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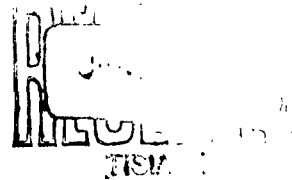
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LIFESPAN MEASUREMENTS IN THE MALE RAT

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by
D. C. Jones
D. J. Kimeldorf



409550

U.S. NAVAL RADIOLOGICAL
DEFENSE LABORATORY
SAN FRANCISCO 24, CALIFORNIA

PHYSIOLOGY-PSYCHOLOGY BRANCH
D. J. Kimeldorf, Head

BIOLOGICAL AND MEDICAL SCIENCES DIVISION
E. L. Alpen, Head

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Eugene P. Cooper
Eugene P. Cooper
Scientific Director

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E. B. Roth
E. B. Roth, CAPT USN
Commanding Officer and Director

ABSTRACT

Measures of lifespan were determined for a population of male Sprague-Dawley derived male rats, comprised of 747 animals from eighteen experiments. Variations in lifespan measures among experiments were found even under stable environmental conditions in a single strain of rats with no known epidemic infections. Measures of central tendency and dispersion appeared to be uncorrelated with each other, and normally distributed among experiments. Within most experiments there was a definite tendency for an excess (above the normal distribution) of shorter lifespans, and in seven experiments this resulted in significant deviations from the normal distribution. On a composite basis, the frequency distribution of lifespans, and the associated survival curve, were not those of a normally distributed variate. Consideration of life expectancies at various ages and age specific death rates revealed that the "force of mortality" declines at advanced ages. These findings indicate the need for caution in selecting statistical procedures for analysis of lifespan information.

NON-TECHNICAL SUMMARY

The Problem

Although the rat has long been used as an experimental animal, there have been essentially no detailed reports of lifespan measures based upon the observation of large numbers of animals maintained throughout life in a stable environment under epidemic-free conditions. In the course of studies concerning the persistent and late effects of radiation upon the male rat, such data has become available from the associated nonirradiated control animals and is described in this report.

The Findings

Among a series of eighteen separate experiments comprising a total of 747 nonirradiated control rats, median lifespans were found to range from 629 to 807 days, with a pooled value of 707 days. Among experiments the slope and position of the central portion of the survival curve along the age axis appeared to vary according to the normal symmetrical statistical distribution, and to be uncorrelated with each other. When the distribution of lifespans within an experiment differs significantly from the normal, the departure results from an excess of shorter lifespans. Whether the data are grouped by experiment or by individual, resulting survival curves still show the excess (above the normal) of shorter lifespans. These findings indicate that some degree of departure from the normal distribution may be the rule rather than the exception for the lifespan of the male rat in a single strain

of animals maintained under stable environmental conditions with no epidemic infections. Further, these findings indicate the need for caution in selecting statistical procedures based upon normal distribution of events for analysis of lifespan information.

INTRODUCTION

In the course of studies concerning the persistent and late effects of ionizing radiation upon the male rat, data relating to the lifespan of a large number of non-irradiated control animals have become available. Based on eighteen separate experiments, extending over a period of some five years, information has been derived pertinent to (1) measures of lifespan for a large population of male rats, (2) variability in these measures with replication, and (3) comparison of measures from specific pathogen-free stock with those from a conventional breeding colony.

METHODS

Animals were of the Sprague-Dawley strain, pen-bred at this laboratory. Within each experiment, animals were born during the same week and were assigned the last day of that week as a common birthdate. Animals of the first seven experiments were from an epidemic-free breeding colony maintained under scrupulously clean but conventional conditions. Those of the last eleven experiments were from a specific pathogen-free breeding colony maintained behind an autoclave-airlock barrier, in which there is continuing assay for pathogenic organisms with negative findings. The original stock was obtained by aseptic caesarian delivery and nursed by germ-free females from the LOBUND project, University of Notre Dame. Litters of from 3 to 12 animals were received at about 25 days of age, and each animal was immediately

placed in an individual suspended mesh cage measuring 7 x 7 x 9 1/2 inches. Animals of a single litter were always in adjacent cages. Average room temperature was 72°F with a range from about 68° to 76°. Relative humidity averaged 40% with a range from about 30 to 60%. Room air turnover was about 10 changes per hour. Rooms were continuously lighted with an average value of 50 foot-candles at the level of the middle cages on the racks. A semi-isolation technique, which involved specific gowns for all personnel entering each room, the routine use of an organic iodine disinfectant solution for hands and shoes, and strict attention to sanitary maintenance, was successful in preventing the occurrence of any epidemic infections during the course of the study. Animals remained in this environment throughout life except for temporary removal for experimental use in one of the programs to be described below. Meal food (Purina Lab Chow) and water were supplied ad libitum. In all cases, age at death was accurate within 3 days. Where death was thought to be precipitated by accident or experimental procedure, the animal was rejected from the study.

