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GENETIC AND ENVIRONMENTAL INFLUENCES ON LIFESPAN AND DISEASES IN HAN:WISTAR RATS*

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SUMMARY

In a longevity study with SPF rats of the Han:Wistar outbred stock, 320 virgin males and 320 virgin females of marked littermates were maintained in a barrier-type animal house under highly standardized conditions from weaning until their natural end of life. Diseased and dead animals were sectioned and examined using histological, bacteriological and virological methods.

The mortality of the rats is low up to the 18th month of life. Thereafter the mortality graph inclines steadily. The course of the graph is determined by very few diseases only, such as pituitary adenomas in both sexes, adenocarcinomas of the uterine glands in the females, and in the third and fourth year nephropathies in the males. Because of the high number of adenocarcinomas of the uterine glands the median life expectancy is between 30 and 33 months for the females, somewhat lower than between the 33rd and the 36th months for the males.

The disease spectrum consists generally of tumor lesions, but out of the large tumor spectrum only single alterations exceed the 5% border. As shown by analysis of variance and estimation of the heritability coefficients (h^2), mortality and the most important tumorous lesions underlie high genetic effects. The cage environments are found to have no influence upon mortality and diseases.

Under the given standardized environmental conditions, the present results can be looked upon – because of the strong genetic effects on mortality and diseases – to be representative for correspondingly selected populations of future generations of the Han: Wistar stock.

INTRODUCTION

Since 1974 we have been engaged on a longevity study to determine the mortality and age-related diseases in rats of the Han:Wistar outbred stock [1]. In this paper a report

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is given on the most important and characteristic diseases in these rats, and the genetic background as well as the variations between single cages influencing the development of these alterations.

MATERIAL AND METHODS

In this experiment we studied 1200 male and 1200 female rats of 15 different age groups, each group containing 80 males and 80 females, taken from an SPF colony started in our institute more than 10 years ago with caesarian-derived animals and bred by a mating scheme for rigorous outbreeding [2]. The breeding colony is controlled routinely and is found to be free of endo- and ectoparasites, protozoa, *Salmonella* and other important pathogenic bacteria, mycoplasma, fungi and eight of the most important murine virus species (Reo virus 3, Sendai virus, pneumonia virus of mice (PVM), LCM virus, Theiler virus, adeno virus, Kilham virus, Toolan's H-1 virus) [3]. From this colony different sibs were selected to be kept from weaning until their natural death in barrier type animal quarters under conditions of 22 ± 1 °C room temperature, 55 ± 5% relative humidity, +15 mm H_2O hyperbaric pressure in relation to external pressure, 12:12 hours light–dark sequence, light intensity of about 300 lux and an air change 20 times per hour. None of the animals were mated. Males and females were maintained separately in groups of five in Macrolon-cages type No. IV (base area 1750 cm^2) on autoclaved softwood granules. Cages and bedding were changed once a week. The rats were marked according to their parentage and assigned to different cages, so that each cage contained animals from five different litters. Dead animals were not replaced by others. All rats were fed an autoclaved (120 °C for 5 min) commercial cereal-based diet, supplemented with vitamins and minerals, with a metabolizable energy content of 10258 J/g, 17.7% crude protein, 4.2% crude fat and 6.8% crude fiber [4]. Acidified (pH 2.5) and pasteurized (95 °C for 15 sec) tap water was always available. All rats were controlled twice daily. Diseased animals which were expected to die within the next hours and all dead animals were dissected. Brain, pituitary gland, salivary glands, thyroid, trachea, lung, heart, aorta, cardia and fundic stomach, duodenum, ileum, cecum, colon, rectum, mesenteric lymph node, liver, spleen, kidneys, adrenal glands, pancreas, bladder, testes, epididymis, seminal vesicles, coagulating glands, prostate glands, ovaries, uterus, vagina, mammary gland, skeletal muscle, as well as all other macroscopically altered organs and tissues were prepared for histological diagnosis. Tissue sections (4 μm) were stained routinely with haematoxylin–eosin. Whenever necessary for diagnostic purposes, special stains were used or bacteriological and virological examinations were undertaken.

