# The Incidence of Diethylstilbestrol-induced Cancer in Reciprocal $F_1$ Hybrids Obtained from Crosses between Rats of Inbred Lines That Are Susceptible and Resistant to the Induction of Mammary Cancer by This Agent

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In mice, the role of an extra-chromosomal or milk factor in the etiology of mammary cancer was demonstrated independently by Little and his coworkers (10) and by Korteweg (8) in the reciprocal $F_1$ hybrids obtained from crosses between mice of strains with a high and a low incidence of mammary cancer. Confirmation of the presence of such a factor in the etiology of other neoplasms in mice has not been obtained, nor has the existence of a similar factor been demonstrated in the etiology of mammary cancer in any other species. Andervont and Dunn (1) and Heston *et al.* (7) have shown that mammary cancers occur spontaneously in mice that do not have the mammary tumor agent, and the former (2) have shown that mammary cancer can be induced by methylcholanthrene in agent-free DBA mice. The induced and spontaneous tumors that have been observed in agent-free mice display a greater variety of histologic types and a greater tendency toward keratinization than are found in mice with the agent. Andervont and Dunn (2) found, furthermore, that estrogenic stimulation had no influence on the occurrence of methylcholanthrene-induced mammary cancers; but Twombly (11) and Shimkin and Andervont (9) found that the foster nursing of agent-free mice by females with the agent greatly increased the proportion that developed estogren-induced mammary cancers. The latter authors (9) also observed that the incidence of stilbestrol-induced mammary cancer in C3H males was reduced when they were fostered by agent-free C57BL females.

An opportunity to test for the presence or absence of a maternally transmitted factor in the etiology of mammary cancer in rats was presented by the demonstration of strain differences in the incidence of experimentally induced mammary cancer. Dunning, Curtis, and Segaloff (5) showed that inbred rats of A × C line 9935 were susceptible to the induction of mammary cancer by the implantation in the subcutaneous tissues of the scapular region of cholesterol pellets containing 4–6 mg. of diethylstilbestrol. In 1948 (6) they showed further that inbred rats of August line 990 were as susceptible to the induction of mammary cancer as the A × C rats and, in addition, were susceptible to the induction of diethylstilbestrol-induced bladder and adrenal cancer. Both reports showed that rats of Copenhagen line 2331 failed to develop mammary cancer in response to similar treatment. Except for the exogenous source of the hormonal stimulation, this situation is analogous to that found in inbred strains of mice with a high and low incidence of spontaneous mammary cancer.

In a more recent publication (4) it was demonstrated that, among 51 hybrids derived from susceptible A × C line 9935 fathers and resistant Copenhagen line 2331 mothers, fourteen (26 per cent) developed mammary cancer in response to the implantation of diethylstilbestrol pellets in the scapular region when the animals were 3–4 months of age. Among 61 reciprocal hybrids that were similarly treated, twenty (35 per cent) developed mammary cancer. The average latent period, another index of susceptibility, was not significantly different in the two groups of hybrid tumor-bearers, being 561 days for those derived from mothers of the resistant strain and 586 for the hybrid tumor-bearers from mothers of the susceptible strain. If susceptibility of the hybrid individual to these induced neoplasms was controlled by a factor transmitted in the milk of the susceptible parent, such as obtains in some inbred strains of mice, the percentage of individuals with induced mammary cancer among the progeny of the mothers of the susceptible strain should have equaled or com-

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pared favorably to that obtained in rats of the susceptible parental strain and should have been zero or relatively low among the progeny of the mothers from the resistant strain. Since the percentage of induced mammary cancer was not significantly different in the two groups of hybrids, no evidence for a maternally transmitted etiological factor was obtained.

This is substantiated by the data to be presented on the occurrence of diethylstilbestrol-induced mammary cancer in reciprocal $F_1$ hybrids obtained from crosses between rats of the same resistant inbred line and rats of another susceptible inbred line.

