Mechanisms of Ageing and Development, 38 (1987) 89–105
Elsevier Scientific Publishers Ireland Ltd.

ANALYSIS OF SURVIVAL DATA ON AGING RAT COHORTS: PITFALLS AND SOME PRACTICAL CONSIDERATIONS

JAN MOS* and CAREL F. HOLLANDER**

TNO Institute for Experimental Gerontology, Lange Kleiweg 151, P.O. Box 5815, 2280 HV Rijswijk (ZH) (The Netherlands)

(Received October 27th, 1986)

SUMMARY

Experimental aging research is very dependent on the determination of the survival characteristics of the animal species or strain under study. Such data are generally inferred from mortality curves of cohorts of animals that are set aside at an early age for aging studies. Rectangular survival curves and the presence of multiple pathological lesions are a prerequisite for aging studies so as to resemble the situation in man.

From 1977 onwards, many rat cohorts have been formed in the Institute for Experimental Gerontology (IVEG) for the study of aging processes. Data from these have been analysed for a period of 5 years up to and including 1982. (Males and females of the WAG/Rij and BN/BiRij strains were used.)

The 50% survival and the maximum survival of cohorts varied considerably, but showed no consistent trend over the years. The median (50%) survival between the cohorts differed by as much as 7.9–10.7 months for the strains and sexes studied. Maximum survival between the cohorts varied from 3.7 to 9.9 months. Median and maximal survival were greater for the females.

Maximum survival and 50% survival correlated significantly, the relation between the two being approximately linear. The effect of removing animals from cohorts on the estimation of 50% survival was only minor, whereas maximum survival was clearly diminished by this procedure.

The wide variation in survival characteristics, even between successive cohorts, cautions against too simple a measure of the animals survival in only one number for median or maximal survival in months.

**Present address*: Duphar B.V., Dept. of Pharmacology, P.O. Box 2, 1380 AA Weesp, The Netherlands.
***Present address*: Laboratoires MSD CHIBRET, Centre de Recherche, Departement d'E.I.M., Route de Marsat, B.P. 134, 63203 RIOM Cedex, France.
All correspondence should be addressed to: Jan Mos, Duphar B.V., Department of Pharmacology, P.O. Box 2, 1380 AA Weesp, The Netherlands.

0047-6374/87/$03.50
Printed and Published in Ireland

An indication of the variance of 50% survival and of maximum survival should therefore be included in scientific publications. Moreover, the 50% survival is the parameter of choice to define cohorts, not only because this can be most reliably estimated with good confidence limits, but also because this measure is the least sensitive to removing animals from the cohorts. As this will often be the case in many research institutions, it might be of practical importance to order old animals from different cohorts since this diminishes the chance of using an extremely short or long lived cohort.

Finally, the analysis revealed that combining intact or incomplete cohorts into larger survival curves resulted in nearly identical graphs. An attempt was made to calculate the minimum cohort size which yields survival curves with constant 95% confidence limits.

Key words: Aging; Rat cohorts; 50% Survival; Maximal survival

INTRODUCTION

Survival curves play an important role in aging research, both in the human situation and in experimental gerontological studies. The rectangularization of the human survival curve in many countries and the presence of multiple pathological lesions are prerequisites that have to be met in animal model studies so that they resemble the human situation [1]. Such rectangular survival curves are usually defined by the 50% survival time and the maximum survival. The 50% survival (also referred to as median survival) is that point in time when half of the original population has died and half is still alive. This median survival is not necessarily identical to the mean or average survival, which is the arithmetic mean of how long the animals have lived. The maximum survival is defined as the time that the longest living individual has survived. The latter parameter is determined by only one animal, whereas the median survival is a parameter reflecting survival of the whole population.

When a population is very large and death rates are recorded sufficiently accurately, the construction of survival curves can be done with adequate precision. These survival curves are needed for both longitudinal and cross-sectional studies. In some types of longitudinal studies the population may be followed over such a long period that the population completes its natural survival. Survival and median survival can then be easily recorded and groups with different treatment conditions may be compared. Reference values for survival are less crucial in this type of experimentation, but these studies are both time consuming and expensive.