The identification of the animals and all pathological findings were registered and evaluated by processing data. Genetic effects and effects of cages on lifespan were calculated by analysis of variance. The heritability coefficient (h^2) was estimated by a method described by Le Roy [5].

The results of this study were based on four age groups of 320 male and 320 female rats. From these, 306 males and 304 females could be considered for histological examination; 14 males and 16 females were rejected because of autolysis and cannibalism.

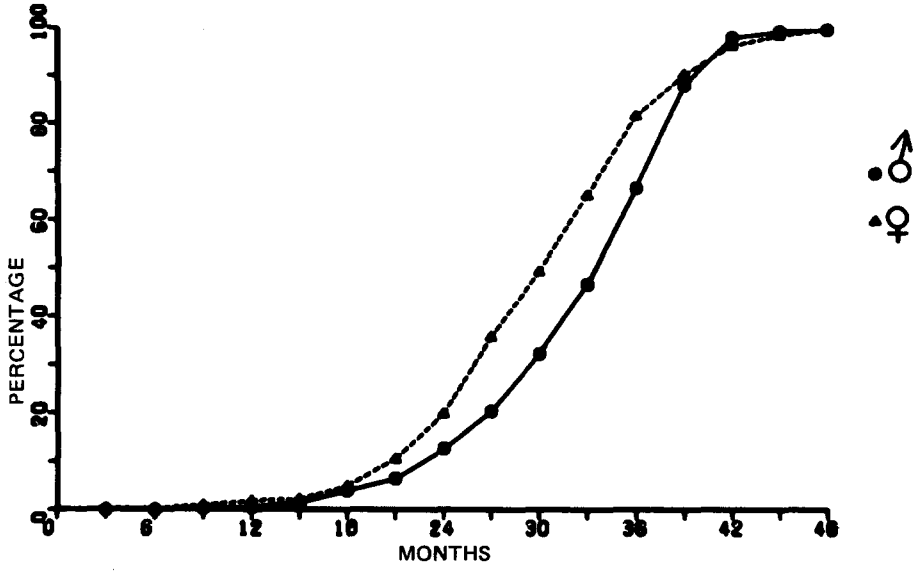

Fig. 1. Cumulative death rate in male and female Han:Wistar rats.

RESULTS AND DISCUSSION

In Fig. 1 the cumulative death rate is shown up to the 48th month, by which time all animals had died of natural causes. In the first year only a small number of animals died, the death rate being 0.6% for the male rats and 1.9% for the females. Even at the end of the second year the mortality rate remains under 20% in both sexes. The median lifespan of the male rats lies between 33 and 36 months: in the females it is somewhat lower, between 30 and 33 months. The mortality rate of the Han:Wistar rats during the first and second year of life is relatively low compared to the findings in the literature; even the median life expectancy is somewhat greater than in most other cases [6–9]. However, the striking difference in the mortality rate between the sexes is noticeable. The greatest difference, approximately 18%, occurs at the end of the 33rd month.

The quarterly death rate (Fig. 2) shows that the peak of the mortality in the females lies in the second half of the third year; in the males this occurs later, in the beginning of the fourth year. The difference in the mortality rate between the sexes is generally caused by the higher death rate of the females between the 18th and 33rd months of age. This is exactly the period in which a high number of adenocarcinomas of the uterine glands are observed. These tumors are indeed responsible for the divergence of the mortality rates between the sexes.