TABLE 1

NO. RATS IN EACH GROUP THAT SURVIVED FOR AT LEAST 150 DAYS, THEIR AVERAGE DOSE OF DIETHYLSTILBESTROL, AGE AT TIME OF PELLET IMPLANTATION, DAYS TO DEATH, TOTAL BODY WEIGHT, AND PITUITARY WEIGHT AT DEATH

| Group | Sex | No. rats | Dose of stilb. | Age at start | Days to death | Body wt. (gm.) | Pituitary weight (mg.) |
|---|---|---|---|---|---|---|---|
| A×C | ♂ | 44 | 6.1 | 168 | 403 | 192 | 115 |
| A×C | ♀ | 29 | 5.4 | 119 | 442 | 173 | 116 |
| August | ♂ | 52 | 11.4 | 105 | 459 | 181 | 138 |
| August | ♀ | 21 | 8.8 | 104 | 370 | 168 | 71 |
| Copenhagen | ♂ | 30 | 8.6 | 109 | 528 | 171 | 64 |
| Copenhagen | ♀ | 27 | 9.0 | 109 | 518 | 150 | 92 |
| Aug.×Copen. | ♂ | 44 | 7.8 | 131 | 553 | 221 | 167 |
| Aug.×Copen. | ♀ | 32 | 6.8 | 122 | 557 | 186 | 162 |
| Copen.×Aug. | ♂ | 25 | 5.7 | 148 | 537 | 238 | 113 |
| Copen.×Aug. | ♀ | 31 | 4.5 | 166 | 419 | 207 | 90 |

## MATERIALS AND METHODS

Reciprocal $F_1$ hybrids were obtained from crosses between rats of Copenhagen line 2331 and August line 990. Rats of the two parental strains and some additional rats of A×C line 9935, the susceptible parental strain used for obtaining the previously reported hybrids, were included for comparative purposes.

Cholesterol pellets containing 4–15 mg. of diethylstilbestrol were implanted subcutaneously in the scapular region when the rats were 4–6 months of age. The laboratory stock diet consisted of Friskie Dog Pellets supplemented with a green vegetable once a week and free access to water. Each rat was weighed and inspected for tumors bi-weekly.

After death, a post mortem examination included a description of every visible tumor, gross sectioning of all mammary glands, inspection of the liver, kidneys, adrenals, pituitary, and sex glands. Representative sections of each of these tissues were preserved and examined microscopically.

## RESULTS

The results are summarized briefly in Tables 1–3. Table 1 shows the number of rats of each group that survived for at least 150 days after implantation of the pellet, their average dose of diethylstilbestrol, age at implantation of the pellet, average number of days survived after implantation, and their average body weight and pituitary weight at death. The average dose of diethylstilbestrol varied from 4.5 mg. for Copenhagen × August hybrid females to 11.4 mg. in August males, but rats with equal doses of diethylstilbestrol were included in each group. There appeared to be no correlation between the actual dose and the development of mammary cancer. The smallest dose of 4 mg. was as effective as the largest dose of 15 mg.

The size of the pituitary varied from 64 mg. for Copenhagen male rats that survived an average of 528 days to 167 mg. for August× Copenhagen male hybrids that survived for 553 days. The relatively low average of 71 mg. for August females resulted, in part, from their relatively short average survival period of 370 days. Many of the August female rats died early with acute uterine infections, but some of those that survived for longer periods had pituitaries weighing 250 and 300 mg. at death. The pituitary tended to be consistently smaller in the Copenhagen rats and in some of the hybrids, in spite of their relatively long survival period, which finding perhaps indicated a lesser degree of hormonal stimulation.

Table 2 shows the percentage of rats of each group with induced cancer of the mammary gland, the number of cancers observed, and the minimum and average latent period. Of 73 treated August rats of the susceptible parental strain, 42 or 58 per cent developed 155 mammary cancers in an average of 485 days. This is essentially equivalent to that observed for the A × C rats used as the susceptible parental strain for the previously reported hybrids (4). Of 73 treated A × C rats, 47 (64 per cent) developed 151 mammary cancers in an average of 429 days. No mammary cancers were observed in 57 similarly treated Copenhagen rats that survived an average of 520 days.

Among 76 treated hybrid progeny from Copenhagen mothers and August males, eighteen (24 per cent) developed mammary cancer in an average of 632 days. The tumor-bearing rats were seven males and eleven females, or 16 per cent of the

## TABLE 2

### No. Rats in Each Group, the Percentage with Induced Cancer of the Mammary Gland, and the Minimum and Average Latent Period in Days

