OCCURRENCE OF CANCER IN RATS TREATED WITH OESTRONE

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Lacassagne (1), Bonser, Stickland and Connal (2), and others have reported the occurrence of mammary adenocarcinoma after treatment with oestrogens in male and female mice of strains showing low susceptibility to spontaneous cancer of the breast. Lacassagne (3) and Loeb et al. (4) have also encountered cancer-like proliferation of the genital tract in mice of refractory strains after treatment with oestrogenic substances. In strains in which spontaneous cancer had never been observed, Lacassagne was unable to produce breast tumours with oestrone within fifteen months.

The experiments to be reported here are concerned with rats, which are of relatively low susceptibility to spontaneous carcinoma. We have previously reported (5) that prolonged subcutaneous injection of oestrone into albino rats has produced benign tumours, multiple mammary milk cysts, and squamous metaplasia of the endometrium. Since malignancy was not encountered in this preliminary study, it was decided to set up further experiments in which various forms of local irritation would be superimposed upon an even more protracted oestrone treatment.

The rats used were of a hooded strain which has been maintained in this laboratory for the past six years, but whose genetic constitution is unknown.
The food used was McCollum's mixture until 1934, when Purina fox chow was substituted. Breeding animals have also been given lettuce or cabbage twice weekly. The oestrone used in the experiments was crystalline folliculin kindly supplied by Dr. A. Girard, Paris.

The first experimental series consisted of 12 female rats of which half were ovariectomized at the age of 58 days. All the animals received daily vaginal applications of a solution of oestrone in corn oil (120γ per c.c.) from the age of 62 days until death. Some of the animals were subsequently subjected to hysterectomy, in order to remove pyometra; some were painted thrice weekly with tar (horizontal retort gas-house tar received from the Gas, Light and Coke Company, London, England) over an area of about three-quarters of an inch in diameter on the interscapular region; in two cases, 30 mg. of dry kieselguhr were inserted into the upper left mamma. The distribution of these forms of treatment in the various members of the group, and the fates of the individual animals are summarized in Chart I.

In this series, Rat no. 2, killed on the 964th day of life, displayed a squamous carcinoma of the vagina (Fig. 1), a precancerous condition of the cervix (Fig. 2), and an adenofibroma of the left inguinal mamma. Rat no. 6, at the age of 751 days, showed a large abscess adherent to the descending colon in the wall of which a squamous-cell carcinoma (Fig. 3) was discovered. Rat no. 12 died at 700 days, with an abdominal cystic mass containing faeces (utero- or vagino-rectal fistula). In other members of the group a cervical papilloma and an ileo-caecal lymphosarcoma were encountered.

In the second series, 10 rats were ovariectomized at the age of 106 days, and at the same time silk threads were laid longitudinally in one or both uterine horns. All of these animals received daily subcutaneous injections...
CHART I. LIFE HISTORIES OF RATS OF SERIES I: DAILY APPLICATION OF OESTRONE IN CORN OIL (120γ PER C.C.)

Continuous lines indicate periods of oestrone treatment, broken lines periods without treatment. Cross-hatched lines indicate periods of tarring, from time, T. C = castration; H = hysterectomy; K* = kieselguhr placed in mammary gland; * = histologic diagnosis of cancer.

CHART II. LIFE HISTORIES OF RATS OF SERIES II (30γ OESTRONE SUBCUTANEOUSLY DAILY) AND THEIR CONTROLS

1 indicates silk thread placed in left horn and b = silk threads in both horns of uterus; Sm = silk thread in mammary gland. Arrows indicate those control rats which were injected with corn oil from the time of castration till death. Other symbols as in Chart I.
of 30y of oestrone in corn oil until death; some of them were also subjected to
tarring or to the placing of silk threads or of kieselguhr in the upper left
mamma. The details are summarized in Chart II.

Rat no. 3, killed on the 293d day of life, showed a precancerous lesion of
the cervix (Fig. 4) and an abdominal lymphosarcoma. An adenocarcinoma of
the right upper breast (Fig. 5) was removed from Rat no. 1A4 at the age of
624 days; homotransplants of this tumour were unsuccessful. In the same
animal, 49 days later, a mass was discovered in the lower abdomen which proved to be an abscess surrounded by a mass of reaction tissue enclosing the uterus and communicating by a fistula with the lumen of the bowel; in the wall of this abscess, squamous pearls of doubtful malignancy (Fig. 6) were observed in an area showing squamous metaplasia and therefore assumed to
be of uterine origin. The same animal also showed an area of squamous metaplasia of doubtful malignancy (Fig. 7) in the left upper mamma, which had been injected with kieselguhr.

