Strain Differences in Response to Estrone and the Induction of Mammary Gland, Adrenal, and Bladder Cancer in Rats

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Previous publications (9, 10) have shown that significant strain differences could be demonstrated in the response of rats to an effective mammary cancer-inducing dose of diethylstilbestrol. In later reports (6, 7) this difference in susceptibility to diethylstilbestrol was shown to be transmitted equally by both parental strains to their reciprocal F_1 hybrid progeny. One of the previous publications (9) showed that the mode of administration of the hormone might be as important as the actual dose of estrogen. The slow continuous absorption of diethylstilbestrol from cholesterol pellets proved much more effective for the induction of mammary cancer than intermittently implanted pellets of much larger doses of crystalline diethylstilbestrol. The more or less contradictory results obtained by other investigators, such as the relatively low frequency of induced mammary cancer obtained by McEuen (20) and Eisen (11) and the high frequencies observed by Geschickter and Byrnes (15), Noble *et al.* (22), and Nelson (21), might be accounted for on the basis of strain differences of the hosts, the choice of estrogen, or the mode of application.

The purpose of this experiment was to study the response of rats of four inbred strains to stimulation by subcutaneously implanted estrone pellets and to compare their response to that which resulted in rats of the same strains from stimulation by subcutaneously implanted cholesterol pellets containing similar doses of diethylstilbestrol.

MATERIALS AND METHODS

Pedigreed rats of August line 990, A × C line 9935, Fischer line 344, and Copenhagen line 2331 were used for this investigation. One or two estrone[1] pellets weighing 8–12 mg. were implanted in the subcutaneous tissues of the scapular region of the rats when they were 2½–6 months of age. The hair was plucked from a three sq. cm. area of the lower back. The pellet was inserted by a nasal forceps through a skin incision in this area, and the incision was closed with a wire staple.

The rats were weighed and inspected for mammary tumors every 2 weeks. They received the laboratory stock diet of Friskie Dog Pellets, supplemented by a green vegetable once a week and free access to water. At necropsy the pellet was removed and weighed. The post mortem examination included a thorough inspection and description of all macroscopic tumors and gross sectioning of mammary glands, thymus, liver, kidneys, adrenals, urinary bladder, sex glands, and pituitary. Representative sections of each were preserved and examined microscopically.

RESULTS

The results are summarized briefly in Tables 1–6 and shown graphically in Chart 1. Table 1 shows the number of rats of each of the four strains that survived for at least 150 days after the implantation of a single estrone pellet, the average age and weight at the start of the experiment, the average amount of estrone absorbed, the average survival period, and the body weight and pituitary weight at death. Twenty-nine Fischer male and female rats absorbed, respectively, an average of 9.4 and 9.6 mg. of estrone in an average of 572 and 578 days, i.e., at an average daily rate of 0.016 and 0.017 mg/rat. In the A × C strain, each of the 30 males absorbed on the average 8.2 mg. in 515 days at the average daily rate of 0.013 mg., and each of the 32 females absorbed an average of 7.5 mg. in 535 days at an average daily rate of 0.014 mg. Rats of the other two strains absorbed the

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TABLE 1

Rats of Each Strain That Survived for at Least 150 Days after Implantaton of a Single Estrone Pellet

Strain	Sex	No. rats	Av. dose of estrone absorbed (mg.)	At start: Age (days)	At start: Body wt. (gm.)	Days to death	At death: Body wt. (gm.)	At death: Pituitary weight (mg.)
Copenhagen	♂	10	5.0	116	203	486	170	174
Copenhagen	♀	9	3.4	119	154	377	146	136
A×C	♂	30	8.2	84	157	615	219	35
A×C	♀	32	7.5	82	127	535	161	84
Fischer	♂	29	9.4	86	168	572	222	21
Fischer	♀	29	9.6	85	132	578	173	24
August	♂	25	3.3	173	260	370	189	124
August	♀	12	3.2	165	183	319	187	120

TABLE 2

Mammary Cancer Induced in Rats with a Single Estrone Pellet

Strain	Sex	No. rats	Per cent rats bearing cancer	No. of cancers	Latent period (days): Minimum	Latent period (days): Av.
Copenhagen	♂	10	0	0		
Copenhagen	♀	9	0	0		
A×C	♂	30	13	10	371	518
A×C	♀	32	9	5	294	347
Fischer	♂	29	7	8	599	680
Fischer	♀	29	10	5	488	603
August	♂	25	36	26	208	385
August	♀	12	42	10	315	360

TABLE 3

Bladder Calculi and Cancer and Adrenal and Spontaneous Tumors Observed in Rats Treated with a Single Estrone Pellet