| Group | Sex | | No. rats | Per cent rats bearing cancer | No. cancers | Av. no. of cancers | Latent period Minimum | Latent period Av. |
|---|---|---|---|---|---|---|---|---|
| A×C | ♂ | | 44 | 57 | 62 | 2.5 | 265 | 436 |
| | ♀ | | 29 | 76 | 89 | 4.0 | 225 | 425 |
| | | sum | 73 | 64 | 151 | 3.2 | 225 | 429 |
| August | ♂ | | 52 | 64 | 135 | 4.1 | 210 | 485 |
| | ♀ | | 21 | 43 | 20 | 2.2 | 224 | 483 |
| | | sum | 73 | 58 | 155 | 3.7 | 210 | 485 |
| Copenhagen | ♂ | | 30 | 0 | 0 | | | |
| | ♀ | | 27 | 0 | 0 | | | |
| | | sum | 57 | 0 | 0 | | | |
| Aug.×Copen. | ♂ | | 44 | 16 | 9 | 1.3 | 328 | 636 |
| | ♀ | | 32 | 34 | 20 | 1.8 | 472 | 631 |
| | | sum | 76 | 24 | 29 | 1.6 | 328 | 632 |
| Copen.×Aug. | ♂ | | 25 | 20 | 5 | 1.0 | 363 | 517 |
| | ♀ | | 31 | 23 | 11 | 1.6 | 376 | 567 |
| | | sum | 56 | 21 | 16 | 1.3 | 363 | 551 |

## TABLE 3

### No. Rats in Each Group and the Percentage with Bladder Calculi and Cancer, Adrenal Tumors, and Tumors of Other Locations

| Group | Sex | | No. rats | Per cent with calculi | Per cent with bladder cancer | Per cent with adrenal tumors | Per cent with spontaneous tumors |
|---|---|---|---|---|---|---|---|
| A×C | ♂ | | 44 | 14 | 7 | 5 | 5 |
| | ♀ | | 29 | 3 | 3 | 3 | 17 |
| | | sum | 73 | 10 | 5 | 4 | 10 |
| August | ♂ | | 52 | 10 | 4 | 29 | 2 |
| | ♀ | | 21 | 38 | 29 | 5 | 0 |
| | | sum | 73 | 18 | 11 | 22 | 1 |
| Copenhagen | ♂ | | 30 | 73 | 53 | 0 | 10 |
| | ♀ | | 27 | 30 | 22 | 0 | 4 |
| | | sum | 57 | 53 | 39 | 0 | 7 |
| Aug.×Copen. | ♂ | | 44 | 30 | 14 | 23 | 9 |
| | ♀ | | 32 | 12 | 6 | 6 | 12 |
| | | sum | 76 | 22 | 11 | 16 | 11 |
| Copen.×Aug. | ♂ | | 25 | 4 | 0 | 16 | 16 |
| | ♀ | | 31 | 6 | 3 | 6 | 10 |
| | | sum | 56 | 5 | 2 | 11 | 12 |

former and 34 per cent of the latter. Among 56 treated reciprocal hybrids from August mothers and Copenhagen fathers, twelve (21 per cent) developed sixteen mammary cancers in an average of 551 days. These tumor-bearers were five (20 per cent) of the males and seven (23 per cent) of the females.

Chart 1 presents graphically for each sex the per cent of tumor-bearers in both parental strains and in both groups of reciprocal hybrids and shows clearly that both groups of hybrids were less susceptible than their susceptible strain parents and more susceptible than their resistant strain parents and were not significantly different from each other.

Table 2 shows further that the hybrid tumor-bearers developed less tumors per rat than their susceptible strain counterparts and that the minimum and average latent periods were longer. August line 990 tumor-bearers had an average of nearly four tumors per rat compared to an average of less than two in the hybrids. Slightly more multiple tumors were observed in the hybrid progeny of the resistant strain mothers than in the hybrids of the reciprocal cross. The minimum latent period for mammary cancer in the August rats was 210 days, compared to 328 and 363 days for the hybrids. The average latent period varied from 485 days in August rats to 551 and 632 days for the cancers that developed in the two groups of hybrids. The longer latent period was observed in the hybrids of the resistant strain mothers, but in both groups there was too much variability for the difference to be significant.

Unlike the mammary cancers reported in agent-free mice, these induced mammary cancers showed very little variation in morphology. Among the total of 351 induced cancers 343 were classified as papillary cyst adenocarcinomas of the type most frequently observed in mice with the agent, one was a solid carcinoma, and seven were mixed adenocarcinomas and squamous-cell carcinomas. The adenoacanthomas occurred in an A × C male, an August female, and five August males. The solid carcinoma was observed in a hybrid female from an August mother.