Many experiments on aging are not conducive to longitudinal designs since ani-

mals must be killed or are otherwise unsuited to prolonged experimentation. In this case, studies have to rely on cross-sectional experimental designs. One of the drawbacks of this type of experiment is the variation in survival characteristics between several groups or cohorts. From the human situation it is clear that birth cohorts vary considerably. Various environmental and genetic factors may contribute to this spread in cohort characteristics. In this respect, inbred strains of rodents show a greater constancy in both their environmental circumstances and in their genome.

In the institute, inbred strains of rats and mice have been maintained for aging studies for many years. It is therefore possible to analyse trends in survival characteristics of cohorts of animals set aside for aging studies. For the purpose of this study, a cohort is defined as a group of rats of the same strain, sex and age which all entered the aging colony at the same time. Usually the cohorts are of limited size. In contrast, cohort size in human studies is generally far greater. In animal studies, the cohort size from which survival characteristics are inferred varies considerably. Sizes range from about as low as 17 animals to several hundreds (studies cited by Walford [2]). Large sample studies result in more reliable estimates of the median survival, while the regular setting aside of cohorts permits the evaluation of drift in survival. Moreover, when faced with the task of maintaining a steady stream of old animals, the cohort system is highly desirable for the planning of experiments.

Surprisingly few studies have addressed the question of cohort variability. Recently Curcio *et al.* [3] described the variation in eight rat cohorts, each originally containing one hundred animals. They found an increase in lifespan over time in outbred retired breeder Sprague–Dawley rats. Thus, they cautioned against the comparison of different cohorts. However, Schlettwein-Gsell [4] found no systematic change over time in lifespan, but did observe significant inter-cohort variation. These data were based on cohort sizes ranging from 156 to 288 male and 135 to 288 female Wistar rats. No attempt was made to investigate the effects of removing animals from the cohorts on survival characteristics. Jones and Kimeldorf [5] found insignificant inter-cohort variations in the mean and median survival of male Sprague-Dawley rats (mean cohort size 42). The mean and median survival varied by 5.3 and 5.9 months respectively.

The present study is mainly intended to be of practical value to investigators of aging phenomena. It is our aim to describe the variability of survival characteristics in small aging cohorts from two rat strains (the WAG/Rij and the BN/BiRij), for both sexes. It will be demonstrated that even inbred strains kept under identical conditions vary considerably in maximum survival and median survival and forms an extension to earlier publications [1,6]. Moreover, the effects of removing animals from the cohorts on survival parameters, were investigated. The variability between cohorts necessitates some special procedures when planning experiments with old animals. Some possibilities will be discussed.

MATERIALS AND METHODS

Male and female rats of the inbred WAG/Rij and BN/BiRij strains were kept under clean conventional conditions [6] in the institute. For a detailed description of husbandry conditions and selection of the animals, the reader is referred to an earlier publication [6]. Cohorts of 17–30 rats were formed 3 weeks after birth. Most of the cohorts comprised groups of 20 rats except for the BN/BiRij females, which had an average cohort size of 30 rats. No weighing factor was allotted to the size of the cohorts. Details about cohort size are given in Table I.

Animals were removed from most of the cohorts for experiments, but a number of cohorts remained intact. These were specifically reserved for the study of pathological changes with aging, but were otherwise kept under identical conditions. The date that an animal died spontaneously or was removed for experiments was precisely recorded. Survival curves were calculated with a correction for the animals at risk. Kaplan-Meier statistics were applied to compare survival curves and determine the 95% confidence limits of the survival parameters under study. Other comparisons of median or maximum survival between groups were performed using Student's t-test.

The present analysis was mainly concerned with the median (50%) survival and the maximum survival of individual cohorts. Whenever a cohort ended by taking away the longest living animal for experimentation, the maximum survival could not be accurately determined, therefore this parameter was not used in every analysis.

