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Spontaneous Tumors and Lifespan of Female NMRI Mice of the Outbred Stock Sut: NMRT During a Lifetime Study

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Summary. A group of 150 female NMRI mice of the outbred stock Sut: NMRT was kept until they died naturally, at which time they were necropsied and examined histologically for spontaneous tumors. The natural life expectancy (median) was 782 days. Life expectancy was markedly reduced by mammary and pulmonary adenocarcinoma, and by tumors of the hypophysis. The spontaneous tumor rate was 58%. That is to say, 87 of the 150 mice had spontaneous tumors: 57 animals each had one tumor, 20 animals each had two tumors, and 10 animals each had three tumors. The organs most commonly affected by tumors were those of the lymphoreticular and haematopoietic systems, followed by the respiratory tract in second place and the breast in third place. Data reported in the literature generally show the same organ distribution, but the total tumor rate given is generally somewhat lower as the animals are seldom left alive until they die naturally (spontaneously).

Key words: Spontaneous tumors – NMRI mice – Life time study – Survival rate

Introduction

It is rare for animal studies to be continued until the last animal in an experiment has died, and untreated animals in parallel control groups are usually sacrificed before they die of natural causes. There are many reasons for this, not least economic considerations. However, particularly for the long-term analysis of toxicological and cancerological studies, it is important to know the natural life expectancy of the animals used, the main diseases and tumors to which male and female animals are prone, and the organs most likely to be affected. Knowledge of the husbandry conditions prevailing during the experiment obviously plays an additional role, and an experimenter planning an animal study must be in possession of these biological data (Tierexperimentelle Forschung und Tierschutz/Deutsche Forschungsgemeinschaft 1981).

Contributions on spontaneous tumors in the literature are concerned mainly with aetiology. More extensive compilations are frequently grouped according to organ and animal species (Madewell 1981), and synoptical tables showing the incidence in individual animal species and strains are rare (e.g., for rats: Nunziata and Storino 1982; for mice: Sher 1974). But even these synopses are only of limited value as far as information on the incidence of spontaneous tumors is concerned, as most of the data presented in this way stem from experiments that were not extended until the natural death of the animals, so that tumors appearing during the last phase of life were not considered. Thus, although Weisse et al. (1975) compiled data on the spontaneous tumors of 2,300 SPF NMRI mice of both sexes, the mice were killed at the age of 730 days, while the natural life expectancy of this strain under standard conditions is well over 800 days. The authors correctly conclude that: "A statement concerning the spontaneous tumor rate in the sense of a thorough biological characterization of the strain used is not possible from this experiment because the animals were not observed until their natural deaths and because not all organs were evaluated histopathologically."

This gap in the literature became obvious to us repeatedly during our own long-term experiments. We therefore maintained 150 female NMRI mice of the outbred stock Sut: NMRT under constant conditions until they died naturally. We report here on the macroscopic findings at necropsy and the results of histopathological evaluation.

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Materials and Methods

1. Animal Strain

We used 150 female mice of an outbred stock, Sut: NMRT, obtained from the NMRI breeding colony of the Süddeutsche Versuchstierfarm, D-7200 Tuttlingen (FRG).

2. Husbandry Conditions at the German Cancer Research Centre

Mice were housed in groups of five in Makrolon type II cages in a closed system under the following climatic conditions: room temperature 22 °C ± 2 °C, relative humidity 40%–70%, excess pressure in animal room compared with outside pressure 0.9–1.5 mbar, 15 air changes per hour, lighting cycle 12 h light/12 h darkness. Parasitological, bacteriological, and virological investigations were performed at the start of the experiment.

An autoclaved soft wood granulate supplied by Altromin, 4973 Lippe, FRG was used as bedding and was changed once weekly.

Pelleted, total pathogen-free (TPF) Altromin standard diet no. 1324 was fed, and sterilized drinking water was provided ad libitum.