In Tables I and II the genetic effects and influences upon the variance of lifespan of males and females in different cages are presented as analysis of variance. The influence of variation from different cages is only a part of the whole environmental variance. Cage influences are determined by social behaviour of the animals' group size, micro-

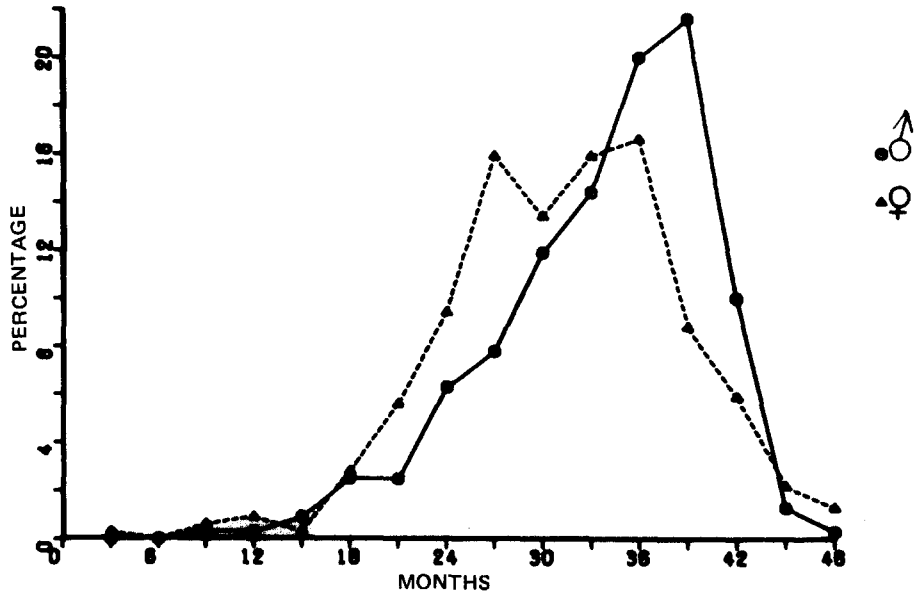

Fig. 2. Quarterly mortality in male and female Han:Wistar rats.

TABLE I

GENETIC EFFECTS ON LIFE SPAN

Source of variation	*SS*	*d.f.*	*MS*	*F*
Age of males in months				
Between brothers	12294.24	192	64.032	1.484*
Within brothers	24154.33	560	43.132	
Total	36448.58	752		
Age of females in months				
Between sisters	14089.67	201	70.097	1.400*
Within sisters	28421.49	568	50.037	
Total	42511.17	769		

SS = sum of squares; d.f. = degrees of freedom; MS = mean square.

*Significant at the 1% level.

climate, microflora, *etc.* The low F value shows that the variances between the cages are not greater than within the cages. On the other hand, highly significant differences exist between the groups of brothers and sisters, demonstrating a comparatively great genetic influence on lifespan. The heritability coefficient reaches 51% in the males and 36% in the females. In domestic animals the degree of heritability regarding their life expectancy seldom lies above 10%.

TABLE II

EFFECTS OF CAGES ON LIFESPAN

Source of variation	*SS*	*d.f.*	*MS*	*F*
Age of the males in months				
Between cages	3560.11	91	39.122	0.822
Within cages	17510.00	368	47.581	
Total	21070.12	459		
Age of the females in months				
Between cages	5921.61	107	55.342	1.087
Within cages	21878.70	430	50.880	
Total	27800.31	537		

Abbreviations as in Table I.

TABLE III

FREQUENCY OF TUMORS

No. of tumors	*Males (n = 306)*	*Females (n = 304)*
1 or more	97.4	98.7
2 or more	72.5	70.1
3 or more	37.9	37.8
4 or more	16.0	18.8
5 or more	3.9	5.9

Values are expressed as percentages.

In an earlier paper [1] we gave a report on the high percentage of tumors causing death in the rats of this experiment. Table III gives a general view of the total numbers of tumorous lesions in these rats: 97.4% of the males and 98.7% of the females showed at least one tumor, more than 70% of both sexes showed at least two tumorous alterations and 3.9% of the male and 5.9% of the female rats harboured five or more tumors in different tissues at the end of their natural life. Metastases have not been taken into account.

Out of a large spectrum of tumors only a small group exceeded the 5% level. From these we have considered the following processes for the estimation of the heritability coefficients (Figs. 3 and 4).

