tested showed positive transfer reactions. These findings, despite their limitations, render untenable the prevailing concept that only delayed forms of hypersensitiveness in man are transferrable by peripheral blood leukocytes.

*Summary.* From a human donor in whom immediate and delayed forms of sensitivity to *Ascaris lumbricoides* antigen had been experimentally induced, only the immediate type of sensitivity was transferred to one of 3 recipients by use of peripheral blood leukocytes. Markedly positive transfer reactions were obtained.

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## Spontaneous Mammary Cancer Developing in Rats Born and Nursed by Mothers Treated with 3-Methylcholanthrene.* (26071)

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In the study of mammary carcinogenesis induced by 3-methylcholanthrene, one aspect of the investigation in this laboratory is concerned with the mechanism of chemical carcinogenesis. Development of mammary adenocarcinoma in rats following oral administration of 3-methylcholanthrene has been reported(1,2,3). These observations have indicated that the neoplasms induced by 3-methylcholanthrene were predominantly mammary cancers. The selective response of mammary tissue to orally administered 3-methylcholanthrene cannot be explained fully, since the mechanism of carcinogenesis is not known. However, Dao *et al.*(4) demonstrated selective concentration of administered polycyclic carcinogens in breast and fat and confirmed the earlier report by Shay *et al.*(5) of excretion of 3-methylcholanthrene into the milk of lactating female rats. These results raised certain questions: (a) If the chemical carcinogen can be excreted in the milk, could mammary cancer be induced in offspring suckled by mothers treated with 3-methylcholanthrene? (b) If 3-methylcholanthrene is administered to pregnant female rats, could it pass through the placental barrier, and induce, by its effect on the early embryo, mammary cancer in the offspring? (c) Can 3-methylcholanthrene induce a "product" which may be transmitted through the milk to the offspring, thus promoting tumor development?

The present article is concerned with some preliminary results of experiments undertaken in an attempt to answer these questions.

*Materials and methods.* In all experiments, 55- to 60-day-old mature female Sprague-Dawley albino rats were used. The animals were considered sexually mature when opening of the vagina and estrous cycles were established. Four groups of experiments were carried out: 1. 3-Methylcholanthrene was fed to the mature female rats at a dose level of 10 mg/ml sesame oil daily through a stomach tube for 6 days in one group and 10 days in the other. Each group consisted of 20 rats. Vaginal smears were taken daily after completion of feeding for 4 days to determine optimal time for mating. The female rats treated with 3-methylcholanthrene were then mated with normal males for a period of

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TABLE I. Effect of 3-Methylcholanthrene on Litter Size and Survival of Newborn Rats.

| No. of mothers | Duration of 3-MC adm. (days) | Litter size | | No. newborn | | No. surviving at weaning |
|---|---|---|---|---|---|---|
| | | Range | Mean | Alive | Dead | |
| 19 | 10* | 3-12 | 6.5 | 85 | 12 | 6 |
| 14 | 6* | 3-11 | 6.7 | 67 | 28 | 6 |
| 14 | 10† | 5-12 | 8.8 | 122 | 3 | 7 |
| 16 | 6† | 4-12 | 8.1 | 131 | 0 | 21 |

* 3-Methylcholanthrene, 10 mg/ml sesame oil, was fed to mothers during pregnancy.
† 3-Methylcholanthrene, 10 mg/ml sesame oil, was fed to female rats before mating with the males.

