Tumor Incidence in Normal Sprague-Dawley Female Rats

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A high incidence of spontaneous tumors in normal Sprague-Dawley rats has been observed during the progress of studies in this laboratory on the chronic toxicity of polonium.[1] However, the data on tumors in the control animals appeared to be of interest *per se* and are being reported separately here.

In this laboratory, 250 normal control rats of the Sprague-Dawley strain had been under observation in a series of experiments for long periods of time. Some of these experiments were life-span studies, and the observations extended over the entire adult life of the rats. As the animals approached the last quarter of their lives, there appeared a greater incidence of tumors than had been expected. A search through the published literature failed to reveal any mention of such information about this rat strain. Thus, it seemed that a critical evaluation of this phenomenon as the animals lived out their life span would be of interest. Accordingly, in four different long-term experiments detailed tumor observations were made.

MATERIALS AND METHODS

Only the female control rats from the group of four long-term experiments, herein designated Groups A (40 rats), B (100 rats), C (50 rats), and D (60 rats), were used for the main tumor incidence study. Males were not used in all experiments; hence, there were much smaller numbers of male controls. The tumor data on the males are therefore not so reliable as the data on females and are reported only as incidental to the main body of data. Accordingly, all subsequent statements will concern the females alone, unless specifically stated otherwise.

Rats in a weight range of 150–200 gm were obtained from Sprague-Dawley, Inc., Madison, Wisconsin. The animals were kept in quarantine until judged acceptable for experimental use, usually a period of 14–21 days. The first group was placed on experiment April 26, 1951, and the second, third, and fourth groups followed 11, 13, and 15 months later, respectively. Since the mean life-span was over 25 months (760 days), the rats of the different groups lived during the same chronological time for a large part of their observed lives. The rats in Groups A, C, and D were fed ad libitum a standard laboratory chow, obtained from the Vitality Mills, Chicago, Illinois. Fresh tap water was available at all times. The Group B rats were maintained on a specially prepared laboratory diet.[2] The animals were housed ten to a cage in air-conditioned rooms under stable temperature and humidity conditions. The temperature was maintained at 74 ± 1° F., with a relative humidity of 52 per cent. The figures used to compute the average life-span for the different groups were taken from the birth date records supplied with the rats at the time of purchase.

Histological studies of tumor tissue were performed at autopsy, and the percentages of benign and malignant tumors were calculated in Groups A and B. Only observations on gross tumors were recorded for the other two groups.

The rats were allowed to live out their life span without tumor biopsies. In many cases large tumors developed in the mammary gland region and later ulcerated and underwent necrosis before the animals died. Consequently, not all tumors histologically examined in Groups A and B could be

[2] Special rat diet formula:

Cellu-Flour	120.0 gm.
Osborn-Mendel salts	40.0 "
Dried brewers' yeast	100.0 "
Wheat germ oil	10.0 "
Cod liver oil	10.0 "
Vitamin K	0.01 "
Mazola oil	200.0 "
Casein	160.0 "
Corn starch	200.0 "
Dextrin	190.0 "
Sucrose	200.0 "
H_2O (Q.S.)	2000.0 ml.

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‡ Mound Laboratory is operated by Monsanto Chemical Company for the United States Atomic Energy Commission under Contract Number AT-33-1-GEN-53.

[1] R. K. Davis, G. T. Stevenson, K. A. Busch, and D. S. Anthony. The Effects of Small Amounts of Polonium on Rats (submitted to *Radiation Research*, 1955).

Received for publication August 26, 1955.

classified as to malignancy. The observed ulceration and necrosis of the larger tumors appeared to be the result of reduced vascularity in the area affected. They seemed to result from the friction of the tumor mass against the cages as the rats moved about feeding. The lesions first appeared on the taut skin covering the tumor and then progressed inward into the tumor in the areas of reduced vascularity. Whether the reduced vascularity was due to pressure exerted on existing blood vessels or to an intrinsic absence of blood vessels in the area is not known. These subjective observations led to the assumption, for purposes of statistical computations, that large necrotic tumors had neither higher nor lower incidence of malignancy than the others.

The rats were classified as either tumor-free or tumor-bearing, regardless of the number of individual tumors any one rat possessed. There were four rats in Group A, each with two benign tumors. These were tabulated as one entry under the classification of the larger of the two. When, in a few cases (two in Group A and three in Group B), the rats were found to have both benign and malignant tumors, they were classed only as malignant tumor-bearing animals. In one case a malignant tumor had extended to another organ, and this secondary tumor resulted in death to the rat; the latter fatal tumor was the one tabulated.