In some experiments the animals were subjected intermittently to various experimental procedures. The types of treatment were as follows:

None. No treatment, continuous residence in home cage.

Negligible. Continuous residence in home cage with intermittent minimal treatments, which included clinical examinations, food

and water consumption measurements, and body weight determinations.

Type A. Psychological tests such as maze learning and lever-pressing behavior, which involved temporary (overnight) food and water deprivation.

Type B. Scheduled home cage food and water consumption measurements, body weight determinations, and residence for a two week period in wheel cages for the measurement of volitional activity.

Type C. Physical fitness tests with the unanesthetized animal in an inactive state. These measurements included heart rate, respiratory frequency, colonic temperature, oxygen consumption, and blood pressure (indirect). In some cases blood samples were withdrawn under light anesthesia.

Type D. Physical fitness tests under conditions of external drive. These measurements included one hour of treadmill exercise at moderate intensity, and two hours residence at a reduced environmental temperature (-10°C) or pressure (15,000 feet altitude equivalent). During these tests heart rate, colonic temperature, and respiratory frequency were recorded in non-anesthetized animals.

The distribution of these treatments among the experiments is shown in Table I. In the case of experiments 1 thru 5, there appeared to be some tendency for decreased longevity in those groups subjected to Type D treatment; however, no consistent differences in distribution

TABLE I. Distribution of animals and treatments

Experi- ment No.	Initial No. Rats	Test Interval	Maximum No. Tests	Age (days) First Test	Type of Treatment
1 ^a	51	6 months	5	83	None, or A, or B, or C and D
2 ^a	50	6 months	5	83	None, or A, or B, or C and D
3 ^a	55	6 months	5	83	None, or A, or B, or C and D
4 ^a	51	6 months	5	83	None, or A, or B, or C and D
5 ^a	49	6 months	5	83	None, or A, or B, or C and D
6	20	-----	1	80	D
7	17	-----	1	80	D
8	45	-----	-	--	None
9	28	-----	-	--	Negligible
10	30	1 month	2	80	C
11	29	9 weeks	7	460	C
12	26	3 months	3	435	D
13	60	-----	-	--	None
14	56	-----	-	--	None
15	44	-----	-	--	Negligible
16	36	-----	-	--	Negligible
17	47	-----	-	--	Negligible
18	53	-----	-	--	Negligible

^aAnimals in these experiments were divided into four sub-groups, each receiving one of four treatments--see text for description of tests.

of individual lifespans among the four treatment groups within each of the five experiments were observed. Further, when the four groups were pooled within each experiment, measures of central tendency and dispersion were comparable to those for other experiments of this study in which animals received more uniform treatment.

RESULTS

Some standard parameters of lifespan for the eighteen individual experiments are shown in Table II. Among the experiments, the mean lifespan averaged 10 days less than median lifespan, with a range from 38 days less to seven days more than the median. Mean lifespan is, of course, highly correlated with median lifespan (Pearson $r = 0.94$, $p < 0.01$). It appears that there is a definite tendency for the mean lifespan to be less than the median since this occurred in thirteen of the eighteen experiments. This, in turn, suggests the possibility of skewness in the distributions of lifespans within the individual experiments (see below).

Neither measure of dispersion, the standard deviation and the $Q_3 - Q_1$ interval, was found to be significantly correlated with its central value. The Pearson r for $Q_3 - Q_1$ interval vs median lifespan was -0.115 , and for SD vs mean lifespan it was -0.194 . Examination of the survival curves for each of the eighteen experiments (not shown) revealed that, in the interval between Q_3 and Q_1 values, the points could be reasonably fitted by a straight line. Therefore, in addition