7 days. After the litters were weaned, female and male offspring were separated in different cages for long-term observation. These rats were examined at frequent intervals for palpable tumors. II. Sexually mature normal females and males were mated for one week. Vaginal smears were taken on the eighth day for a few days until pregnancy was ascertained. 3-methylcholanthrene was then fed to these pregnant rats at a dose level of 10 mg/ml sesame oil daily for 6 days in one group and 10 days in the other. After litters were weaned, male and female offspring were separated for observation of tumor formation. III. Normal male and female rats were mated. Five days after litters were born, 10 mg of 3-methylcholanthrene in 1 cc of sesame oil was fed to the lactating mothers daily for 15 days. Litters were weaned on the 21st day after birth. During the feeding, great care was taken to prevent ingestion of maternal feces by the infant rats. Mothers and offspring were housed in cages with bottoms of wide wire mesh that allowed the feces to drop from the cage. Also, these cages were frequently inspected to remove fecal material that failed to drop through the meshes. After weaning, female and male offspring were separated for observation. IV. Fresh mammary tumors removed from the rats were handled in a sterile manner and processed promptly. A 20% W/V tissue mince suspension in cold Ringer's solution was prepared. It was homogenized in an Omnimixer homogenizer for 10 minutes with the container immersed in an ice bath. The homogenate was centrifuged in a refrigerated centrifuge at 3000 G at 0°C for 30 minutes and supernatant fluid collected and passed through bacterial filters (Selas 02). *E. coli* (broth cultures) were added to the supernatant fluid prior to filtration to check the integrity of the filters.

Subcutaneously 0.1 ml of the cell-free filtrate was injected into the newborn rats less than 24 hours old. After weaning, male and female offspring were housed separately for observation.

*Results.* The effect of 3-methylcholanthrene on litter size and survival was summarized in Table I. When 3-methylcholanthrene was fed to the pregnant rats, incidence of stillbirth was high but it did not seem to affect the litter size significantly. When 3-methylcholanthrene was fed to the female rats some time before they became pregnant, incidence of stillbirth was greatly reduced. Survival of offspring of mothers treated with 3-methylcholanthrene was low in both situations. Most newborn rats died around 2 weeks after parturition; only 40 rats survived until time of weaning (21 days).

Results are summarized in Table II. Among a total of 28 surviving (7♂ and 21♀) offspring of mothers treated with 3-methylcholanthrene before pregnancy, 4 female rats developed spontaneous mammary cancers at the age of 49, 85, 90, and 90 days. These tumors were biopsied when they reached a size

TABLE II. Development of Spontaneous Mammary Carcinoma in Offspring of Mothers Treated with 3-Methylcholanthrene.

| 3-MC treatment of mothers | No. of mothers | Total No. surviving offspring ♂ | ♀ | Appearance of tumors in daughters |
|---|---|---|---|---|
| Before mating | 30 | 7 | 21 | 4/21 |
| During pregnancy | 23 | 3 | 9 | 1/9 |
| After pregnancy, during lactation | 10 | 9 | 11 | 0 |

of about 1 to 1.5 cm in diameter. Histologically, 3 are mammary adenocarcinoma and 1 an undifferentiated carcinoma. Only 1 mother had mammary cancer; the other 3 did not have tumors. One female rat among a total of 12 (3♂ and 9♀) offspring of mothers treated with 3-methylcholanthrene during pregnancy developed mammary cancer at the age of 200 days. Biopsy showed that it was cancer. In the experiment in which 3-methylcholanthrene was fed to the lactating rats, 20 offspring (11♀ and 9♂) from 10 lactating mothers survived for long-term observation. No tumors have been observed in these offspring at the end of 10 months. A total of 73 newborn rats was injected subcutaneously with a cell-free filtrate prepared from the mammary cancer induced with 3-methylcholanthrene. No mammary or other tumor has been detected at the end of one-year observation.

*Discussion.* We have not observed spontaneous mammary cancers in more than 1000 untreated female rats of the Sprague-Dawley strain used in this laboratory. Davis *et al.* (6) reported observation of spontaneous mammary cancer in 6 Sprague-Dawley female rats among a total of 250 animals observed over a long period. These tumors developed after the age of 540 days. Bullock and Curtis(7) reported observation of 521 spontaneous tumors in 489 animals from a total of more than 2450 rats autopsied. Among 521 spontaneous tumors, 94 were breast tumors, of which 7 were considered malignant and the rest were mostly benign fibroadenomas. Only 4 of these 7 malignant lesions were epithelial in origin, and they had appeared at 337, 354, 544, and 624 days of age. Benign fibroadenomas do occur in the breast of some of the female rats of several strains, but do not develop until the animals are very old(6,8). Furthermore, in those strains in which the females did develop malignant lesions in the breast, such tumors did not develop until well beyond the age at which they were manifested in these 4 Sprague-Dawley rats.