Whenever practicable, all rats were autopsied immediately after death and examined for grossly evident tumors. When tumors were found, tissue samples were taken and immediately fixed and processed for histopathological examination and subsequent tumor classification. In view of the recognized difficulty in the distinction between benign and malignant growths, a review of the various criteria used in this study is given. Tumors were classified as malignant when they met several of the following criteria: (*a*) centrifugal invasion with invasion of the vessels, (*b*) prominent destruction of tissue, (*c*) evidence of rapid growth, (*d*) probable metastasis, and (*e*) anaplasia. In respect to the individual cell architecture, if, in addition to anaplasia, the tumor cells demonstrated (*a*) an increased nucleocytoplasmic ratio, (*b*) an infiltrative growth of cells, (*c*) anisocytosis of the nuclei, (*d*) poikilocytosis of the nuclei, (*e*) an irregularity and thickening of nuclear membranes, (*f*) clumping of chromatin and hyperchromasia of the nuclei, (*g*) prominence (size and number) of the nuclei per cell, they were considered malignant growths. In contrast to these criteria for malignant tumors, tumors were considered benign if (*a*) they were usually encapsulated, (*b*) they grew by centripetal expansion (usually slow growth), (*c*) there was no invasion of vessels, (*d*) they demonstrated no metastases, (*e*) the tumor cells were differentiated, and (*f*) there was no great amount of tissue destruction, unless the tumor had become large and the peripheral and central tissue had an inadequate blood supply due to pressure atrophy of the tumor.

TABLE 1

TUMOR INCIDENCE DATA ON NORMAL FEMALE SPRAGUE-DAWLEY RATS

Group	Date born	Av. life span (days)*	Range life span (days)	No. rats	Tumor incidence (per cent)†	Tumors classified mammary (per cent)	Tumors classified benign (per cent)	Tumors classified malignant (per cent)
A	4/30/51	739±59	337–1100	40	65	100	87.5	12.5
B	4/ 8/52	744±30	251–1071	100	80	94.8	88	12
C	5/ 8/52	780±52	205–1087	50	56	87.5		
D	7/10/52	792±47	193–1064	60	51	97		

* The ± value represents the 95 per cent fiducial interval.

† Per cent of tumor incidence is defined as the total number of tumor-bearing rats × 100 divided by the total number of animals.

Within the benign tumor classification (Table 2) it was debatable whether to omit the classification of fibroma and class these tumors with the adenofibromas where they might more usually be placed. It was recognized that adenofibromas are the most common benign tumors of the breast, but in these particular cases the tumors showed little if any recognizable glandular tissue, and it was on this basis that the present classifications were used.

RESULTS AND DISCUSSION

A high incidence of spontaneous tumors, averaging 57 per cent of all animals, was observed in three of the four groups (Table 1). Group B had an even higher tumor incidence of 80 per cent. This was significantly greater than the average for the other three groups. The greater tumor incidence in Group B must have been due to the dietary influence, since the diet was the only variable factor among the other three groups, environment and heredity being identical.

A variety of supporting information was obtained on the time sequence of tumor appearance, the effect of tumors on life span, origin of tumor tissue, and incidence of malignancy. It was found

that 87 per cent of all tumors occurred after the rats were 540 days old. It was also found that the mean "residual" life-span, that is, the number of days a rat lived after a tumor was first observed, was 140 days, regardless of whether the tumor was benign or malignant. It is felt that this residual life span reflects the rate of development of the tumor mass up to a size that seriously interfered with the rat's ability to take nourishment. Furthermore, it was estimated that about 60 per cent of the tumor-bearing rats died sometime after 73 and before 209 days following the first tumor observation. These observations proved to be true regardless of whether the animals developed a tumor early or late in life, over the age range of from 494 to 798 days at time of first tumor observation. A possible exception was observed in very old animals. There was an indication that the average residual life-span of tumor-bearing rats whose tumor appeared after 850 days of age was significantly shorter than 140 days (about 60 days at 860 days of age). This reduction in the residual life span of these rats is interpreted as being due to their advanced age at the time of tumor appearance and the fact that death normally intervened before the average residual time of 140 days could occur.

TABLE 2

CLASSIFICATION OF TUMORS FROM NORMAL FEMALE SPRAGUE-DAWLEY RATS

	Mammary tumors				
Group	Adenoma	Adenofibroma	Fibroma	Adenocarcinoma	Others
A		7	7	2	
B	1	39	11	4	One sarcoma of skin One carcinoma of maxillary gland One carcinoma of lung*

* This rat also had an adenocarcinoma of the mammary gland, but because the lung lesion was the primary cause of death, it is classified only as carcinoma of the lung.

In all but one instance, the death patterns were similar. The rats became emaciated and greatly debilitated and appeared enervated by their tumors. At autopsy no specific organ damage, other than the usual lung congestion, was observed that could be ascribed as the direct cause of death. The autopsy findings were indistinguishable from those observed for old nontumor-bearing rats. The one exception was the rat that died as the result of carcinoma of the lung (Table 2). This rat had developed an adenocarcinoma of the mammary gland that spread to the lung, producing the fatal lesion.

