse reaction which involved an increase in the number of collagenous connective tissue fibers in the subcutaneous tissue, with small localized areas of lymphocytic infiltration. There were occasional areas of acute inflammation (polymorphonucleated cells), with numer-
ous macrophages, some of which had phagocytized crystals of the carcinogen.

Some of the first pellets that were implanted ulcerated out of the subcutaneous tissue. However, with proper care in making and inserting pellets of methylcholangthrene, the only reaction seemed to be the formation of small granulomatous nodules which soon regressed to form firm connective tissue capsules about the pellets (Fig. 45). There was an appreciable inflammation between the connective tissue capsules and the pellets (Fig. 48) which also was often present in certain areas outside the capsule (Figs. 46 and 51). This condition persisted for several months. There was no evidence that the pellets were so effectively walled off that this carcinogen could not get out to the tissues. Crystals of methylcholangthrene placed in the omentum usually became surrounded by basophilic fibroblasts. Oral and intravenous administration was without effect. Gross lesions which occurred when methylcholangthrene was injected and painted on the ear are shown in Fig. 7. These lesions never became malignant, and when injections and painting were stopped they eventually healed, even though a pellet was placed in the same region.

When the breasts were treated with methylcholangthrene, numerous granulomatous masses formed, many of which became inflamed, broke down and ulcerated. The ulcerated areas were eventually healed by extensive formation of scar tissue (Figs. 2 and 3). Often the nipple became undercut by fibrous connective tissue so that presumably the lymphatic drainage was interfered with, and the nipple became extremely edematous, swelling to 4 or 5 times its normal size (Fig. 2). A great increase in the amount of collagenous connective tissue usually occurred in the subcutaneous tissue of the nipple. The other cutaneous and subcutaneous changes were the same as the general reactions described above except that there was an accumulation of hyaline material. There was usually a very marked metaplasia of the lactiferous and interlobular ducts (Fig. 13). The most extreme metaplasia of the mammary ducts occurred when the carcinogen was injected directly into the lactiferous ducts. This was evidently due to the carcinogen since injection of the oil solvent alone did not produce these changes in the breast. The intralobular connective tissue appeared quite normal following injection of the carcinogen, but the interlobular connective tissue was very dense, and usually there was almost a complete absence of fat in the interlobular spaces. However, these changes never progressed any further, even when treatment was continued up to 8 years. Oil cysts were found in the control breast which was treated with sesame oil, and lymphocytes and macrophages were also frequently present.

When the carcinogen was instilled into the cervix or injected into the uterine cavity, there was a distinct metaplasia of the cervical glands and hyalinization of the cervix that appeared to be identical with the metaplastic changes in the cervixes of estrogen-treated animals, with the possible exception that changes were not as marked. Injections into the wall of the cervix or beneath the vaginal epithelium caused the same inflammatory changes in the tissues as were described for the skin and subcutaneous tissue. Rather large inflammatory lesions were produced (Figs. 23 and 24). The endometrial changes reflected the sex hormone levels, but there was some hyalinization which was presumably caused by the carcinogen. Hyalinization was apt to be more extreme in the myometrium which usually also showed a definitely increased fibrosis.

Dibenzanthracene.—The effects of dibenzanthracene were essentially the same as those described for methylcholangthrene. The necrosis and ulceration, with the resultant scarring, was certainly as great with either painting or injection (Figs. 1, 8 and 10). However, it did not seem that the deeper tissues were as greatly affected. The scarring from applications to the breasts usually resulted in interference with the lymphatic drainage, with the result that the nipple became edematous (Fig. 1). The metaplasia of the alveolar ducts was not as great as with methylcholangthrene, and there was usually an appreciable amount of fat in the gland. The general diffuse inflammation (Fig. 49) was about the same, and collagenous fibers were very numerous. The lesions around the ears (Fig. 4) were very distinct. The single animal that received pellets under the serosa of the stomach showed no gross changes. There were the usual fibrous connective tissue capsules around the pellets of carcinogen.

Benzpyrene.—Benzpyrene caused much less fibrosis than did methylcholangthrene and dibenzanthracene. Even so, palpable nodules were detected as early as 7 days following the first treatment with the carcinogen. The reaction was essentially the same as that described for the other two carcinogens. The difference seemed to be that the fibrous reaction was not so great, although in some cases extreme, yet well organized, fibrosis was present (Fig. 44). See Figs. 1-3 and 9 for gross changes in the breasts, and Figs. 5 and 6 for gross changes in the skin of the ears. Hyperkeratinization and the subsequent dermal changes occurred (Fig. 20).
There was probably somewhat less necrosis and ulceration, but both chronic and acute inflammation were present in the subcutaneous tissue. Scar tissue was quite evident. The inflammatory reaction in response to implantation of benzpyrene pellets was not as great (Figs. 47 and 50) as described for methylcholanthrene, but otherwise the capsule formation was the same. Metaplasia of the lactiferous ducts of the mammary glands (Fig. 15) occurred quite readily, with prickle cell formation and piling up of epithelium, and occasional formation of intraductal papillomas which, however, never gave any indication of malignancy. The terminal ducts were relatively unaffected (Fig. 14), and the alveoli seemed to be actively stimulated by benzpyrene (Fig. 16).