Twelve control rats were subjected to the same treatment except that they received no oestrone: 6 were injected with corn oil alone; 6 were given no injections. Rat no. 3, at the age of 753 days, disclosed an abscess of the left uterine horn which contained a squamous carcinoma (Fig. 8). It is noteworthy that the vaginal smears of this rat were oestric from the 584th day onward. The possibility of accidental contact of this and of certain other control animals with oestrone must be taken into account. It so happens that this animal was handled daily immediately after a series of oestrone injections had been completed, and it is impossible to prevent transference of small amounts of oil to the operator's fingers and thence to the fur of the animals. Rat No. 2A1 also presented an abscess of the left uterine horn in the walls of which squamous cells were seen (Fig. 9): it is impossible to say, from the histologic findings, whether or not this lesion is malignant.

The third series consisted of 12 females, 6 of which were ovariectomized at the age of 116 days. All received daily subcutaneous injections of 30γ of
oestrone in corn oil. Fifteen milligrams of kieselguhr was placed in the left uterine horn of each animal, and 35 mg. in the peritoneal cavity of some of them (Chart III). Rat No. 3 of this series developed a tumour of the left upper breast which was diagnosed at biopsy, at the age of 553 days, as a malignant scirrhus (Fig. 10); homotransplants failed. One hundred and four days after biopsy the tumour had reached a large size and the injections of oestrone
were stopped. The tumour continued to grow rapidly for three weeks and then more slowly for eleven weeks, when a second biopsy showed a scirrhous adenomatous tumour of doubtful malignancy. Oestrone treatment was then resumed and 25 days later, when the tumour was seen to be again growing rapidly, the animal was killed at the age of 781 days. Sections taken from three different portions of the tumour, which weighed 33 grams, all showed adenofibromatous tissue with no evidence of malignancy (Fig. 11). Owing to the loose and lobulated flat nature of the mass, it was not possible to make caliper measurements. Our impression is, nevertheless, that the withholding of oestrone administration coincided with a slower growth rate. The stage of cancer formation may thus have been still reversible when oestrone was stopped. Rat No. 1, killed at the age of 397 days, presented a large peritoneal abscess and, distinct therefrom, the left uterine horn was ballooned with faeces, indicating a utero-rectal fistula. Another member of this group died with an abdominal lymphosarcoma.

The controls for this third series received the same treatment as the experimental animals except that they were not injected with oestrone or corn oil. Abdominal lymphosarcoma was encountered in one case, but no cancer was found in any of these rats. It may be observed that fistulae between the uterus or vagina and the rectum were encountered only in oestrone-treated rats.

In considering these results it is clear that those rats which failed to survive to the earliest age at which we have detected cancer—that is 500 days—may be left out of account. Out of 12 rats in the three series described above, which were treated with oestrone and survived 500 days or longer, 4 developed
cancer; while of 18 rats from the controls for series 2 and 3, living to an age of 500 days or more, only one had cancer. Application of the Chi-square test to these figures shows that the probability of such a distribution arising by chance alone is about 1 in 25. It must be clearly understood that throughout this work the diagnosis of malignancy has been made on the basis of histologic examination alone. The figures here quoted supplement and replace those published in a preliminary note (6) and record our findings up to Feb. 10, 1938.

It is relevant to discuss here all other cases of possible malignancy which have been encountered in this colony. First, in an uncompleted experiment, in which 6 male rats received daily 30γ of oestrone subcutaneously after the introduction of kieselguhr into one testis, all the animals survived to an age of 500 days and one developed a spindle-cell tumour in the left flank (Fig. 12) at the site of oestrone injection; this tumour has been transplanted through 15 generations during six months. A second uncompleted experiment involved the feeding of a crude but potent oestrin product, approximately 1000 to 1500 international units per day per rat, to a series of rats of which 47 survived seventeen months. Of these, one showed a squamous-cell cancer in the wall of an abscess enclosing the uterus, and two rats which were repeatedly tarred developed squamous-cell cancers of the skin (Figs. 13 and 14), one of which metastasized to the axillary nodes. In a third group of rats, repeatedly injected with testosterone, one male animal was found to have metastatic adenocarcinoma in cervical lymph nodes at the age of 699 days; the primary tumour was not discovered.