TABLE II. Numeric parameters of lifespan

Experiment No.	Birth Date	Number Animals	Parameter (Days)						
			Median Lifespan	Q_3	Q_1	$Q_3 - Q_1$ Interval	Mean Lifespan	SD	SE
1	10/19/57	51	703	657	782	125	707	103	14
2	11/9/57	50	716	646	812	166	713	130	18
3	11/30/57	55	696	604	771	167	696	119	16
4	12/21/57	51	699	594	786	182	695	122	17
5	1/11/58	49	698	579	781	202	675	153	22
6	5/31/58	20	636	579	729	170	634	143	32
7	6/21/58	17	629	524	692	168	626	116	28
8	7/26/58	45	744	638	832	194	721	139	21
9	10/4/58	28	665	609	722	113	665	108	20
10	10/11/58	30	717	613	782	169	707	126	23
11	11/1/58	29	715	594	773	179	677	160	30
12	11/8/58	26	756	660	831	171	732	114	22
13	12/27/58	60	690	580	774	194	672	160	21
14	1/31/59	56	689	612	776	164	675	154	21
15	6/27/59	44	669	589	790	201	676	139	21
16	12/12/59	36	807	730	854	124	779	126	21
17	12/26/59	47	697	572	828	236	688	197	29
18	1/9/60	53	724	610	815	205	724	132	18
Combined Experiments		(18)	699	611	785	174	692	136	22
Combined Individuals		747	707	611	790	179	696	141	5

to its use as a measure of dispersion about the median, the $Q_3 - Q_1$ interval was used as an index of slope of the survival curve during the period of mortality of the middle half of the animals.

The disparity from a normal distribution of lifespans (see below) was tested by computing the g_1 and g_2 values (Snedecor, 1948) for each experiment. These are shown in Figure 1. Significant skewness ($p < 0.05$) was found in two experiments (Nos. 5 & 6) and both skewness and kurtosis were significant ($p < 0.05$) in five experiments (Nos. 8, 12, 13, 14 & 16). In addition, there was a significant correlation between skewness and kurtosis (Spearman $r = 0.79$, $p < 0.01$) among the eighteen experiments. Skewness was not correlated with mean lifespan (Spearman $r = 0.073$, $p > 0.05$).

A comparison of lifespans among litters was made in three experiments where sufficient animals within litters were available for statistical testing. Kruskal-Wallis one way analysis of variance revealed that in Experiments 8 (45 animals, eleven litters) and 14 (56 animals, eleven litters) there were significant ($p < 0.05$) differences in mean lifespan among litters. In Experiment 13 (60 animals, twelve litters) the p value was between 0.10 and 0.05.

In order to compare measures of the lifespan for the eighteen experiments with respect to order of birth, the medians and those time points which include 70 and 90 percent of the animals are illustrated in Figure 2. The horizontal axis is divided in order to group

those experiments where there were relatively short intervals between successive birthdates (less than four weeks - Table II). While there is obviously some variability from experiment to experiment in all three of these measures, and in symmetry of the 70 and 90 percent ranges about the median, no consistent trends with date of birth are apparent over a period of observations of about five years. Also, there appears to be no discernible relationship between these measures of lifespan and season of birth.

Examination of Table II and Figs. 1 and 2 also reveals no appreciable difference between the groups from the conventional breeding colony (Expts. 1-7) and those from the specific pathogen-free colony (Expts. 8-18). Analysis of variance revealed no significant difference ($p > 0.25$) between the lifespans of the animals from the two breeding colonies.

In order to establish a measure of variability for the overall survival curve of the eighteen experiments, the ages at various percentage survivals for each of the experiments were determined. Since the distributions of the survival times for 100, 50 and 0% among the experiments did not deviate significantly ($p > 0.05$) from the normal, a parametric measure of variability was considered to be appropriate. The survival curve so constructed is shown in Figure 3. Ninety-five percent confidence intervals for each of the points and for the slope ($Q_3 - Q_1$ interval) were established by multiplying the standard devia-