Adenomas and adenocarcinomas of the anterior lobe of the pituitary gland were observed in both males and females. In 34.9% of the males and 60.3% of the females this common alteration of rats was to be seen by macroscopic and histological examination. In most cases it was responsible for the death of the tumor-bearing animal.

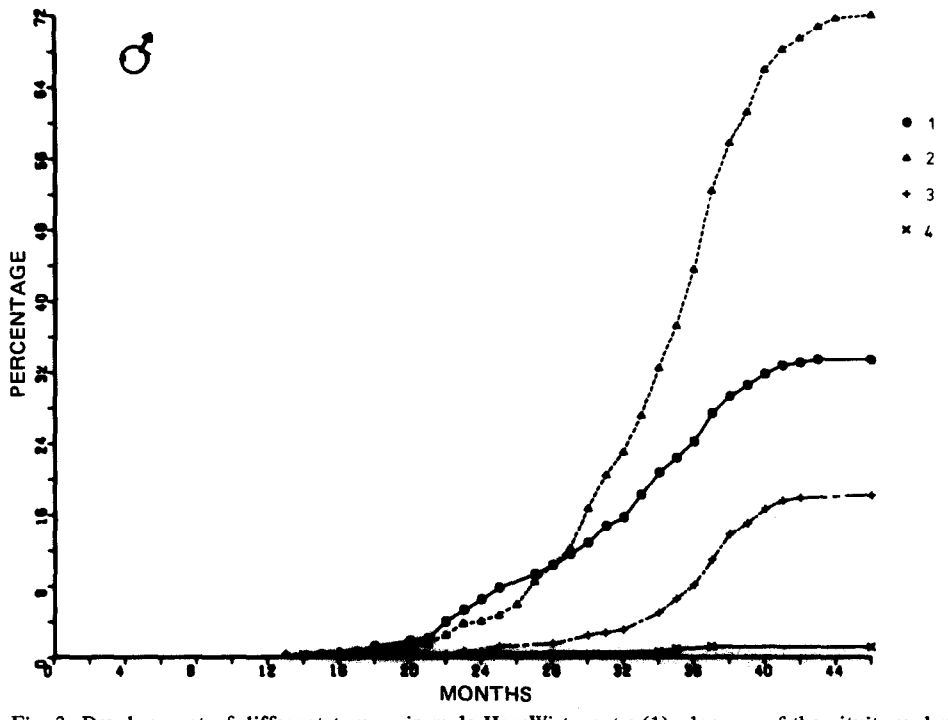

Fig. 3. Development of different tumors in male Han:Wistar rats: (1) adenoma of the pituitary gland; (2) lymphangioma of the mesenteric lymph node; (3) Leydig cell tumor; (4) thymoma.