In order to obtain more information as to the occurrence of spontaneous cancer in rats of this colony, 121 animals were set aside without treatment. Of these, 55 females and 27 males survived 500 days or more. A submaxillary gland carcinoma was seen in one of the 67 rats of this large group which were kept in the colony rat room, while in the 54 rats which were expressly kept elsewhere, neither cancer nor lymphosarcoma has been encountered.
Since 1933, the attendants in the rat room have been repeatedly instructed to look out for and to report any subcutaneous or intra-abdominal “lumps.” At that time the colony of this strain numbered some 2000 rats; it increased to about 4500 in 1935. There were constantly on hand at least 50 male and female animals a year and a half to two years of age. Among animals in the colony breeding cages, since 1932, we have found one malignant epithelial tumour surrounding a kidney, one uterine cancer, and two cases of mammary cancer. In addition, one female from a litter cage presented a subcutaneous tumour, from the histology of which one could only conclude that it was possibly malignant. All of these tumours have come to light since February 1936.

Up to 1935, benign tumours were occasionally met with; these were, almost without exception, small fibro-adenomata of slow growth. Since the beginning of 1936, a lymphosarcoma originating in the ileocaecal mesenteric lymph nodes has made its appearance in the colony, and has been observed in 65 female and 10 male rats up to the present time. Most of these tumours have been found in the colony breeding-cages, although some have been encountered among various experimental animals. No particular emphasis, therefore, is placed upon the appearance of this tumour in the experimental series with which this paper is concerned. Since early in 1937, a number of examples of adenofibromata of large size and rapid growth have been encountered; the largest weighed 281 grams and was actively secreting milk (Fig. 15).
We are unable to account for the increase in the incidence of spontaneous tumours in this colony during these years. It does not seem that it can be attributed to a higher proportion of rats reaching the cancer age. It is just conceivable that there should be taken into account the possible dissemination about the cages of ground food containing oestrin, as supplied to the rats in the uncompleted experiment mentioned above.

Mention should be made of the incidence of rat carcinoma as recorded by other observers. Woglom (7) refers to a fibro-epithelioma of the tongue found by Stahr, the Flexner-Jobling adenocarcinoma, and the mammary carcinoma of Lewin and Michaelis. Morris (8) recorded a basal-cell epithelioma in a male rat five months old. Bullock and Curtis (9) for ten years maintained a colony which reached a maximum of 10,000 rats, and during that period observed more than 2400 animals with tumours of the liver attributable to infestation with *Cysticercus fasciolaris*, and 489 rats with 521 neoplasms which could not be directly connected with the parasite and were therefore called by them "spontaneous." Of these, 309 were malignant, 227 being sarcomata and 63 carcinomata. They comment on the fact that they obtained only 4 malignant tumours of the breast. Of interest with reference to our findings of uterine abscess, utero-rectal fistulae, and uterine cancer, is their statement that "in all except two rats with uterine cancer, the involved uterus or horn was the seat of a chronic suppurative inflammation which probably antedated the appearance of the tumours and provided the irritation which seems to play an important rôle in the production of certain neoplasms." They also report 78 cases of sarcoma of mesenteric lymph nodes, apparently similar to the lymphosarcoma encountered by us.

Heim and Schwartz (10) make the following statement: "McCoy examined 100,000 wild rats, of which 103 or about 1 per mille showed tumours. Wolly and Wherry, out of 23,000 wild rats, found 22 tumours, also about 1 per mille. These observers were unable to make any statement as to the age of the animals examined. Statements of percentage occurrence without regard to age are almost valueless. Bullock and Rohdenburg, in an examination of 15,000 laboratory rats of an average age of three to eight months, found in all
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4 tumours; another group of 4,300 adult rats of mixed strains showed 21 tumours. McCoy's tables show 30 sarcomas against 10 carcinomas, whereas Wolly and Wherry report 7 sarcomas, 1 endothelioma and no epithelial tumours. Bullock and Rohdenburg report 4 sarcomas and 2 carcinomas."

Cameron and Meltzer (11) observed that the rat is extremely resistant to tarring. Among 10 rats treated with ordinary gas-works tar they obtained 2 tumours which proved histologically to be cancer after eighteen and a half months of treatment. This result is comparable to our own finding of 2 skin cancers in tarred oestrin-fed rats. There is evidence (12) that in the mouse external application of oestrone increases the susceptibility of the skin to the local carcinogenic action of benzpyrene.

SUMMARY

Rats were treated for long periods with oestrone, usually in conjunction with various forms of local irritation. The occurrence of tumors histologically diagnosed as malignant was found to be more frequent in these animals than in controls or in rats of the same colony subjected to other procedures, used for breeding, or set aside without treatment.

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REFERENCES