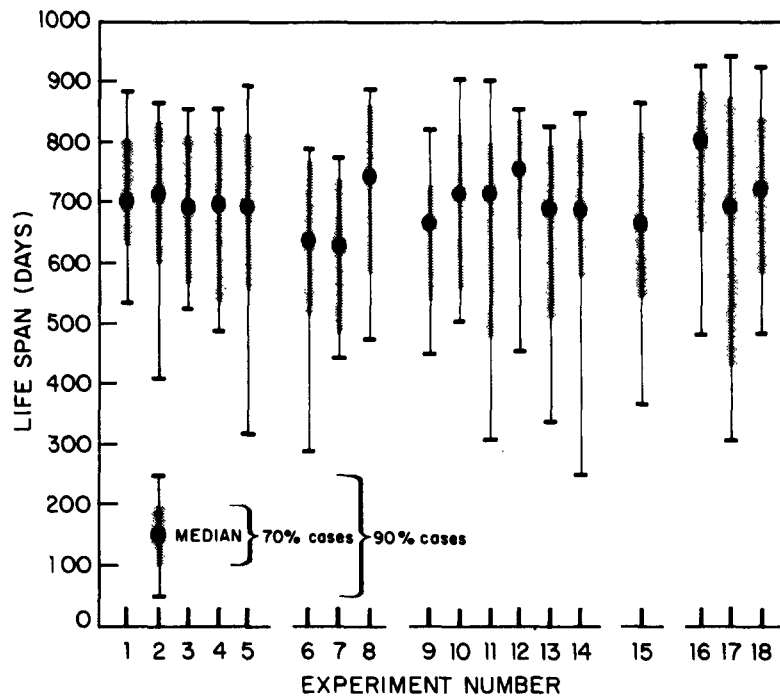


Fig. 2 Median lifespans for groups of male rats. Points including 70 and 90% of the animals are indicated.

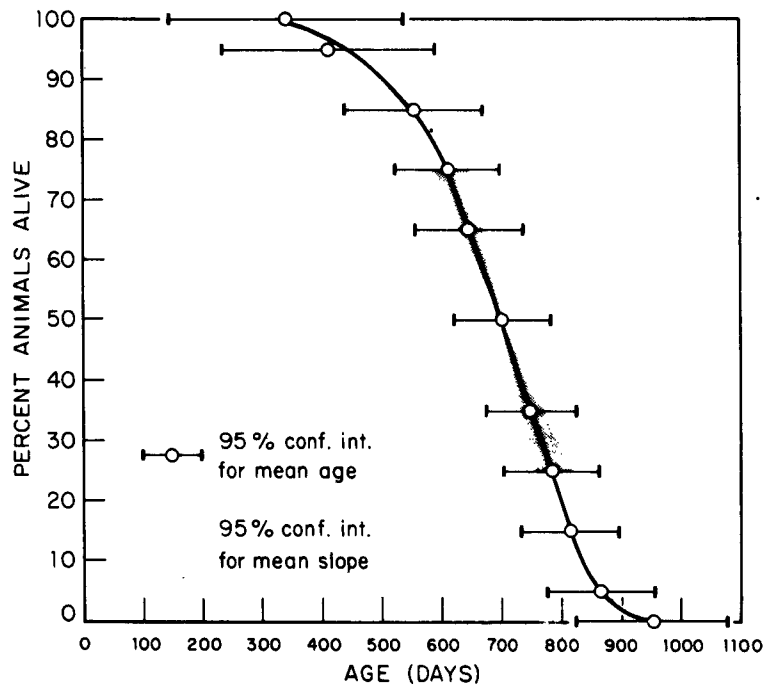


Fig. 3 Composite survival curve for male rats, with 95% confidence limits for each point and for slope, based upon grouping by experiment. (No. of experiments = 18, total number of animals = 747).

tion by 1.96. It should be noted that these confidence limits apply to each of the points and the slope individually, and not to the curve as a whole.

Another, perhaps more usual, method of establishing the overall survival curve is to pool all 747 animals and treat them as one homogeneous group. Before treating the data in this way the feasibility of considering animals of these eighteen experiments as one population was evaluated by several methods. There was no significant heterogeneity in mean or median lifespans among the eighteen experiments (Kolmogorov-Smirnov One Sample Test, $p > 0.02$). With a multiple range test (Harter, 1960), which takes into account the within-experiment variation, it was found that the animals from one experiment (No. 16) had a chance probability of less than 0.05 of being from the same statistical population as the rest of the animals. However, neither the standard deviation nor the Q_3-Q_1 interval for this experiment were atypical. On the basis of this evaluation animals from all eighteen experiments were pooled and treated subsequently as one group, since in this manner a more conservative estimate of variability is obtained.