TABLE I

DATA CONCERNING SIZE AND SURVIVAL CHARACTERISTICS OF COHORTS

	WAG/Rij ♀	*WAG/Rij* ♂	*BN/BiRij* ♀	*BN/BiRij* ♂
Total no. of cohorts	59	15	32	34
No. cohorts $N = 20$	50	11	2	30
No. cohorts $N > 20$	7	4	30[a]	3
No. cohorts $N < 20$	2	0	0	1
No. of intact cohorts	19	3	6	13
Total no. of animals				
Intact cohorts	427	60	177	266
Incomplete cohorts	696	236	779	383
% of animals removed before median survival time[b]	65	69	80	79

[a]Almost all cohorts comprised groups of 30 animals because an increased demand for animals was expected at the time the cohorts were formed.

[b]These data are expressed as percentages of the number of animals removed, which was not the same for different sex and strain.

Nonetheless the median survival time of these cohorts was processed together with that of other cohorts where this was appropriate.

Cohorts from which more than 70% of the animals were removed were excluded from further analysis. In total, 59 cohorts of the WAG/Rij females, 15 of the WAG/Rij males, 32 of the BN/BiRij females and 34 of the BN/BiRij males were available for statistical analysis.

RESULTS

50% Survival of individual cohorts over time

Figures 1A–D summarises the variability in the median survival time of the individual cohorts for both strains and sexes. This variability is quite large, ranging from 27.2 to 36.0 months and 21.9 to 29.8 months for the WAG/Rij females and males respectively and from 27.2 to 35.1 months and 23.5 to 34.2 months for the BN/BiRij females and males respectively. Mean and 95% confidence limits are given in Table II, together with a separation of the data for intact and incomplete cohorts.

Between 1977 and 1981, no apparent change was observed in the median survival

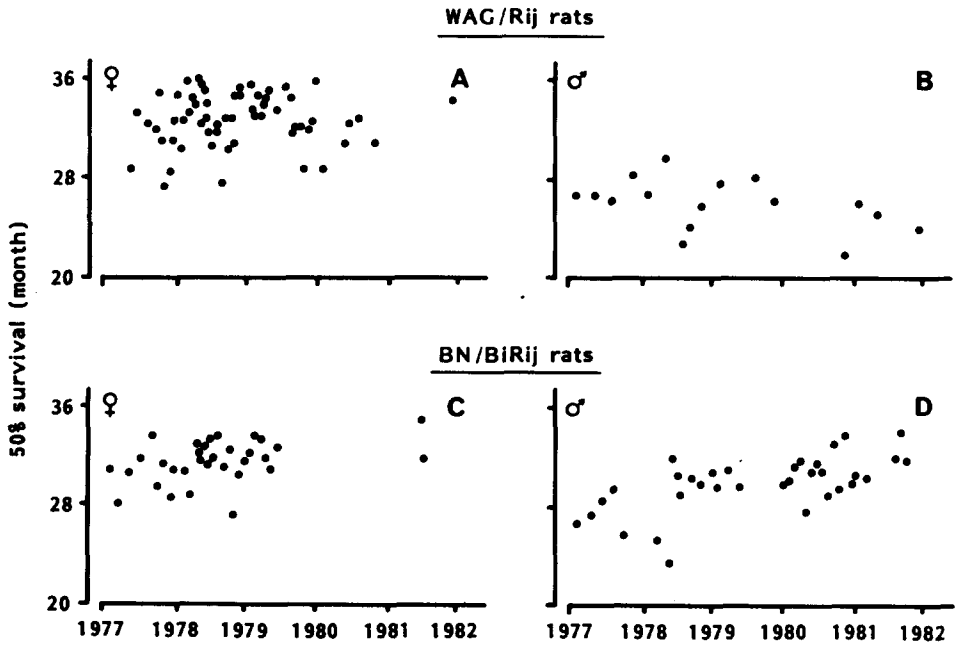

Fig. 1. The 50% survival of successive cohorts is plotted against the time of birth for the females and males of the WAG/Rij and BN/BiRij rat strains. Each point represents one cohort, intact or incomplete. No significant regression over time was found, except for the BN/BiRij males when the data for 1977 were included.