Several of the subcutaneous nodules resulting from benzpyrene treatment were extremely well vascularized (similar to Figs. 41 and 42 following methylcholanthrene treatment) and occasionally contained lymphoid nodules. One striking difference from the response to treatment with the other carcinogens was the frequent occurrence of brown pigmentation in the skin of the areas treated with benzpyrene. Sometimes this was temporary and at other times it remained until the area became extensively scarred.

Although benzpyrene was placed in the uteri of 7 monkeys, evidence of cervical metaplasia was seen in only one animal, which was also on chronic estrogen treatment. The principal reaction in the uterus was a general fibrosis of the myometrium. The endometrium was usually compact, with short straight glands, if the host had been castrated.

**Combination of treatment.**—As may be seen in Table I, 7 monkeys received estrogen and methylcholanthrene, 9 received estrogen and benzpyrene, one received estrogen and dibenzanthracene, 6 received methylcholanthrene and benzpyrene. Four animals from the last 2 groups received all 3 carcinogenic hydrocarbons. The reactions to the carcinogenic hydrocarbons were purely local and were independent of any other carcinogen applied to another site. Therefore, the effects of the various carcinogenic hydrocarbons can be compared in the same animal, as for example, by treating one breast with benzpyrene and the other with methylcholanthrene or by treating one with dibenzanthracene and the other with benzpyrene, etc. A comparison of the effects of the various carcinogens on similar organs in the same animal confirms the finding when different animals are compared. The fundamental action was the same with all of the carcinogenic hydrocarbons. Methylcholanthrene caused the most pronounced changes; dibenzanthracene was almost as effective, and both produced more fibrotic changes than did benzpyrene. There was absolutely no evidence of any enhancement of the reaction when more than one carcinogen was present in the same animal.

Estrogen gave a systemic as well as a local effect in the doses employed. It is interesting that while estrogen and carcinogens were found to have similar effects on certain structures in the monkey, a combined treatment did not seem to give either an additive or an augmentive effect. Estrogen stimulated normal alveolar growth of the mammary glands without metaplasia of the ducts. Methylcholanthrene or benzpyrene caused a marked metaplasia of the ducts but only a moderate alveolar reaction, which was typified by a relatively low epithelium, yet there was a certain amount of secretion of an amorphous staining substance. With the combined injections the metaplasia of the ducts was the same as with methylcholanthrene or benzpyrene alone, and the alveolar reaction could not be distinguished from that produced by the estrogen alone. In the uterus, where estrogen, methylcholanthrene and dibenzanthracene all cause metaplasia of the cervical glands, and fibrotic changes in the rest of the uterus, the combination of estrogen and methylcholanthrene or estrogen and dibenzanthracene produced no greater effect than could be obtained by estrogen alone. Since it was necessary to apply the carcinogenic hydrocarbon directly to the uterus, the presence of the carcinogen could usually be detected by the inflammatory reactions. Otherwise, it is doubtful that its effect could have been distinguished from that of estrogen in histological preparations of the tissues.

**Animals still alive.**—Four monkeys are still alive and in good health after from 5 to 10 years of treatment with estrogens and carcinogenic hydrocarbons. In the 5 years since pellets of carcinogens replaced other methods of application the extreme hyperkeratinization and the ulcerated lesions have completely cleared up, leaving a great amount of scarring where the ulcers were located. The larger fibrotic masses and papillae are still present but are somewhat smaller in size and less firm in consistency than when injections and paintings were stopped. The animals with the extreme reactions shown in Figs. 2, 3, 6 and 7 are still alive and, despite the fact that pellets of carcinogens have been present in the tissue around the lesions since these pictures were made, more than 6 years ago, all lesions are healed, and the skin has taken on a healthy appearance. The general fibrosis and the granulomatous nodules never completely dis-
appear, but they gradually take on the feel and texture of the surrounding tissue. On careful examination the numerous scars can be seen, and some fibrosis can be palpated along with the small subcutaneous nodules. The latter are appreciably smaller than when treatment was changed and have been in this condition for some time. Small papillae are also present on the skin, but they are of a soft, pliable nature in contrast to the very hard, coarse, and brittle condition observed at the time injections and paintings were discontinued and the pellets implanted. Biopsies of the old fibrosed areas show that the coarse collagenous fibers become less and less obvious with time, despite the presence of pellets of carcinogen in the nearby tissue. There is a gradual accumulation of fat in the formerly granulomatous nodules, so that after 3 or 4 years fat may replace a large portion of the nodule. It becomes extremely difficult to tell these areas histologically from the normal tela subcutanea. The papillae are very similar to the normal skin of the area and seem to represent a mechanical inability of the skin to return to its original shape rather than any altered histology. The reactions to newly implanted pellets continue to be the same as those already described for each of the carcinogens. Pellets which have been present for long periods of time show only the connective tissue capsule.