3. Observation of the Animals

All animals were checked once daily; any dead mice were necropsied and 25 organs or tissue samples were taken routinely, together with any tissues showing macroscopic changes indicative of disease. These organs and tissue samples were fixed in 10% neutral formalin, and paraffin wax sections obtained from them were stained with haematoxylin and eosin (HE).

4. Documentation

All findings were protocolled on record sheets and, using special programs developed by the Biostatistical Department of the German Cancer Research Centre, entered into a computer and evaluated. Median values and the corresponding confidence limits (5%, or alpha = 0.05) were computed (Dunn 1964; Kruskal and Wallis 1952).

5. Parasitological, Bacteriological, and Virological Investigations

Before starting the experiment, a control group of mice was investigated for the pathogens listed below. All findings were negative:

Endoparasites and ectoparasites
Pasteurella multocida, Pasteurella pseudotuberculosis, Pasteurella pneumotropica
Listeria monocytogenes
Erysipelothrix rhusiopathiae, Bordetella bronchiseptica
Salmonellae
Haemolytic streptococci
Leptospira
Ectromelia virus
Mycoplasmas

We are grateful to Dr Pol of the Government Veterinary Investigation Centre in Aulendorf for performing these investigations.

Results

1. Survival Times

Three mice died during the first year of life, and a total of 50 had died before reaching 2 years of age. The median lifespan was 782 days and the confidence limits (alpha = 0.05) were 750–815 days.

Survival times grouped according to tumor type and organ affected are presented in Fig. 1. The median survival time of tumor-free animals dying spontaneously was 844 days, with confidence limits of 772–893 days. Where tumors did occur, only in the case of mammary and pulmonary adenocarcinoma and tumors of the hypophysis, adrenals and Harderian glands was the survival time markedly shortened. Tumors of the lymphoproliferative system, hepatic adenomas, and uterine haemangiomas had no effect on survival time.

2. Tumor Spectrum

Table 1 shows the tumor spectrum together with the organs and organ systems affected by tumors. Tumors occurred most frequently in the lymphoreticular system, followed by the respiratory organs, mammae, reproductive organs, and endocrine glands. Tumors of the digestive organs were much less common, and very few tumors were seen in the skeletal system and skin. The central and peripheral nervous systems were always tumor-free.

3. Concomitant Diseases

Pneumonia and nephropathy were additional important causes of reduced lifespan, and toxic fatty liver was occasionally observed. As far as the heart and circulatory system was concerned, one case of pericarditis was diagnosed.

Discussion

1. Influence of Spontaneous Tumors on the Life Expectancy of Female Sut: NMRT Mice in a Long-Term Experiment

The purpose of long-term animal studies is generally the observation, in some way, of a side-effect. When planning and conducting such studies, it is important to know to what extent deaths caused by spontaneous tumors might influence the results and their evaluation. This evaluation is facilitated by comparing the findings in test animals with those in parallel control groups. With experimental groups of about 20–30 animals, however, there is always a possibility that differences between groups may lead to false conclusions.

In our study of 150 female mice, life expectancy was considerably reduced in animals with mammary adenocarcinoma (median 500 days), pulmonary adenocarcinoma (median 612 days), and hypophyseal adenoma (median 619 days). Mice with granulosa cell tumors of the ovaries had a life expectancy of 702 days (median).

These figures should be borne in mind when performing long-term studies with this strain of mouse.