The frequency distribution of deaths for the pooled group of 747 animals is shown in Figure 4, together with a normal curve of similar variability. Note that there is a significant negative skewness and positive kurtosis. The corresponding survival curve is shown in Figure 5, where the goodness of fit of the straight line from 75 to

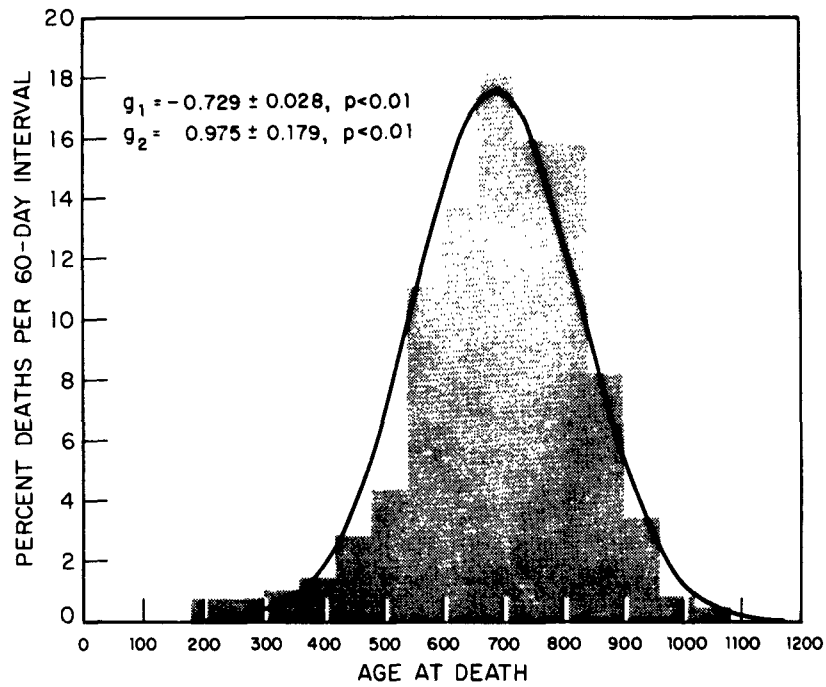


Fig. 4 Frequency distribution of deaths for a single composite group of 747 male rats. Measures of skewness (g_1) and kurtosis (g_2) with their standard deviations and probabilities of departure from the normal curve are indicated, together with a normal curve of similar variability.

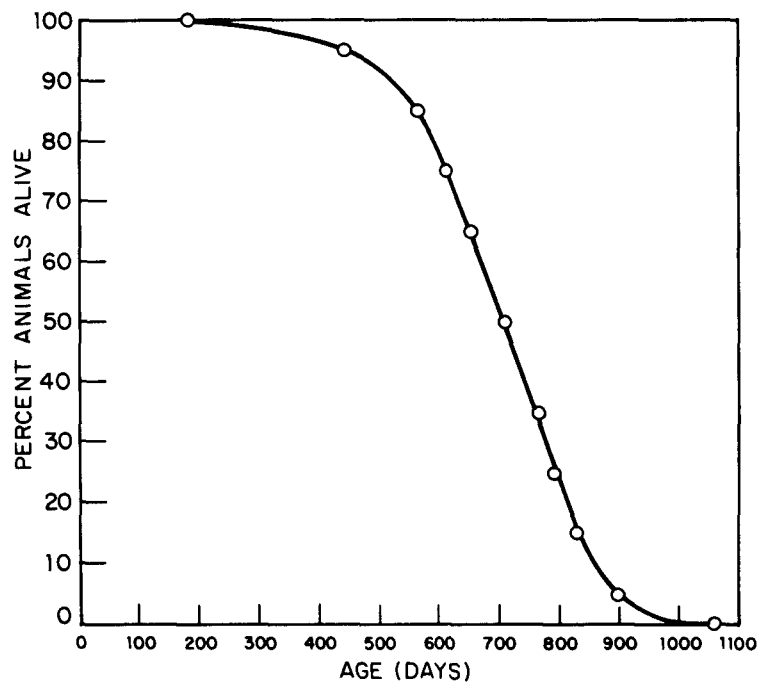


Fig. 5 Survival curve for a composite group of 747 male rats.

at least 25 percent survival is apparent and the excess (over the normal distribution) of shorter lifespans which resulted in the negative skewness is quite evident. With skewness of this degree, neither probit-linear nor probit-logarithmic plots of these data result in satisfactory rectification of the survival curve for purposes of analysis.