**DISCUSSION**

It is evident from the results just described that malignant tumors have not been produced in the monkey by chronic treatment with the female sex hormones and the 3 most commonly used carcinogenic hydrocarbons, methylcholanthrene, benzpyrene and dibenzanthracene. These findings are made more striking by the fact that the carcinogenic compounds have been supplemented in some animals by numerous and extreme inflictions of trauma. Moreover, periodic acute inflammation has been superimposed upon the chronic inflammation that occurs at the sites of application of the carcinogen. This has been especially true when the substance was injected subcutaneously in sesame oil (Fig. 49).

Negative results are usually considered rather unsatisfactory since their validity is limited in scope. They apply much more rigidly to the experimental conditions than do positive findings. Therefore, a critical analysis of the extent to which the conditions of the present study adhere to the generally accepted requirements for the induction of malignancy is advisable. Information on all types of experimentally produced tumors indicates that the following factors are of importance: age of host, period of treatment, proper treating agent, method of application, and dosage. The actual age of these animals was not always accurately known. However, about one-third of the group of monkeys were at the age when the menstrual cycle was just being established (3 to 4 years), and about half were well developed, sexually mature animals whose ages were estimated to be at least 8 years. Of the mature animals, 4 were treated for from 7 to 10½ years, which would bring their ages up to rather more than the average life expectancy (16 years) of monkeys reared in captivity or under laboratory conditions. Therefore, it can be assumed that an adequate age range has been covered and that treatment has been continued over a sufficiently long period of time (up to 10½ years). Also, since treatment was continued over a period from adolescence to old age, it is to be assumed that any relationship of malignancy to age would be covered. It is true that only 4 of the animals meet the extreme of these two factors. However, there are 9 other animals, treated from 3 to 6 years, whose distribution over the age range greatly lessens the possibility of having failed to treat the animals at the right time.

It would seem that the requirements concerning methods of application have been met quite adequately since almost all the methods that have been employed to obtain cancer in the smaller laboratory mammals have been used. The carcinogens have been applied to the skin in benzene (in one experiment in methyl salicylate; they have been injected intradermally, subcutaneously, and intramuscularly; and have also been injected into the mammary glands, intracervically, into the uterine cavity and into the myometrium. They have been injected in solution in sesame oil and olive oil, and in normal saline suspension. Crystals of the carcinogens have been placed in the various viscera surgically, and pellets of 12 to 15 mgm. have been implanted subcutaneously and into the various organs most subject to malignancy in man. Intravenous and oral applications have also been made. Injection directly into the lymphatic system was not done because of the difficulty of execution, and it was felt that this route would be adequately covered by the other methods of application. Nor was the carcinogen put into the brain or cranial cavity, since it was felt that if lesions of the type produced in other areas developed, they would kill the animal and it would not be known whether neoplasms would have eventually appeared.

Dosage does not seem to be as important as other factors, except in the case of estrogens, which
must be given in fairly large doses over prolonged periods, as has been done in these experiments. The carcinogenic hydrocarbons were applied so that, with minor exceptions, each animal received from 2 to 6 mgm. weekly until the animal came to autopsy. Thus several animals received a total of around a gram of methylcholanthrene plus a half gram of benzpyrene or dibenzanthracene. The largest doses given were 1.615 gm. of methylcholanthrene and 700 mgm. of benzpyrene to one animal; and 380 mgm. of methylcholanthrene, 430 mgm. of benzpyrene and 5.323 gm. of dibenzanthracene to another. There is evidence from tissue culture studies (3) that too high a concentration of carcinogenic hydrocarbon retards the growth of cancer cells. However, the slow rate of absorption of the pellets of carcinogen and the fact that there has been no evidence of inhibition of cellular activity in the immediate vicinity of the pellets or crystals of carcinogenic hydrocarbons, even when granulomatous changes were occurring, seems to rule out such an interpretation as the cause of the failure to induce cancer in the monkey.

The results of painting the carcinogens on the skin are in agreement with those obtained by Bonne, Lodder and Streef (2) following the painting of coal tar on the skin of the Javanese monkey (Macaque cynomolgus). In the latter experiments the animals received cutaneous applications of tar twice weekly for periods up to almost 2 years. Hyperkeratinization, necrosis, ulceration and proliferative growth such as occurred in the present experiments were readily produced. On the basis of histological structure the authors diagnosed these lesions as epithelial carcinomas but pointed out that they never metastasized. Moreover, the lesions regressed following cessation of the tar painting and often showed regressive changes while painting was being continued.