The formation of calculi in the urinary bladder and associated cancer of the bladder mucosa in diethylstilbestrol-treated rats has been previously reported (5). Table 3 shows, for the rats used in the presently reported experiments, that thirteen of the treated August rats and 30 of the Copenhagen rats were found to have urinary calculi and that cancer of the bladder had developed in eight of the former and in 22 of the latter. The tendency was present in both parental strains and would, therefore, be expected in their hybrid progeny. Bladder calculi were observed in seventeen of the hybrid progeny from Copenhagen mothers and in three of the hybrid progeny of August mothers. Cancer was present in eight of the former and in one of the latter. In the previously reported (4) reciprocal $F_1$ hybrids from Copenhagen and A × C crosses, bladder calculi were found to be more frequent among the male progeny of Copenhagen females than among their female progeny, and it seems also to be true of these hybrids from Copenhagen mothers and August fathers. Thirteen of 44 treated hybrid males from Copenhagen females were found to have calculi, while only four of 32 hybrid females developed calculi. Calculi were observed in only one of 25 hybrid males from August females. This suggests the possibility that the Copenhagen

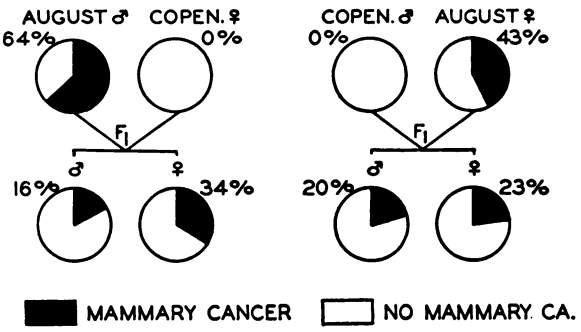


CHART 1.—The percentage of mammary cancer in both sexes of parental strains and in the reciprocal $F_1$ hybrids.

mother transmits some infective agent that more readily gains access to the male urinary bladder to act as a nidus for the deposition of magnesium ammonium phosphate.

The two parental strains, as previously reported (6), differed also in their tendency to develop adrenal tumors following treatment with diethylstilbestrol. As shown in Table 3, sixteen (22 per cent) of the treated August rats were found to have adrenal tumors. These occurred more frequently in males, only one having been observed in a female. No adrenal tumors were observed in treated Copenhagen rats of either sex. On the basis of morphology, the tumors were classified as adenomas, although two of similar morphology were found to have generalized dissemination to the lungs. Twelve (16 per cent) of the hybrids from Copenhagen mothers were found to have adrenal tumors, and six (11 per cent) of the reciprocal hybrids had adrenal tumors. In both groups the adrenal tumors were more frequent in males than in females. Since the higher percentage of tumors was observed among the male offspring of Copenhagen or resistant strain mothers, no evidence for the opera-

tion of a maternally transmitted etiological factor was obtained.

Spontaneous neoplasms of types previously reported (3) for rats of this colony were observed in 27 rats. These included nine lymphosarcomas that arose in the lymph nodes of the ileocolic mesentery. One of these neoplasms was observed in a A × C female, one in a Copenhagen male, four occurred in hybrids from Copenhagen females, and three in hybrids from August females. Tumors of the uterus were observed in six rats. These included one carcinoma in an A × C female; two sarcomas, one each in a Copenhagen female and a hybrid from a Copenhagen mother; and three mixed tumors, one in an A × C female, one in a hybrid from a Copenhagen mother, and one in a hybrid from an August mother. Two A × C females and one Copenhagen male had tumors of the thymus. Two A × C males and a hybrid female from a Copenhagen mother had subcutaneous sarcomas of the face and neck, and one hybrid male from an August mother had a liposarcoma of the chest wall. One hybrid male from a Copenhagen mother had a papillary carcinoma of the testis, the only unusual tumor of the group. Lymphatic leukemia occurred in four rats—one August male, one Copenhagen male, and one male and one female hybrid from August mothers.

## SUMMARY

1. Diethylstilbestrol-induced mammary adenocarcinoma occurred in an average of 632 days in eighteen (24 per cent) of 76 hybrid progeny obtained from crosses between resistant strain male and susceptible strain female rats.

2. Among 56 similarly treated hybrids from reciprocal crosses, twelve (21 per cent) developed mammary adenocarcinomas in an average of 551 days.

3. Induced adrenal tumors were observed in twelve of the hybrids from resistant strain females and in six of the hybrids from the reciprocal crosses. Adrenal tumors were more frequent in the males than in the females of both groups of hybrids and the susceptible parental strain.

4. Diethylstilbestrol-induced bladder calculi and cancer occurred in both parental strains and in both groups of hybrids but were most frequent in the male progeny of Copenhagen line 2331 females.

5. Spontaneous neoplasms, probably unrelated to the treatment, occurred with equal frequency in both hybrid groups.

6. The $F_1$ hybrid progeny from reciprocal crosses between diethylstilbestrol-resistant and diethylstilbestrol-susceptible rats showed an equal susceptibility to tumors induced by this agent.

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