Many investigators choose to represent pooled data of this type as a straight line when the logarithm of some computation of death rate is plotted vs age at death (Gompertz function). From Figure 6, however, it does not appear that a straight line is an adequate representation of the situation when the present data is plotted in this manner. It is possible that the present data may fit a logistic formula (Beard, 1959) when death rate vs age is the relationship under consideration.

Another commonly used treatment of pooled data is to compute the residual lifespan, or life expectancy, at various ages. In view of the lack of normality the median (rather than mean) residual lifespans, and the 70 and 90% ranges were determined, and are illustrated in Fig. 7. Note that asymmetry in distribution of life expectancy is apparent and that the rate of decrease in life expectancy falls with increasing age up to about 960 days.

DISCUSSION

Even under the stable environmental conditions of the present study, variations among experiments in several measures of lifespan

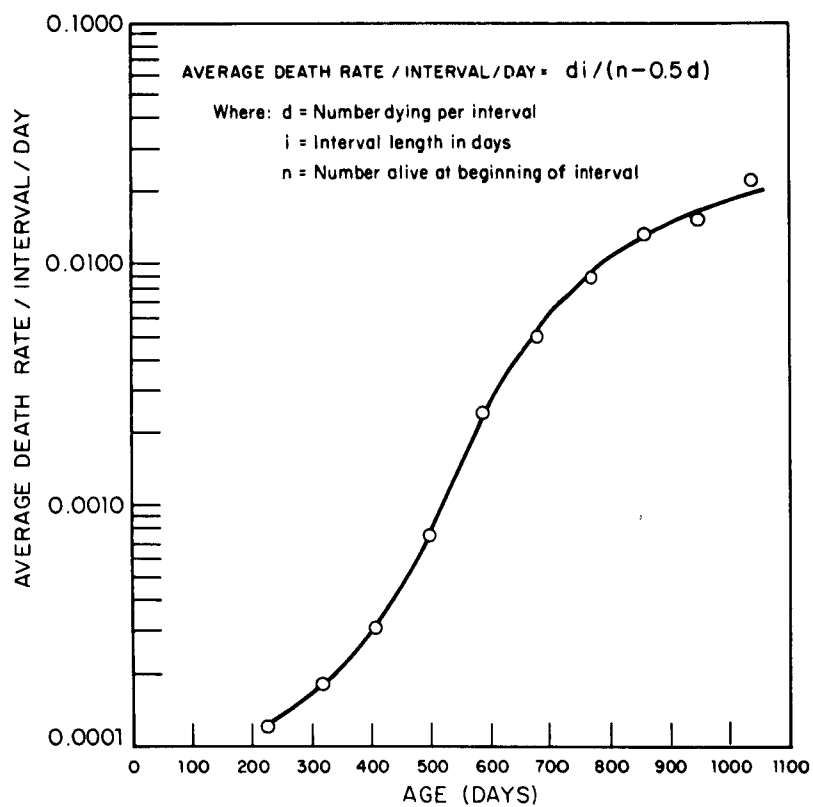


Fig. 6 Age-specific death rate for a single composite group of male rats.

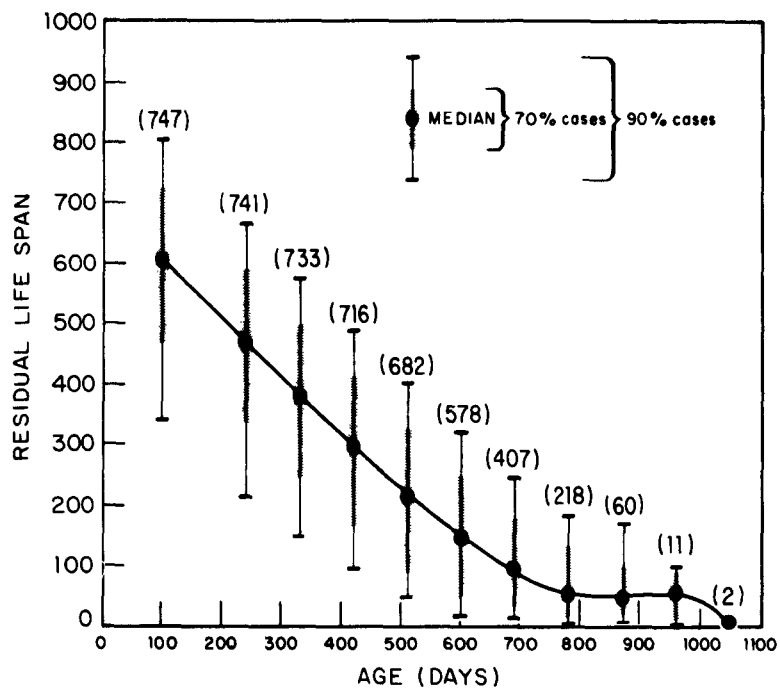


Fig. 7 Life expectancy of a composite group of 747 male rats at various ages. Points including 70 and 90% of the animals are indicated. Values in parentheses indicate number of animals alive at each age.