A comparison of the action of the carcinogens in the monkey with those in the lower mammals indicates that the lesions obtained in the monkey, especially the relatively unorganized proliferation of the epithelium and the marked metaplasia of certain epithelia, are very similar to the precancerous lesions in other animals. It seems that in the monkey the proliferative stimulus can be induced in appreciable intensity, but the tissue never completely loses its power of organization and cannot progress into the truly neoplastic state. The latter may be the reason why we are able to see differences in the response to the three carcinogenic hydrocarbons, an observation which apparently has not hitherto been reported. The essential reaction to each hydrocarbon was the same, but benzpyrene caused less fibrotic change than did methylcholanthrene and dibenzanthracene. If the primary lesions had gone on to the neoplastic state, these differences would not have been so readily seen; on the contrary, because of the failure to reach the neoplastic condition, one could not determine in the monkey whether early regressive changes are a prerequisite to cancer formation, as evidence from lower animals seems to suggest (9, 11).

It has been shown in the lower mammals that action of the carcinogens does not necessarily involve any irritation or inflammatory reaction (9). The findings on the monkey are probably in agreement with this, despite the fact that inflammation to a greater or lesser degree is usually present. Epithelial metaplasia and a certain amount of fibrotic change can occur without any inflammatory reaction. It seems clear, moreover, that the reaction to the carcinogen is something more than a foreign body reaction, even though the response of the deeper structures is a general, diffuse fibrosis or the production of a nodule of typical granulation tissue. These granulomas pass from the early epithelioid cell and leukocyte state to a condition where immature fibroblasts predominate and finally relatively coarse collagenous fibers appear. Infections certainly play a prominent role in the extreme ulceration observed. However, when the direct source of the trauma is removed, the ulcerated areas slowly heal, even though the carcinogen is applied in another area or placed as a pellet in the nearby un-ulcerated tissue. The healing process and scar formation are identical with the classical description of these processes (12). The failure of the general fibrosis and the gross nodules to completely disappear is due to mechanical factors. The tissue composing these areas is, however, replaced by as nearly normal tissue as the mechanical factors will allow. The fact that there is a minimum of reaction to implanted pellets, if care is used in preparing and inserting them, also indicates that the inflammation and ulceration are secondary reactions.

The findings regarding the response of the monkey to chronic estrogen treatment are in complete agreement with those of Engle, Krakower and Haagensen (4). At the time the present experiments were begun in 1936 the concept, now supported by many investigators, that the role of estrogen in the production of mammary cancer is only to develop the mammary gland to the state at which other factors, if present in sufficient force, can cause neoplastic growth, was not in existence. Since mammary cancer has not been reported in breeding female Rhesus monkeys, this concept would have
argued against the chance of success from attempts to induce mammary cancer by the administration of estrogen, unless, possibly, the estrogen were given in combination with carcinogenic hydrocarbons. In view of the fact that the hydrocarbons have now been shown to be ineffective themselves, the results obtained are to be expected. The incidence of tumors in monkeys can only be roughly guessed. Ratcliffe (13) has reported that at least 8 tumors of all types occurred in the primates of the Philadelphia Zoological Gardens during the period from 1901 to 1932 but that this incidence was lower than for any other order of mammals. Only 2 of these tumor-bearing animals belonged to the genus Macaca and none were found in the Rhesus monkey. However, Bagg (1) has described an osteogenic sarcoma, and Engle and Stout (6) a prostatic carcinoma, in the Rhesus monkey (Macaca mulatta). The low incidence of tumors in primates kept in zoological gardens (13) as compared to other mammals is somewhat offset by the fact that the primates had the shortest average exhibition period. The tumor bearers among the primates had an average exhibition period (111.8 months) which was more than 6 times the average for all the primates. Among the family Caropithecidae (the old world monkeys), the average exhibition period for tumor-bearers (124.3 months) was about 8 times that for all animals in this group. These findings strongly indicate that monkeys are so susceptible to the common diseases of man that they seldom live to the tumor-bearing age. This is particularly true of the monkeys in the zoological gardens, at present our main source of data on this subject, since they are continually exposed to crowds of people. The cost of keeping monkeys under laboratory conditions has usually prevented studies on old monkeys, except as they may occasionally come into the laboratory as recognized old animals. It is also unlikely that monkeys live to a very old age in their native wild condition. These factors make it particularly difficult to determine anything about tumor incidence, yet the fact that the occasional tumors that are found are of quite diversified types, makes it seem probable that the monkey is not particularly immune to cancer in the hereditary sense. Therefore, one is led to suspect that the monkey has a very good protective mechanism against the carcinogenic hydrocarbons. The fact that the so-called precancerous lesions occur at the site of application, that the carcinogen when in a vehicle which is painted on the skin or injected by various means is more effective than when introduced as a pellet or dispersed crystals, and that the carcinogens even in tremendous doses have no systemic effect, all suggest that such a protective mechanism is in operation. The systemic protective mechanism is apparently much more effective than the local, yet the fibrotic changes around the applied carcinogens are extremely limited. This protective mechanism can hardly be explained on the basis of a simple walling off of the carcinogen because histologically this reaction is no more marked than it is with estrogen, where the systemic effect is strongly evident, or than in the mouse where the carcinogens are highly effective. Moreover, the carcinogens gradually disappear from the pellets, indicating that they are being absorbed. It may be that they are destroyed as rapidly as they are absorbed and, of course, one would link such a phenomenon with the liver. It is of interest, therefore, that the livers of monkeys, getting even the chronic treatments, show little if any impairment.