Strain	Sex	No. rats	Per cent with bladder calculi	Per cent with bladder cancer	Per cent with adrenal tumors	Per cent with spontaneous tumors
Copenhagen	♂	10	70	60	0	0
Copenhagen	♀	9	33	11	11	0
A×C	♂	30	0	0	3	13
A×C	♀	32	0	0	6	12
Fischer	♂	29	0	0	0	14
Fischer	♀	29	0	0	3	17
August	♂	25	16	4	36	0
August	♀	12	0	0	17	17

TABLE 4

Rats of Each Strain That Survived for at Least 150 Days after Simultaneous Implantation of Two Estrone Pellets

Strain	Sex	No. rats	Av. dose of estrone absorbed (mg.)	At start: Age (days)	At start: Body wt. (gm.)	Days to death	At death: Body wt. (gm.)	At death: Pituitary wt. (mg.)
Copenhagen	♂	31	9.4	102	191	444	157	181
Copenhagen	♀	26	7.7	119	164	412	142	160
A×C	♂	28	5.7	127	204	304	165	280
A×C	♀	28	4.4	125	150	243	142	292
Fischer	♂	27	5.1	146	201	269	186	259
Fischer	♀	19	3.5	140	143	194	159	253

TABLE 5

Mammary Cancer Induced in Rats with Two Estrone Pellets

Strain	Sex	No. rats	Per cent of rats bearing cancer	No. of cancers	Latent period: Minimum	Latent period: Av.
Copenhagen	♂	31	0	0		
Copenhagen	♀	26	0	0		
A×C	♂	28	25	10	266	308
A×C	♀	28	18	9	195	218
Fischer	♂	27	7	2	236	289
Fischer	♀	19	16	3	146	175

TABLE 6

Bladder Calculi and Cancer and Adrenal and Spontaneous Tumors Observed in Rats Treated with Two Estrone Pellets

Strain	Sex	No. rats	Per cent of rats with following lesions: Bladder calculi	Bladder cancer	Adrenal tumors	Spontaneous tumors
Copenhagen	♂	31	48	23	0	3
Copenhagen	♀	26	19	15	0	8
A×C	♂	28	25	7	0	8
A×C	♀	28	4	0	4	4
Fischer	♂	27	0	0	0	0
Fischer	♀	19	11	5	0	0

hormone at a somewhat slower rate. On the average, the ten Copenhagen males each absorbed 5.0 mg. in 486 days, and the nine females each absorbed 3.4 mg. in 377 days. The daily rate of absorption was, respectively, 0.010 and 0.009 mg. Each of the 25 August males absorbed an average of 3.5 mg. in 370 days at a daily rate of 0.009 mg., and each of twelve females absorbed 3.2 mg. in 319 days at a daily rate of 0.010 mg. In spite of smaller average doses of estrone, the rats of the two latter strains had the larger average pituitary weights. The average pituitary weight for Copenhagen males was 174 mg. and for Copenhagen females, 136 mg. The pituitaries averaged 120 mg. in August rats, only 35 mg. in A × C males, and 21 and 24 mg., respectively, in Fischer male and female rats.

Similar data are given in Table 4 for rats of three of these strains that received two pellets of estrone simultaneously. The Copenhagen rats of this series nearly doubled their absorption of estrone without materially affecting their survival period or pituitary weight. Thirty-one Copenhagen males absorbed an average of 9.4 mg. of estrone in an average of 444 days, while 26 Copenhagen females absorbed an average of 7.7 mg. in an average of 412 days, i.e., at the daily rate of 0.021 and 0.019 mg/rat. The double dose of estrone more than halved the survival period of the rats of the two other strains and significantly increased their pituitary weights. Twenty-eight A × C male and female rats absorbed, respectively, 5.7 and 4.4 mg. of estrone in an average of 304 and 243 days. Their average daily rate of absorption was 0.019 and 0.018 mg. Their pituitaries averaged, respectively, 280 and 292 mg. Twenty-seven Fischer male and nineteen Fischer female rats absorbed 5.1 and 3.5 mg., respectively, in an average of 269 and 194 days, the daily rate of absorption being 0.019 and 0.018 mg/rat from two simultaneously implanted pellets. Their pituitaries averaged 259 and 253 mg., compared to 21 and 24 mg. for rats of the same strain in the series with single estrone pellets.