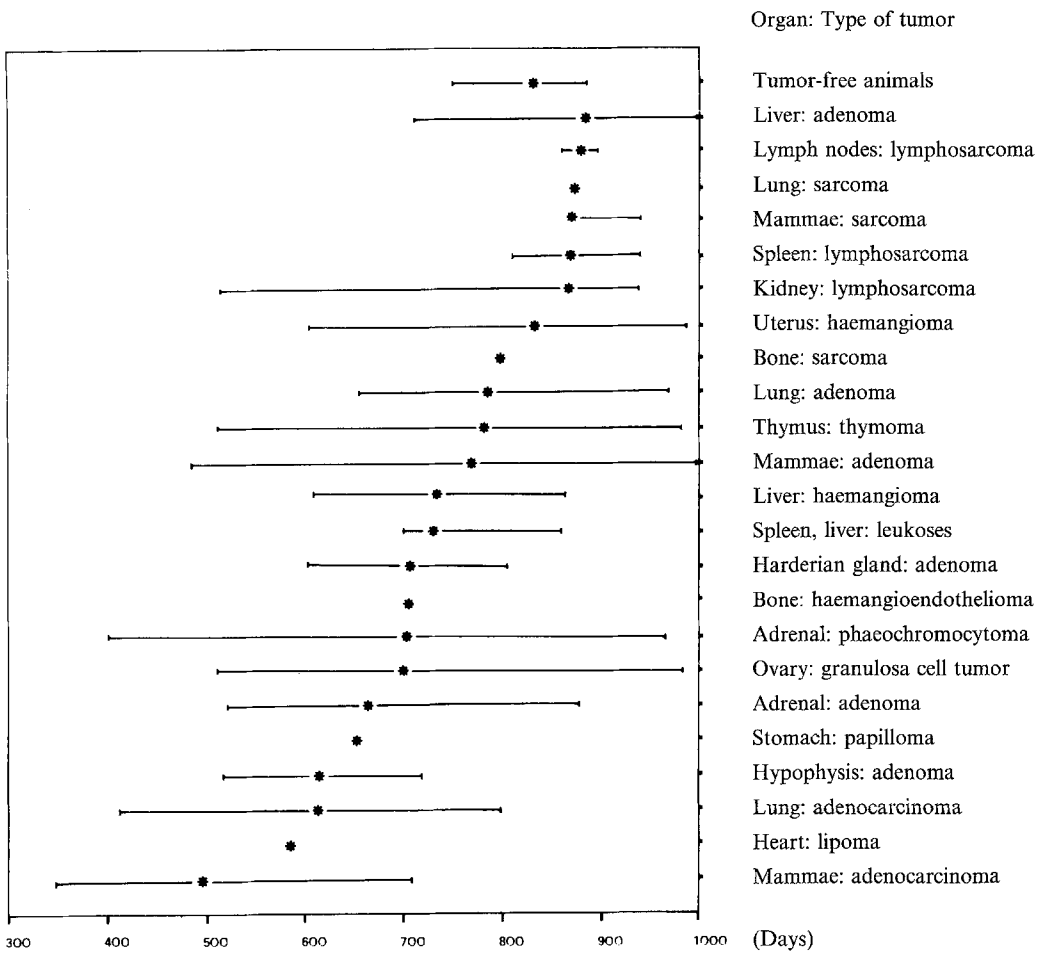

Fig. 1. Survival time of NMRI mice (Stock: Sut: NMRT) [median with confidence intervals ($\alpha = 0.05$)]

2. *Incidence of Spontaneous Tumors and Organs Affected in Female Sut: NMRT Mice*

Whenever any substance is tested for a toxic side-effect or for its capacity to produce tumors, the question of its target organ is raised. In other words, tropisms must be considered and recognized. In addition to the organ which is directly affected, it is quite possible that other organs or systems may become involved. Moreover, as well as direct effects on the genetic substance, substances may also advance the neoplastic process by promoting tumor growth.

We have compared our data on spontaneous tumor formation during a long-term study of 150 female Sut: NMRT mice with data in the literature, particularly those in the tables compiled by Sher (1974). We considered only the findings in animals which survived for longer than 750 days, and compared these data with our own.