SUMMARY

Fifty monkeys received treatment with estrogens and 3 carcinogenic hydrocarbons (methylcholanthrene, dibenzanthracene and benzpyrene) for periods up to 10 years. Almost all organs and tissues that frequently become cancerous in the human being were treated, including the abdominal and pelvic viscera. The breasts received the most chronic treatment, although multiple sites were used in all animals. Carcinogens were also injected intravenously and administered orally. Methylcholanthrene and dibenzanthracene caused more pronounced local reactions than did benzpyrene. Following injections in oil these consisted of firm inflammatory or non-inflammatory fibrotic masses, areas of granulation tissue and encapsulated cysts of the injected material. Following painting on the skin, they consisted of hyperkeratinization, alopecia, cystic hair follicles, cystic sebaceous glands and papillomatous masses or wart-like excrescences. Pellets became encapsulated by dense fibrous connective tissue surrounded by numerous lymphocytes and phagocytes. This reaction sometimes progressed until firm non-inflammatory fibrotic masses were produced, but usually the capsule gradually became indistinguishable from that surrounding any foreign material. The lesions usually became infected and ulcerated, but if the injection site was changed, they slowly healed with typical scar formation. Sometimes there was disorientation of the tissues near the ulceration with marked papillary downgrowth of the surface epithelium. The general fibrosis consisted of large well differentiated collagenous fibers; the non-ulcerative tissue masses were typical grani-
lomas with varying amounts of chronic or acute inflammation.

Metaplasia of the mammary ducts and of the epithelium of the cervical glands occurred with both estrogen and the carcinogenic hydrocarbons, but did not progress beyond this condition. Despite the relatively unorganized proliferation or marked metaplasia and the formation of granulomas, a true neoplastic state was never reached. The proliferative stimulus was apparently present in appreciable amounts, but the tissue never completely lost its power of organization. All lesions gradually reverted toward the normal when treatment at the site was stopped, even though pellets of carcinogen were present in the nearby tissue.

The findings are negative for cancer, but it is felt that most of the requirements of a crucial test have been met.

ADDENDUM

Since the manuscript of this article was submitted for publication, a spontaneous oral carcinoma in a male Rhesus monkey has been described by Klüver and Brunschwig (Cancer Research, 7:627-633. 1947). This led to the finding in the literature reports of two other spontaneous oral carcinomas in Rhesus monkeys (Steiner, P. E., Klüver, H., and Brunschwig, A., Cancer Research, 2:704-709. 1942; and Zuckerman, S., Proc. Zool. Soc., London, 1930, pp. 59-61.) suggesting that this is the most frequently occurring type of spontaneous tumor in the Rhesus monkey.

REFERENCES

DESCRIPTION OF FIGURES 1 TO 7

Fig. 1.—Breasts of monkey No. 66 after 3½ years of treatment with carcinogens. The left breast was treated with dibenzanthracene (1 mgm. in 0.1 cc. of sesame oil weekly, injected subcutaneously). The right breast received weekly injections of benzpyrene (1 mgm. in 0.1 cc. of sesame oil). It can be seen that the reaction to the dibenzanthracene is more intense than to the benzpyrene, the skin around the left nipple showing much greater proliferation. There have been areas of necrosis which are now represented by the rather marked scarring suggestive of keloid formation. There is a greater loss of hair and greater enlargement of the nipple, presumably due to interference with the lymphatic drainage of the nipple by the scarring at the base of the nipple. Fibrous tissue formation is quite marked in both breasts, with definite masses fixed to either the underlying fascia or to the epithelium. This is a chronic condition.