Almost all the tumors in all four groups of animals were mammary tumors. Specifically, 95 per cent of all tumors classified were mammary. There was no significant difference in the percentage classified as mammary tumors in the different groups (Table 1, column 7) when the χ^2 test was applied. Despite the difference in total tumor incidence which appeared in the two rat populations examined histopathologically (Groups A and B), there was no significant difference in their respective proportions of benign to malignant tumors when the χ^2 test for a 2×2 contingency table was applied to these data. The above data were obtained by histological examination and classification of about one-half of the 165 tumors observed. Only tumors that were observable by gross examination at autopsy were processed for histological examination. There may have been metastatic tumors or other small tumorous masses in otherwise normal-appearing organs which were overlooked. In Group A, tumors from sixteen of the 26 tumor-bearing rats were examined and classified histologically. All the tumors so examined in Group A were found to involve mammary gland tissue, and two of these were malignant (adenocarcinomas). In Group B all but three of the 57 tumors examined and classified were mammary. The nonmammary tumors were: sarcoma of the skin, carcinoma of the submaxillary salivary gland, and carcinoma of the lung. Only in the carcinoma of the lung was the lesion the apparent primary cause of death.

The most interesting aspect of the tumor classification data is the similar proportion of malignant and benign tumors observed in Groups A and B (Table 1), even though the tumor incidence in the two groups differed sharply. Apparently the high fat diet of Group B affected only the number, not the type of tumor.

Additional tests were made to determine whether Group B differed in other respects from Groups A, C, and D. The first of these tests was to determine whether Groups A, C, and D were a single population for statistical purposes, and they were found to be homogeneous in every case. The body weight patterns of Groups A, C, and D were indistinguishable from one another. Food and water intake in these groups was likewise very similar. Group B was then compared with the others with respect to growth rate (as evidenced by body weight gains), food and water intake, and life span. It was evident that Group B rats when $1\frac{1}{4}$ years of age were 25 per cent heavier than their counterparts in the A, C, and D groups. This was not unexpected, because of their fat-rich special diet. The life spans of all four groups were tested for equality by analysis of variance, and no significant difference could be detected. While Group B had a life span that was shorter than average,

the difference was not great enough to be significant (Table 1). The average of Groups A, C, and D was 765 days, with a range of 193–1100 days. The average life span for Group B was 744 days, with a range of 251–1071 days. The over-all average life span of the four groups of female rats was 760 ± 21 days (95 per cent fiducial interval).

Additional data obtained included some information on tumor sizes and sex susceptibility. In general, the tumors ranged from diameters of 1–1.5 cm. to sizes of 12 × 10 × 4 cm., but the latter size predominated. In regard to sex susceptibility, a comparison of tumor incidence in males and females within Group A rats indicated that the over-all incidence of tumors, regardless of type, was 5 times greater in females than in males. In the group of 40 control male rats available for observation (additional Group A rats), five, or 12.5 per cent, developed tumors. Four of these were studied histologically. There was one case of bronchiogenic carcinoma, one medullary carcinoma of a mammary gland, one carcinoma of a mammary gland, and one fibroma of a mammary gland. While it is recognized that these are too few samples upon which to base a broad generalization, it seems to indicate that the fraction of tumors that are malignant is greater in male rats than in females. These data also point up the fact that, if naturally occurring tumors are to be avoided, the male rat is the animal of choice.

SUMMARY

A tumor incidence of 57 per cent was observed in 150 female rats allowed to live out their life-span as normal, control animals on standard laboratory chow. One hundred similar rats on a special fat-rich diet developed an 80 per cent tumor incidence.

Ninety-five per cent of the tumors observed involved mammary gland tissue. Twelve per cent of all tumors observed were of a malignant type. Eighty-seven per cent of all tumors appeared after the rats were 540 days of age, and the mean life-span after a tumor was first observed was 140 days.

Related studies with a smaller number of animals indicated that male rats of the same strain were 5 times less susceptible to tumor formation than females. However, three of the four tumors that were examined histologically in the males were classified as malignant.

The mean life span of normal female Sprague-Dawley rats in this laboratory was 760 ± 21 days, with individuals ranging in life span from 193 to 1100 days.

ACKNOWLEDGMENTS

The authors wish to acknowledge the excellent histological preparations by Misses G. L. Norris and Marietta Miller, the expert tissue diagnoses of Mr. R. N. Cowden and Dr. R. E. Zipf, and the extensive help given by Dr. D. S. Anthony in preparing and editing this report. Their services are deeply appreciated.

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Cancer Res 1956;16:194-197.