were found in one strain of rats with no known epidemic infections. In general, these variations appear to be nonsystematic in animals born over a period of about three years. Among experiments the slope and position of the central portion of the survival curve along the age axis appear to vary according to a normal distribution, and to be uncorrelated with each other. In addition, both are independent of significant departure of lifespan distribution from the normal. Thus a lesser slope or greater average lifespan apparently does not depend simply upon a greater proportion of longer lifespans. When the distribution of lifespans within an experiment differs significantly from the normal, the skewness is negative and kurtosis positive, resulting from an excess (over the normal distribution) of shorter lifespans. Although significant departures from the normal distribution were found in only seven of the eighteen experiments, thirteen of the experiments contained one or more animals with lifespans less than 400 days. Whether the data are grouped by experiment or by individual, resulting survival curves are not normal. These considerations indicate that some degree of departure from the normal in lifespan distribution may be the rule rather than the exception for the male rat, at least under the described control conditions.

Present data are quite consistent with lifespan information reported by other investigators using large number of male rats. Berg and Harmison (1957) reported average longevity in their strain of

Sprague-Dawley rats to be about 750 days, with a maximum of 1200 days. From the report of Gilbert and Gillman (1958) it appears that median longevity for their Wistar-derived rats was about 750 days. Verzar (1959) has reported a 50 percent survival at 23.5 months (705 days) with maximum longevity of 39 months (1170 days) for Sprague-Dawley rats of his colony. In view of these similarities among various colonies of male rats, presumably raised under a variety of conditions, the lack of a consistent significant difference in lifespan measures among the four treatments in Expts. 1-4 and between conventionally bred and specific pathogen-free bred animals of the present study is not surprising. Although there are differences in lifespan measures from experiment to experiment, and from laboratory to laboratory, it appears that for groups of male rats these differences in average longevity may extend over only a relatively limited range of values. Most of the sources of inter- and intra-experiment variation in lifespan measures are unknown, although some portion appears due to genetic and/or possible neonatal differences among litters. Further consideration of the clinical examination and autopsy data collected in some of the present experiments may aid in evaluating the relative importance of various factors associated with death at different ages.

The asymmetric distribution of lifespans for the rats of the present study has also been reported for the mouse (Lindop, 1961). These distributions for rodents are not unlike those reported for

humans. For human populations it has been hypothesized that many early deaths are not senescent in nature, but are associated with departure from optimum environmental conditions and behavior (Benjamin, 1959). In the case of the present study, and presumably also for the mouse data (Lindop, 1961), where environmental conditions are stable, it is difficult to see how such a departure could be a factor. It appears that at least for two species of rodents, the distribution of life-spans is generally asymmetric even under stable conditions of environment.

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Naval Radiological Defense Laboratory USNRDL-TR-646 LIFESPAN MEASUREMENTS IN THE MALE RAT by D. C. Jones and D. J. Kimeldorf 9 May 1963 30 p. tables illus. 8 refs. UNCLASSIFIED Measures of lifespan were determined for a population of male Sprague-Dawley derived male rats, comprised of 747 animals from eighteen experiments. Variations in lifespan measures among experiments were found even under stable environmental conditions in a single strain of rats with no known epidemic infections. Measures of (over) <u>UNCLASSIFIED</u>	Naval Radiological Defense Laboratory USNRDL-TR-646 LIFESPAN MEASUREMENTS IN THE MALE RAT by D. C. Jones and D. J. Kimeldorf 9 May 1963 30 p. tables illus. 8 refs. UNCLASSIFIED Measures of lifespan were determined for a population of male Sprague-Dawley derived male rats, comprised of 747 animals from eighteen experiments. Variations in lifespan measures among experiments were found even under stable environmental conditions in a single strain of rats with no known epidemic infections. Measures of (over) <u>UNCLASSIFIED</u>
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