Fig. 2.—Breasts of monkey No. 92 after chronic treatment for 3½ years. The right breast was treated with benzpyrene (1 mgm. in 0.1 cc. of sesame oil) injected subcutaneously at weekly intervals. The left breast was painted biweekly with 1 mgm. of methylcholanthrene in benzene. Firm connective tissue masses are present in both breasts. One large mass, 1 X 1 cm. in diameter, can be seen adjacent and medial to the right nipple. The painted area is characterized by loss of hair and an epithelial reaction similar to that shown in Fig. 13. The extreme edema of the nipples is presumably due to undercutting by scar tissue which interferes with the lymph drainage.

Fig. 3.—Breasts of monkey No. 705 after chronic treatment for 2 years. The right breast was injected subcutaneously with benzpyrene (1 mgm. in 0.1 cc. of sesame oil weekly). The left breast was injected with 1 mgm. of methylcholanthrene weekly and painted with methylcholanthrene in benzene twice weekly (2 mgm. weekly). Firm connective tissue masses are present in both breasts. The painting of the left breast produced thickening and scaling of the epithelium with multiple punctate areas of proliferation. The scaling of these irregular macules and papules is seen most clearly lateral to the left nipple.

Fig. 4.—Left ear of monkey No. 66. Dibenzanthracene (1 mgm. weekly) had been injected subcutaneously for 3½ years. Injections were started on the helix and antitragus of the pinna, but ulceration forced a shift to the area just anterior to the tragus. This area also ulcerated after 6 months, leaving the several sinuses seen in this illustration. The injection site was then moved to a location slightly more anterior. The mass shown in the photograph is clearly circumscribed and is made up in part of proliferated subcutaneous fibrous tissue and in part of proliferated epithelium. The old site of injection on the pinna is readily visible as a scarred, desquamated and proliferated area.

Fig. 5.—Right ear of monkey No. 66. Benzpyrene (1 mgm. weekly in sesame oil) had been injected subcutaneously for 3½ years. There is little effect at the injection site on the pinna except for a condensation of connective tissue which prevented retention of the carcinogen. Note the thickening of the anterior helix. Anterior to the tragus (the last site of injection) a well circumscribed mass similar to that described in Fig. 4 is seen. The reactions here differ from those following dibenzanthracene in that they are not so intense, and there is no tendency to ulcerate.

Fig. 6.—Right ear of monkey No. 705 which had received the same treatment and shows the same reaction as described for Fig. 5. Compare this reaction to benzpyrene with the effect which methylcholanthrene produced on the left ear of this same animal, shown in Fig. 7.

Fig. 7.—Left ear of monkey No. 705. For 2 years methylcholanthrene had been applied to the pinna of the ear and the skin anterior to the tragus by subcutaneous injection (1 mgm. weekly) and by painting (2 mgm. weekly). The first injections were at the helix and antitragus, but ulceration soon forced a change in the injection site to the anterior helix and the epithelium anterior to it. Note that not only is there a clearly circumscribed fibrous mass at the latter injection site, but there is also a marked epithelial reaction which may be due in part to the combined injection and painting. The old sites are marked by definite scars. The reaction is much more extreme than that following benzpyrene, shown in Fig. 6.
Figs. 1–7
DESCRIPTION OF FIGURES 8 TO 12

Fig. 8.—Left breast of monkey No. 10 after treatment for 1 year with approximately 6 mgm. of dibenzanthracene weekly. The carcinogen was injected subcutaneously in sesame oil. Note the ulcerated area just lateral to the nipple. Other areas of ulceration have healed with scar formation, seen among the dermal papillae. The entire area shows extreme hyperkeratinization with some of the fibrotic masses visible as larger elevated areas.

Fig. 9.—Breasts of monkey No. 61. The left breast had been injected with 2 to 4 mgm. of benzpyrene weekly for 15 months. Note the numerous ulcerated areas just lateral to the nipple which has become edematous due to scar formation at its base. There are some fibrotic masses in the connective tissue of the breast, but they are not as extreme as those found following treatment with methylcholanthrene and dibenzanthracene. This is about the most extreme reaction to benzpyrene observed in the breast region.

Fig. 10.—Breasts of monkey No. 42. The left breast had received injections of dibenzanthracene (2 mgm. weekly) for 1 year. The right breast had received control injections of oil of sesame. Note the areas of inflammation, ulceration, hyperkeratinization, and papilla formation, as well as the clearly evident subcutaneous fibrotic masses. The local effect is as extreme as with the much higher dose (see Fig. 8).

Fig. 11.—Male monkey No. 5 which had been injected over the sacrum with 1 mgm. of methylcholanthrene weekly. Note the papillomatous-like mass which developed at the base of the tail. Autotransplants to several abdominal sites failed to grow.