Tables 2 and 5 show the percentage of rats of each group that developed mammary cancer, the number of cancers observed, and the minimum and average latent period. No mammary cancers were observed in Copenhagen rats of either series, while 4 (13 per cent) of the A × C male and 3 (9 per cent) of the A × C female rats developed mammary cancer in an average of 518 and 347 days, respectively, in the series that received one estrone pellet. Twice as many, or 25 and 18 per cent, respectively, of A × C male and female rats that received two estrone pellets developed mammary cancers, in an average of 308 and 218 days. Two Fischer male and three Fischer female rats of each series developed mammary cancers, but the latent period was considerably reduced for the tumors in the series that received the double dose of estrone. The average latent period for the induced tumors in Fischer males of the first series was 680 days, compared to 289 days for the tumors of the second series. In Fischer females the average latent period was 603 days and 175 days, respectively, for tumors of the two series. The

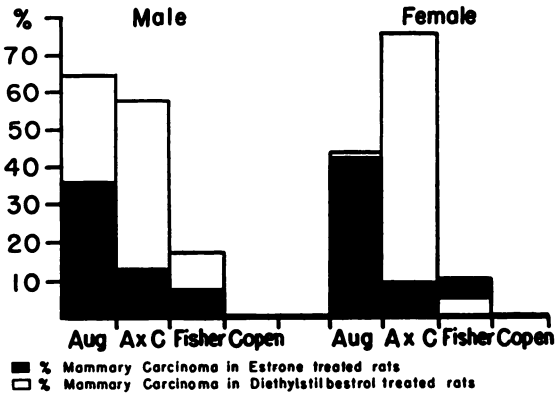

CHART 1.—Comparison of percentages of induced mammary cancers in male and female estrone- and diethylstilbestrol-treated rats of four strains. Designations represent: August line 990, AXC line 9935, Fischer line 344, and Copenhagen line 2331.

highest percentage of induced mammary cancer was observed in rats of the August strain. Because these rats reacted unfavorably to a single pellet of estrone, none were treated with the double dose. Most of the treated August females died in less than 150 days after implantation of the pellet, but, of the twelve that survived, 5 (42 per cent) developed mammary cancers. Among 25 August males, 9 (36 per cent) developed mammary cancers. The average latent period was 385 days for tumors in males and 360 days for tumors in females.

A total of 26 mammary cancers was observed in the nine tumor-bearing males of the August strain, and eight independent tumors were found in the two Fischer male tumor-bearers of the same series. However, multiple tumors were not so frequently encountered in these estrone-treated rats as has been previously reported for diethylstilbestrol-treated rats of the same strains. The tumors of this series were classified as 84 papillary cyst adenocarcinomas and four squamous-cell cancers. The four exceptional tumors were observed in an A × C male that received one estrone pellet, an A × C female, and a Fischer male and female that received two estrone pellets.

Chart 1 compares the percentage of induced mammary cancers in rats of the four strains that received one 8–12-mg. pellet of estrone with the previously observed (6, 9) percentage of induced mammary cancers in rats of the same strains that were treated with 4–15 mg. of diethylstilbestrol in cholesterol pellets. This chart shows that, for male rats of each strain, diethylstilbestrol is a much more effective cancer-inducing agent. This is also true for A × C female rats. Previous experiments (8) with A × C female rats on various synthetic diets indicate that they are nearly 100 per cent susceptible to the induction of diethylstilbestrol-induced mammary cancer. Two A × C rats and many of their F_1 hybrid progeny bearing transplants of the spontaneous functional ovarian tumor described by Iglesias, Sternberg, and Segaloff (17) developed primary mammary gland adenocarcinomas in response to the mixture of estrone and estradiol produced by this tumor. The slow continuous absorption of a similar dose of estrone from the pellet, however, initiated only a small percentage of tumors. The larger dose of estrone increased the percentage of induced cancers but disproportionately reduced the survival period. August female rats succumbed rather early to the toxic effects of both hormones, but those that survived long enough in both groups developed mammary cancers.

Copenhagen rats proved to be as resistant to estrone-induced mammary cancer as they had been to diethylstilbestrol-induced mammary cancer and equally susceptible to the formation of bladder calculi and cancer. Tables 3 and 6 show the percentage of rats of each group that developed bladder calculi and cancer, adrenal tumors, and tumors of other locations. Seven of the ten Copenhagen males that received one estrone pellet had bladder calculi, and six of these had papillary or squamous-cell cancer of the bladder. Among the females, three had calculi, and one of these had a papillary carcinoma. Fifteen (48 per cent) of the Copenhagen males that received the double dose of estrone had bladder calculi, and seven of these had cancer of the bladder. Five (19 per cent) of the females of this group had bladder calculi, and cancer was present in four. Bladder calculi or cancer were not so frequent in rats of the other strains, but calculi were observed in four August males that received one estrone pellet and in seven A × C males and one female and in two Fischer females that received two estrone pellets. Bladder cancer was present in one August male, two A × C males, and one Fischer female. This is not unlike the frequency of bladder calculi and cancer observed previously in diethylstilbestrol-treated rats of the same strains.

Tumors of the adrenal cortex were also previously reported (10) for rats of these strains that had been treated with diethylstilbestrol. These tumors were found to be most frequent in rats of the August strain. Nine (36 per cent) of the estrone-treated August males had adrenal tumors, and two were observed in the August females. One A × C male in each group, one Copenhagen female, one Fischer female, and two A × C females also had adrenal tumors. With one exception these tumors were classified as benign adenomas, although some others were probably malignant. The adrenal tumor that was found in a Copenhagen female showed metastases to the lungs and was classified as an adenocarcinoma. Morphologically, however, this tumor resembled the others.