TABLE II

DATA OF THE SURVIVAL CHARACTERISTICS OF THE COHORTS

	WAG/Rij ♀	*WAG/Rij* ♂	*BN/BiRij* ♀	*BN/BiRij* ♂
50% survival intact cohorts (mean + range)	33.1 30.5–35.5	26.7 26.1–27.7	31.2 28.8–32.8	30.0 23.5–33.2
50% survival incomplete cohorts (mean + range)	32.5 27.2–36.0	25.8 21.9–29.8	31.7 27.2–35.1	30.5 25.4–34.2
Max. survival intact cohorts (mean + range)	41.5 37.2–44.5	34.5 34.8–36.2	39.0 37.4–40.4	37.2 34.6–39.9
Max. survival incomplete cohorts (mean + range)	39.0 34.6–43.6	35.5 32.8–36.5	38.2 31.8–41.3	36.4 33.2–39.9
50% survival all cohorts ± 95% confidence limits	32.6 ± 0.6	26.0 ± 1.1	31.6 ± 0.6	30.0 ± 0.8
Max survival all cohorts ± 95% confidence limits	40.0 ± 0.8	34.7 ± 0.9	38.4 ± 0.9	36.8 ± 0.7

values for the WAG/Rij males and females ($r = -0.45$, $P = 0.08$; $r = 0.07$, $P = 0.61$, respectively), nor was there any appreciable change in variability. The median survival of the BN/BiRij females did not change significantly over time ($r = 0.33$, $P = 0.07$), though BN/BiRij males tended to have an increased 50% survival over the years ($r = 0.63$, $P < 0.01$). however, if 1977 is omitted (because of exceptionally low values), a non-significant correlation ($r = 0.34$, $P = 0.08$) was seen. It is also clear from this figure that large differences in 50% survival time may occur in successive cohorts.

Mean values of the 50% survival of the females were significantly higher than the males ($P < 10^{-3}$ WAG/Rij and $P = 0.0003$ BN/BiRij Student's t-test). The number of cohorts that had to be excluded differed over time. Most cohorts for the WAG/Rij females were available during the early years, while most of the data for the BN/BiRij males were gathered during the later period. After 1981, the use of old animals increased so much that data for another study of this type would probably not be available.

Maximum survival in individual cohorts over time

Similar data, but now for the maximum survival of the individual cohorts, are given in Figs. 2A–D. These data were obtained from a smaller number of cohorts

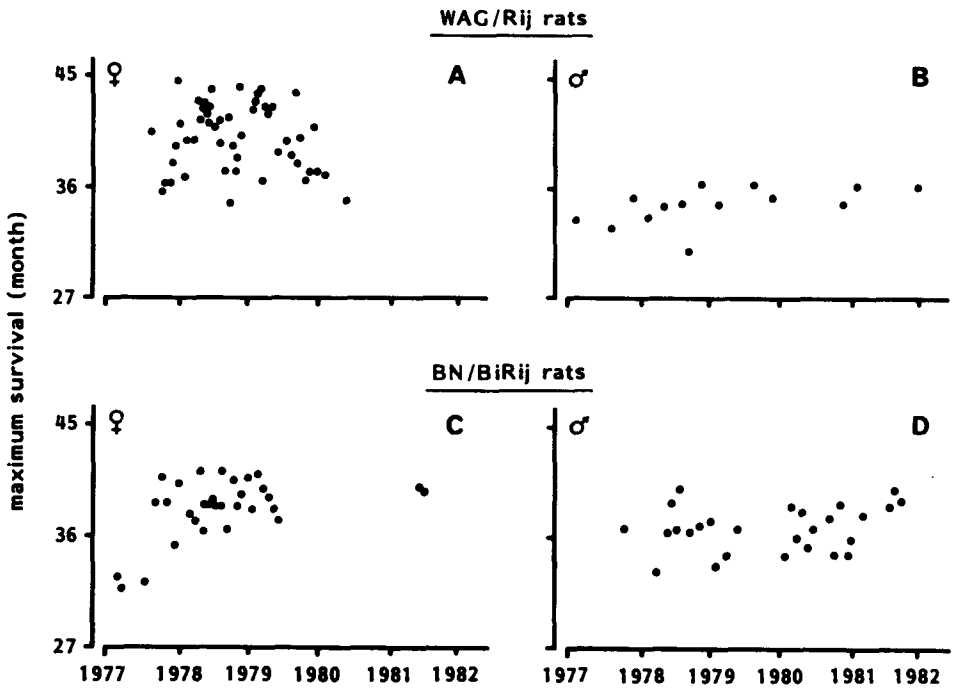

Fig. 2. The maximum survival of successive cohorts is plotted against the time of birth for the females and males of the WAG/Rij and BN/BiRij rat strains. Each point represents one cohort, intact or incomplete. No significant regression over time was observed, except for the BN/BiRij females.