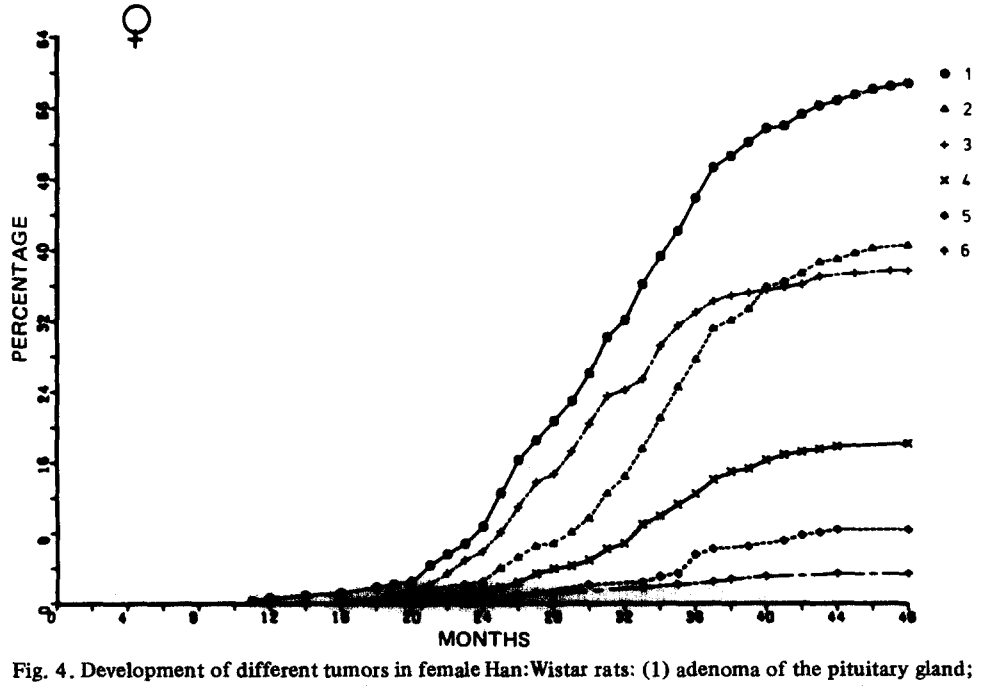

Fig. 4. Development of different tumors in female Han:Wistar rats: (1) adenoma of the pituitary gland; (2) lymphangioma of the mesenteric lymph node; (3) adenocarcinoma of the uterus; (4) fibroadenoma of the mammary gland; (5) granulosa cell tumor; (6) thymoma.

Lymphangioma of the mesenteric lymph nodes (Figs. 5 and 6) was the most frequent tumor, occurring in 73.4% of the male rats. In the females it could be observed in 43.3% of the animals. But only in a few cases did extreme growth and bleeding from this essentially hemorrhagic tumor prove fatal.

Besides the pituitary gland adenomas, malignant adenocarcinomas of the uterus (Figs. 7 and 8) mainly influenced the course of the mortality graph, as mentioned above. This generally metastasizing tumor could be seen in 39.0% of the females. Adenocarcinomas of the uterine glands are typical processes of the non-breeding rats. In a corresponding longevity study with retired breeders of the Han:Wistar stock, we have not yet observed this tumor at all [10].

Adenocarcinomas of the uterus in such a high percentage as well as the lymphangiomas of the mesenteric lymph nodes have not yet been observed in other strains and stocks of rats. These lesions seem to be a special problem in Han:Wistar rats of our breeding colony.

All other tumors seen in Figs. 3 and 4 used for estimating genetic effects and environmental influences are alterations also common in other rate colonies: fibroadenomas of the mammary glands were observed in 19.7% of the females, granulosa cell tumors of the ovary in 8.8% of the females, and Leydig cell tumors of the testes in 18.4% of the third and fourth years of life. Besides these, we have considered another tumor which was observed only in a small percentage of the animals: thymomas were diagnosed in 1.0% of the males and 3.6% of the females.

The high genetic influence on lifespan has already been mentioned before. As seen in Table IV, there are also marked genetic effects on the pituitary gland tumors, the granulosa cell tumors and thymomas. Even relatively high heritability coefficients have been estimated for the adenocarcinomas of the uterine glands, the Leydig cell tumors and the lymphangiomas of the mesenteric lymph nodes. In domestic animals comparable values reach approximately 5%. No genetic effect has been evaluated for the mammary gland fibroadenomas. There are no cage influences either on the lifespan or on any of the tumors mentioned. The environmentally produced rest variance is caused by coincidental effects of the biological, physical and chemical environment, which, however, are not part of the cage effects.

CONCLUSIONS

Under favourable maintenance conditions the spontaneous mortality rate in Han: Wistar outbred populations is very low until the 18th month of life. Thereafter, the mortality graph inclines steadily. The course of the mortality graph is determined by very few diseases only, such as pituitary tumors in both sexes, uterine gland adenocarcinomas in the females and, after the 24th month, nephropathies in the males, as shown in an earlier paper [11]. Because of the high number of adenocarcinomas of the uterine glands and the higher number of pituitary gland adenomas, the median life expectancy is between

Fig. 5. Endometrial carcinoma with widespread intracoelomic metastases.