Fig. 12.—Monkey No. 19 which had been injected with 1 mgm. of methylcholanthrene weekly on the under surface of the tail or between the base of the tail and the anus. Note the granulomatous masses which have partly broken down and ulcerated.
Figs. 8-12
DESCRIPTION OF FIGURES 13 TO 16

Fig. 13.—Low power magnification of a section through the skin and mammary duct of monkey No. 43. Methylcholanthrene had been injected at the base of the nipple. Note the hyperkeratinization of the hair follicle and the metaplasia of the mammary ducts, seen in the center of the photograph. There is an appreciable amount of chronic inflammation in the subcutaneous tissues. Mag. X 31.

Fig. 14.—Terminal duct of the right mammary gland of monkey No. 66, showing increased connective tissue fiber formation and absence of metaplastic changes in the terminal duct. This breast had received subcutaneous injections of 1 mgm. of benzpyrene weekly. Mag. X 140.

Fig. 15.—Lactiferous duct of the breast described for Fig. 14. Note the extreme metaplasia. Mag. X 140.

Fig. 16.—High power magnification of mammary gland showing the maintenance of active alveoli by the benzpyrene. From the breast described for Fig. 14. Mag. X 280.
Figs. 13–16
DESCRIPTION OF FIGURES 17 TO 22

Fig. 17.—Monkey No. 61. Normal skin from an area at some distance from any site of administration of carcinogen. Mag. × 100.

Fig. 18.—Monkey No. 145. Skin after a relatively short period of injection of methylcholanthrene. Note the increased fibrosis and the absence of hyperkeratinization or effect on the hair follicles and sebaceous glands. Mag. × 100.

Fig. 19.—Monkey No. 43. Skin after painting with methylcholanthrene in benzene. Note the extreme hyperkeratinization of the surface epithelium which extends into the hair follicles and sebaceous glands, causing alopecia. At the extreme right center of the photograph is a cystic follicle greatly distended by sloughed keratinized epithelium. Just to the left and inferior to it is a cystic sebaceous gland which is also distended with sloughed keratinized epithelium. At the upper left is a distended hair sheath. This condition also occurs following the deeper reaction which results from injection of the carcinogen but is obtained more quickly by painting. Mag. × 60.

Fig. 20.—Skin of monkey No. 61. Broad pegs of epithelium growing down into the corium following prolonged treatment with benzpyrene. These downgrowths occurred after the necrosis and ulceration of the deeper areas described for Fig. 9. Mag. × 100.

Fig. 21.—Skin of monkey No. 96 following prolonged painting with 2 mgm. of methylcholanthrene in benzene weekly. The extreme epithelial downgrowth illustrated here occurred following a primary extreme hyperkeratinization and ulceration. Most of the surface epithelium is greatly disorganized or sloughed. Mag. × 100.

Fig. 22.—Monkey No. 66. A small segment of a cystic hair follicle to illustrate the extremely active hyperkeratinization. From the right breast described for Fig. 1. Mag. × 140.
Figs. 17-22
DESCRIPTION OF FIGURES 23 TO 26

Fig. 23.—Monkey No. 96. Papillary downgrowth of the cervical epithelium; 2 mgm. weekly of methylcholanthrene in oil had been instilled into the cervical canal. Extreme epithelial reaction was observed. At first this was followed by necrosis and erosion. Note the great disorientation of the surface epithelium, the marked surface inflammation and the characteristic papillary downgrowth with occasional pearl formations. Mag. × 100.

Fig. 24.—Vagina of monkey No. 96. Note the marked cornification of the vaginal epithelium and the papillary downgrowths from it. The downgrowths show a peculiar infiltration with leukocytes. Mag. × 100.

Fig. 25.—Low power view of cervical glands of monkey No. 6. This monkey had been ovariectomized and had received 0.15 mgm. of theelin daily for 6 months. Note the moderate metaplasia of the cervical glands and the pearl-like cysts in the lining epithelium. Mag. × 75.

Fig. 26.—Higher magnification of a portion of the tissue shown in Fig. 25, illustrating the beginning of metaplasia of the cervical glands. Mag. × 280.
Figs. 23-26
DESCRIPTION OF FIGURES 27 TO 34

Fig. 27.—A sagittal section of the cervix of monkey No. 7, which had received 0.4 to 0.6 mgm. of theelin weekly for 2 years, showing adenomas and cyst formation. Mag. × 10.

Fig. 28.—Higher magnification of cervical glands showing considerable metaplasia; from the section shown in Fig. 27. Mag. × 75.

Fig. 29.—Oil immersion photomicrograph showing a small nest of metaplastic cells replacing the tall columnar epithelium of the cervical glands. The tall columnar cells can be seen at the bottom edge of the photograph. Some of the nuclei of the columnar cells still remain at the edge of the metaplastic area, which is seen at the extreme left. From monkey No. 7. Mag. × 1,500.

Fig. 30.—Oil immersion photomicrograph showing the formation of the metaplastic cells and the way in which they displace the normal cervical epithelium. From monkey No. 7. Mag. × 1,500.