The mechanism by which these tumors develop is of considerable interest. Implication of genetics in the carcinogenic action of hydrocarbons has been made by many investigators(9,10,11). Strong(9) reported induced and spontaneous development of gastric lesions in mice. He observed that malignant gastric and other lesions were produced in descendants of mice treated with methylcholanthrene during several generations; these descendants themselves had never received any methylcholanthrene for 6 generations. Strong interpreted his results as due to a germinal mutation induced by the carcinogenic polycyclic hydrocarbon.

The meagerness of our present data does not seem to warrant any further interpretation. However, several possibilities can be considered.

First, these results may be interpreted as due to a germinal mutation induced by 3-methylcholanthrene, but this is a rather remote possibility. If this "mutational" change were germinal, one might expect a similar tumor incidence in both female and male offspring. Our results showed that the spontaneous tumors developed entirely in females and no tumors were observed in male offspring. However, it must be noted that since ovarian hormones play an important role in the induction of mammary cancers in rats treated with 3-methylcholanthrene, failure of development of spontaneous tumors in male offspring may conceivably be due to a lack of ovarian hormones.

The second possibility is a somatic mutation in the young receiving traces of 3-methylcholanthrene through the milk even days after feeding it to the mother. Our earlier results indicated that 3-methylcholanthrene could persist in the mammary tissues in measurable quantities for a considerable length of time(4,12). It would appear, therefore, that the mutation may have occurred in the embryonic stage of development. Spontaneous development of mammary cancer in a female offspring of a mother receiving 3-methylcholanthrene during her pregnancy indicates that the carcinogenic chemical is able to pass the placental barrier. This observation would favor the hypothesis that somatic mutation takes place in the embryonic stage of development.

Thirdly, it could be speculated that 3-methylcholanthrene induced a "product" which

was transmissible through the milk to the offspring to cause spontaneous tumor development. The failure to induce mammary tumors in the rats receiving a cell-free filtrate prepared from the tumors induced by 3-methylcholanthrene does not seem to favor this speculation. Further investigations are now in progress.

*Summary.* Spontaneous mammary cancers were observed in 4 female rats among a total of 28 (7♂ and 21♀) offspring of mothers treated with 3-methylcholanthrene before pregnancy. These tumors developed at the age of 49, 85, 90, and 90 days. Histologically, 3 were mammary adenocarcinoma and 1 an undifferentiated breast cancer. One female rat among a total of 12 (3♂ and 9♀) offspring of mothers treated with 3-methylcholanthrene during pregnancy developed spontaneous mammary cancer at the age of 200 days. No mammary cancer was observed in rats whose mothers were fed 3-methylcholanthrene during lactation. Also, injection of cell-free filtrate prepared from the mammary cancer induced with 3-methylcholanthrene to the normal newborn rats of less than 24 hours old failed to induce mammary tumors in these animals. These observations are of great interest for further investigation.

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## Estrogen Antagonisms: Effects of a Series of 19-Nortestosterone Derivatives on Vaginal Changes Induced by Estrone. (26072)

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Many chemical relatives of 19-nortestosterone are active as antagonists of uterine growth produced in mice by estrone and as progestational agents in Clauberg tests. There appears to be general correspondence between potencies in these tests, although no perfect correlation exists. Comparison of biological activity with chemical structure permitted conclusions regarding effects of structural alterations on activity(1). Since other studies had suggested remarkable differences in responsiveness of uterus and vagina to mixtures of steroid hormones(2,3,4,5), confirmation of these structure-activity relationships in vaginal smear tests became desirable. Comparable studies were possible only with discovery that progesterone would block estrogens administered over a 4-day period(4,6). This paper studies effects of derivatives of 19-nortestosterone as antagonists of estrone effects on the vagina.

*Materials and methods.* Assay methods employed here have already been described (4,6,7). Briefly, adult female Rockland mice were spayed and allowed 2 weeks to recover from effects of operation. They were then integrated into a colony and animals were chosen at random from colony for test-

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