because those cohorts from which the longest living animal had been removed for experimentation, were excluded from the analysis. Here again, a large variation was found between the survival of successive cohorts. Maximum survival ranged from 34.6 to 44.5 months and 32.8 to 36.5 months for the WAG/Rij females and males, respectively.

For the BN/BiRij females and males these values were 31.8–41.3 months and 33.2–39.9 months, respectively. Mean and 95% confidence limits are given in Table II. With the exception of BN/BiRij females ($r = 0.48$, $P = 0.01$), no significant trend over time was observed (WAG/Rij females $r = -0.13$, $P = 0.39$; WAG/Rij males $r = 0.54$; $P > 0.05$; BN/BiRij males $r = 0.24$, $P = 0.22$). Maximal survival values were higher for the females ($P = 0.008$ WAG/Rij and $P < 10^{-3}$ BN/BiRij, Student's t-test).

Relationship between 50% survival and maximum survival

Fifty percent survival of individual cohorts are plotted against maximum survival in Figs. 3A–D. With the exception of WAG/Rij males ($r = 0.12$, $P = 0.68$), the graphs all show a linear correlation; the higher the median survival, the higher the maximum survival (WAG/Rij females $r = 0.48$, $P = 0.0005$; BN/BiRij females

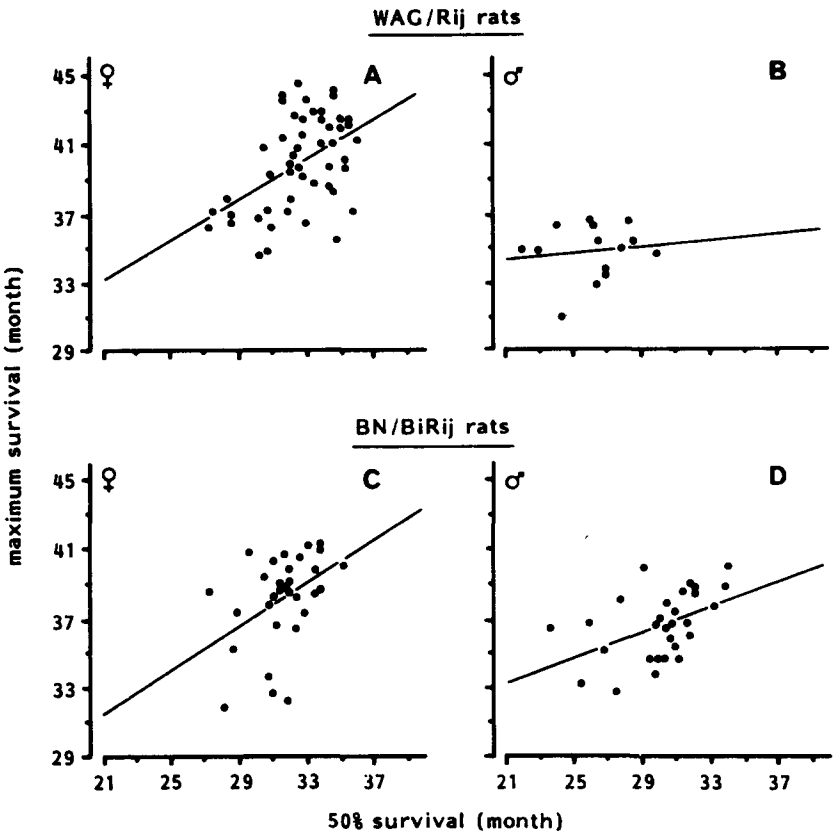

Fig. 3. The maximum survival is plotted against the 50% survival for the rat strains used in the study. With the exception of WAG/Rij males, the median and maximum survival correlated significantly.