In our outbred NMRI stock, Sut: NMRT, we observed a tumor rate of 58%. This figure lies at the upper limit of the spontaneous tumor rates so far reported (cf. Charles River female, between 19.9% and 25.6%; Swiss female between 47.3% and 62%), but we have included the results of histological examination, which is not the case with most other publications. It is therefore probable that the figures quoted by other authors are too low, particularly in view of the fact that few of the studies presented by Sher were extended until natural death. Weisse et al. (1975) investigated a large number of mice (2,300, being 1,150 of each sex) and found a lower incidence of tumors, but the mice were only observed up to an age of 730 days.

It is important to group spontaneous tumors according to the organs affected. Again, we consulted the tables of Sher (1975), compared the organs most frequently affected, and listed them according to tumor incidence. With our own results, we thus had usable data for a total of 13 strains; tumors of the haematopoietic and lymphoreticular systems topped the list in 10 of these, tumors of the lungs being most

Table 1. Tumor spectrum in 87 of 150 female Sut: NMRT mice (outbred stock) observed until natural death[a]

Rank	Organ system/organ	Tumor classification/histopathology	Number of tumors observed	% of all mice/ % of all mice with tumors
1.	Lymphoreticular and haemopoietic system		*41*	*27.3/47.1*
	Blood formation	Leukoses	25	16.7/28.7
	Lymph nodes	Lymphosarcoma	5	
	Thymus	Thymoma	10	
	Spleen	Lymphosarcoma	1	
2.	Respiratory tract		*31*	*20.7/34.5*
	Lungs	Adenoma	21	14.0/24.1
		Adenocarcinoma	9	6.0/10.3
		Sarcoma	1	0.7/ 1.1
3.	Mammary glands		*19*	*11.4/21.8*
		Adenoma	6	4.0/ 6.9
		Adenocarcinoma	10	6.7/11.5
		Sarcoma	3	0.7/ 1.1
4.	Genital tract		*14*	*9.4/16.1*
	Ovaries	Granulosa cell tumors	10	6.7/11.5
		(Cysts)	(7)	
	Uterus	Haemangioma	4	2.7/ 4.6
		(gland. cyst. hyperplasia of endometrium)	(7)	
	Vagina	(epithel. dysplasia)	(5)	
5.	Endocrine glands		*12*	*8.0/13.8*
	Adrenal gland	Adenoma	4	2.7/ 4.6
		Phaeochromocytoma	3	2.0/ 3.4
	Hypophysis	Adenoma	3	2.0/ 3.4
		(hyperplasia of anterior lobe)	(4)	
	Harderian glands	Adenoma	2	1.3/ 2.3
6.	Digestive tract		*6*	*4.0/ 6.9*
	Stomach	Fibroepithelioma	1	0.7/ 1.1
	Liver	Adenoma	3	2.0/ 3.4
		Haemangioma	2	1.3/ 2.3
7.	Sustentaculum		*2*	*1.3/ 2.3*
		Haemangioendothelioma	1	0.7/ 1.1
		Sarcoma	1	0.7/ 1.1
8.	Heart/blood vessels		*1*	*0.7/ 1.1*
	Heart	Myocard. lipoma	1	0.7/ 1.1
9.	Integument		*1*	*0.7/ 1.1*
		Sarcoma	1	0.7/ 1.1

[a] Numbers of preneoplasms are shown in parentheses

frequent in 4 (identical incidence in 1 strain). Second place was occupied by lung tumors in 7 cases, by mammary tumors in 3, by tumors of the lymphoreticular system in 1, and by tumors of the haematopoietic system in 2 cases. In third place, mammary tumors appeared 3 times, and tumors of the lungs, lymphoproliferative and haematopoietic systems once each. Our findings for tumor incidence thus correspond exactly, for the three most commonly affected organs, to the average of results appearing in the literature. The particular incidence of skin tumors reported by Weisse et al. (1975) seems to be valid only for this one special strain.

In this report we have presented exact data on the spontaneous tumors, including organ distribution, of female NMRI mice of the stock Sut: NMRT that we use in our experimental work. It is hoped that similar data will be published for other species and strains

widely used for long-term studies. This would greatly facilitate comparisons of different experiments and the results obtained in them.

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