Figs. 31 and 32.—Cervix of monkey No. 90. This animal had received 0.4 mgm. of theelin weekly for 1 year and 8 months. Metaplasia is present in only the tips of the glands. The remaining portions of the glands show normal estrogen stimulation. Mag. × 95.

Fig. 33.—Cervix of monkey No. 15. This animal had received 0.4 to 0.6 mgm. of theelin weekly for 8½ months. There is appreciable metaplasia, but the cervical glands are not particularly well developed. Mag. × 75.

Fig. 34.—Monkey No. 90. Oil immersion photomicrograph showing metaplastic plugs protruding into the lumina of the cervical glands. Mag. × 500.
Figs. 27-34
DESCRIPTION OF FIGURES 35 TO 38

Fig. 35.—Low power photomicrograph showing the adenomatous nature of the cervix of monkey No. 90. Mag. × 10.

Fig. 36.—Low power view of the squamous epithelial portion of the cervix of monkey No. 90, showing the extreme cystic nature of the glands in this area. Mag. × 70.

Fig. 37.—Cervix of monkey No. 59. This animal had received 4 mgm. of theelin weekly for 15 months plus repeated cervical trauma. Note the extreme atrophy of the cervical glands. There is no epidermization. Mag. × 10.

Fig. 38.—Hyperplastic vagina taken from the same animal as shown in Fig. 37. The epithelium is fairly thick with frequent epithelial bulbs along the basal layer. Mag. × 10.
DESCRIPTION OF FIGURES 39 TO 44

Fig. 39.—Monkey No. 146. Early granuloma following one month of subcutaneous injection of 2 mgm. of methylcholanthrene weekly. Mag. × 140.

Fig. 40.—Section of a biopsy sample of the granulomatous nodule shown in Fig. 39, taken 30 days later. Mag. × 140.

Fig. 41.—Subcutaneous nodule from monkey No. 5, the same animal as shown in Fig. 11. Note the dense, well vascularized connective tissue (after 25 months of treatment). Mag. × 140.

Fig. 42.—Different area from the same nodular mass as shown in Fig. 41. Note the extreme vascularity and chronic inflammation of this area. Mag. × 140.

Fig. 43.—Monkey No. 19. Section of a nodular mass after 4 years of treatment with 1 mgm. of methylcholanthrene weekly. Note the dense but well organized connective tissue comprising this area, with small localized areas of chronic inflammation. Mag. × 140.

Fig. 44.—Section through a subcutaneous nodular mass from the right breast of monkey No. 66 (see Fig. 1) after approximately 5 years of treatment. Mag. × 140.
DESCRIPTION OF FIGURES 45 TO 51

Fig. 45.—Monkey No. 92. The capsule around a pellet of methylcholanthrene 65 days after implantation. There is a fairly wide area of inflammation between the pellet and the connective tissue capsule. In some areas there is a marked inflammation outside the connective tissue capsule (as illustrated in detail in Fig. 46), but for the most part the tissue beyond the capsule is relatively unaffected. Mag. × 40.

Fig. 46.—Enlarged section of the pellet capsule shown in Fig. 45. Note the extreme reaction adjacent to the pellet as well as the area of inflammation immediately external to the connective tissue layer. Pellet surface is at the top. Mag. × 100.

Fig. 47.—Monkey No. 92. Capsule around a benzpyrene pellet in the same animal, for the same length of time, as described for Fig. 45. There is much less tissue reaction adjacent to the pellet as well as external to the connective tissue capsule. This capsule is not as thick as the one around the methylcholanthrene pellet. Mag. × 40.

Fig. 48.—An enlarged section of the pellet capsule shown in Fig. 45 in an area where only a mild reaction extends beyond the connective tissue capsule. This photograph illustrates quite well the inflammatory reaction adjacent to the pellet. Mag. × 100.

Fig. 49.—Monkey No. 42. Oil cysts in muscle following treatment with 3 mgm. of dibenzanthracene weekly. Note the chronic inflammation around the cysts. This is not markedly greater than was found around the sesame oil without the carcinogen. Mag. × 140.

Fig. 50.—Monkey No. 92. A section through the benzpyrene pellet capsule shown in Fig. 47. Note that there is only a slight reaction adjacent to the pellet. The capsule resembles that found around an estrogen or androgen pellet. Mag. × 100.

Fig. 51.—A section of the capsule shown in Fig. 45. This shows that in certain places, even with methylcholanthrene pellets, there are only small localized areas of tissue reaction beyond the connective tissue capsule. There is, however, no evidence that the pellet is being so effectively walled off that the carcinogen cannot get at the tissues. Mag. × 100.
Attempts to Produce Cancer in Rhesus Monkeys with Carcinogenic Hydrocarbons and Estrogens

Carroll A. Pfeiffer and Edgar Allen

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