In some strains of mice, extreme hyperplasia of the adrenal cortex and adrenal cortical carcinomas result from gonadectomy (13, 24, 25) at birth or an early age, and these tumors can be prevented by the administration of estrogens (18, 26). Studies by Frantz and Kirshbaum (12) relative to the hormonal secretions of the tumors, however, indicate that they may produce either estrogens or androgens or a mixture of both and that in some strains the complete withdrawal of gonadal endocrine secretion is not essential for the appearance of the tumors. Studies by Huseby and Bittner (16) based on the transplantation of adrenal tissue from different inbred lines of mice into adrenalectomized, gonadectomized F_1 hybrids show that the responsiveness of adrenal tissue to gonadectomy is probably inherent in the adrenal tissue itself.

Spontaneous adrenal cortical adenomas are relatively rare in rats of our colony, only four having been observed in 7,727 previously reported (3) untreated rats over 19 months of age. One of these was found in a Copenhagen line 2331 rat, and none were observed in rats of the three other strains used in this experiment. During the two succeeding decades, thirteen additional adrenal tumors have been described. One was a carcinoma of the medulla, two were cortical carcinomas, and the others were probably benign adenomas of the cortex. Five were observed in A × C rats, three in Fischer rats, two in August rats, one in a Copenhagen rat and two in hybrids of other strains. While adequate population data are not available in tabular form, the number of rats of tumor age would exceed 20,000. There seems little doubt that most of the seventeen adrenal tumors reported here and the 37 previously reported (6) resulted from excessive, exogenous estrogenic stimulation.

Twenty-four of the rats had additional neo-

plastic diseases that were probably unrelated to the treatment. These included seven with lymphatic leukemia and five with lymphosarcomas that arose in the lymph nodes of the illeo-colic mesentery. There was one rat with each of the following tumors: squamous-cell carcinoma of the ear and cervix, papillary carcinoma of the cervix and ovary, fibrosarcoma of the uterus, leg, and lung, interstitial-cell carcinoma of the testis, adenoma of the kidney, fibroadenoma of the mammary gland, thymoma, and one squamous-cell cancer of undetermined origin. The leukemias occurred, two each, in A × C male and female rats treated with one estrone pellet and in three Fischer females of the same series. The five lymphosarcomas were observed in a Fischer female, an A × C male and female with one pellet, and an A × C female and a Copenhagen female with two pellets.

While estrogens appear to increase or accelerate the occurrence of lymphoid tumors (1, 2, 14, 23) in mice, and orchidectomy and adrenalectomy (19) increase the incidence of lymphoid leukemia in C58 mice, there were probably no more lymphoid tumors reported in this series of rats than would be expected to occur spontaneously. There are no reliable data on the occurrence of lymphoid leukemia in rats of this colony, but spontaneous tumors of the thymus are fairly frequent in rats of the Copenhagen strain, and lymphosarcomas of the mesenteric lymph nodes are the most frequent neoplasm observed in untreated rats of the Fischer and A × C strains (3–5).

SUMMARY

1. One hundred and seventy-six rats of four different inbred strains survived a minimum of 150 days after the implantation of an estrone pellet weighing 8–12 mg. in the subcutaneous tissues of the scapular region.

2. One hundred and fifty-nine rats of three of the same inbred strains survived a minimum of 150 days after the simultaneous implantation of two similar estrone pellets.

3. Absorption of the hormone was more rapid from two pellets than from one, and in two of the three strains tested the survival period of the rats with two pellets was only about half as long as for rats of the same strain with one pellet.

4. Mammary cancers developed in rats of three of the four inbred strains tested with one estrone pellet but not in so high a percentage of the rats as was observed for rats of the same strains treated with a similar dose of diethylstilbestrol.

5. The minumum and average latent period for the induced mammary cancers was considerably shorter in the rats with two estrone pellets than in rats of the same strain with one estrone pellet. The percentage of rats with induced mammary cancers was increased by the larger dose of estrone in one strain but not in the other because of the shortened survival period.

6. No mammary cancers were induced in rats of one strain by either dose of estrone. This was the same strain that proved to be resistant to the induction of mammary cancers by diethylstilbestrol.

7. The induced mammary cancers were classified as 84 papillary cyst adenocarcinomas and four squamous-cell cancers

8. Bladder calculi and cancer were a frequent finding in rats of the strain that proved to be resistant to the induction of mammary cancer and occurred in a small percentage of the estrone-treated rats of the strain with the highest percentage of induced mammary cancer. Adrenal adenomas or cancer were most frequent in rats of the latter strain but occurred in a small percentage of the rats of each strain.

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