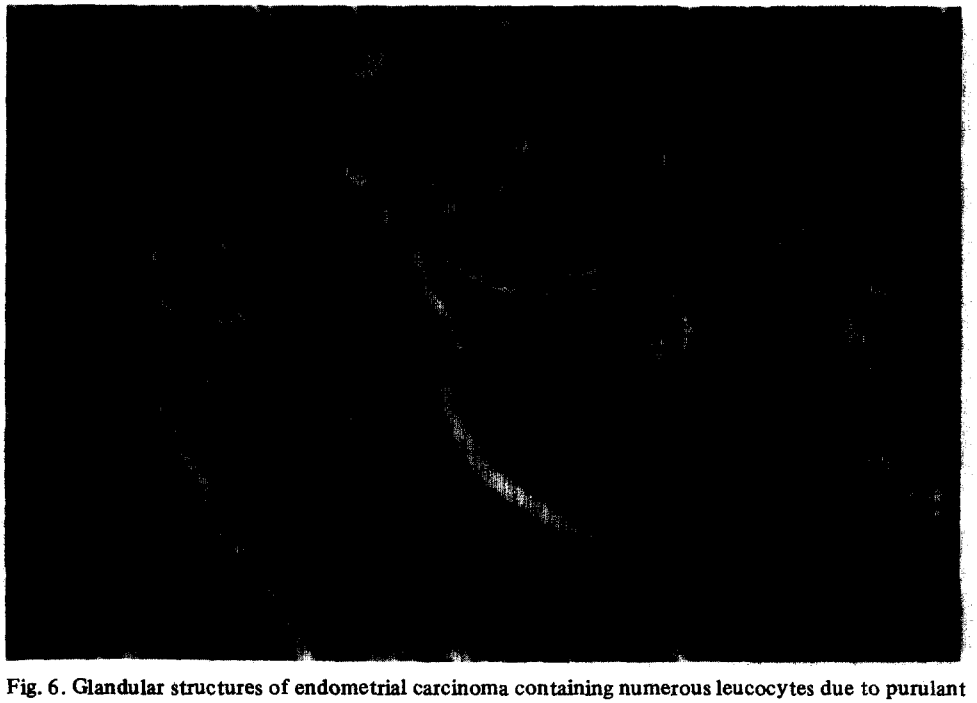

Fig. 6. Glandular structures of endometrial carcinoma containing numerous leucocytes due to purulant endometritis generally observed in connection with the tumor growth.

Fig. 7. Blood-filled lymphangioma of the mesenteric lymph node.

Fig. 8. Common histological structure of lymphangioma of the mesenteric lymph node.

TABLE IV

PERCENTAGE OF GENETIC EFFECTS (h^2) AND CAGE EFFECTS (c) OF THE TOTAL VARIANCE

	Males		*Females*	
	h^2	c	h^2	c
Age at death in months	51	0	36	0
Adenomas and adenocarcinomas of the pituitary gland	61	0	37	0
Lymphangiomas of the mesenteric lymph node	20	0	23	0
Carcinomas of the uterine glands	–	–	20	0
Fibroadenomas of the mammary gland	–	–	0	0
Leydig cell tumors	17	0	–	–
Granulosa cell tumors	–	–	36	0
Thymomas	42	0	40	3

the 30th and the 33rd month in the females, somewhat lower than between the 33rd and the 36th month for the males.

A variety of neoplasmas dominate in the range of spontaneous diseases, but only very few develop in more than 5% of the animals. The estimation of genetic influences and single cage effects on the mortality rate and different diseases was made possible by the choice of an outbred stock, the examination of brother and sister groups and the distribution of these groups among different cages. The cage environments calculated as single cage effects are found to have no influence on the mortality rate or the development of diseases, *i.e.* these are spread equally among all the animal groups examined. On the other hand, high genetic influences determine mortality rate and particular diseases.

Under the given standardized environmental conditions, the present results can be looked upon – because of the strong genetic effect on mortality and diseases – as representative for correspondingly selected populations of future generations of the Han:Wistar stock.

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