$r = 0.42$, $P = 0.02$; BN/BiRij males $r = 0.47$, $P = 0.01$). This points to a parallel shift in the survival curves rather than the curves being markedly different in shape. As an example, the survival curves of the longest and the shortest living cohorts of the intact WAG/Rij females is shown in Fig. 4. Essentially, the only change is a parallel shift in the curves with no other significant differences.

Influence of removal of animals on 50% survival

Cohorts set aside for the study of aging processes do not usually remain intact so that animals are allowed to die spontaneously. Animals tend to be removed from the cohorts for experimental purposes. Removing animals from cohorts may have implications for such survival characteristics as the median survival or maximum survival. Figure 5 summarises the data on the median survival of our cohorts with respect to the removal of animals.

Up to 70% removal of animals had no significant effect upon the estimated

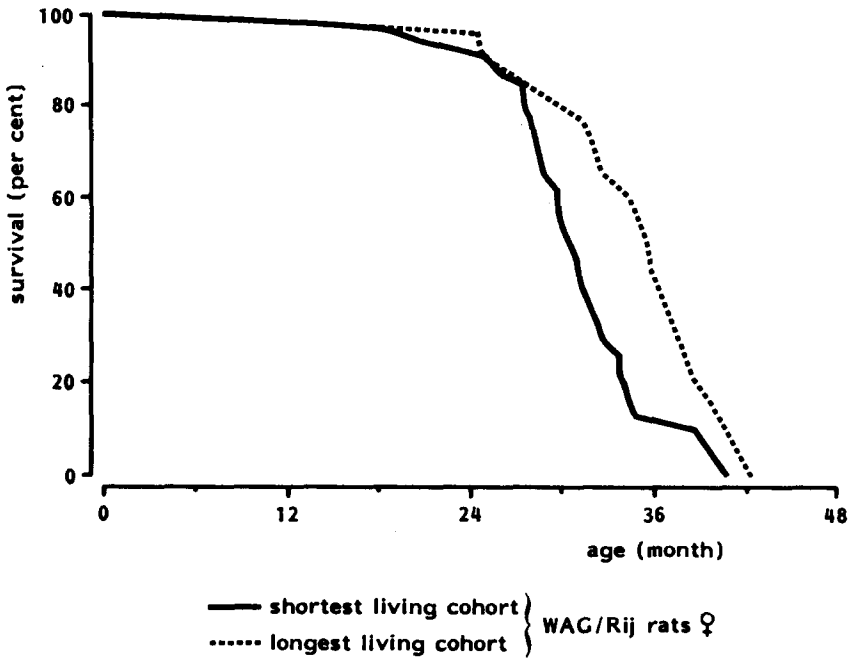

Fig. 4. Plots of the survival curves for the shortest and longest living intact cohort, based on the 50% survival time (WAG/Rij females). The shortest living cohort comprised 31 animals and the longest living, 20 animals. Both were intact cohorts. Essentially, there is only a parallel shift to the right of the longest living cohort.

median survival of the cohorts (i.e. no negative correlation between 50% survival time and the percentage of animals removed).

Correlation coefficients were: $r = -0.19$, $P = 0.15$ for the WAG/Rij females, $r = -0.14$, $P = 0.46$ for the BN/BiRij females, and $r = -0.10$, $P = 0.58$ for the BN/BiRij males. Only the WAG/Rij males showed a significant negative correlation ($r = -0.56$, $P = 0.02$). WAG/Rij males also appear to be an exception in so far as no correlation was found between median and maximum survival, even though they were maintained under identical conditions to the other groups that were studied. We have no explanation for this.

The lack of correlation between median survival and the percentage of animals removed is not surprising since it might be expected that most animals will be removed during the last part of the survival curve. Retrospective analysis, however, revealed that 74% of all animals were removed before the median survival had been reached and only 26% after the median survival. Therefore, although the lack of association between the median survival and the percentage of animals removed may seem trivial, this is not the case in view of the numbers removed before and after the 50% survival. The 50% survival is a good characteristic of the survival curve and this apparently remains so even after 74% of the animals have been removed. Moreover,

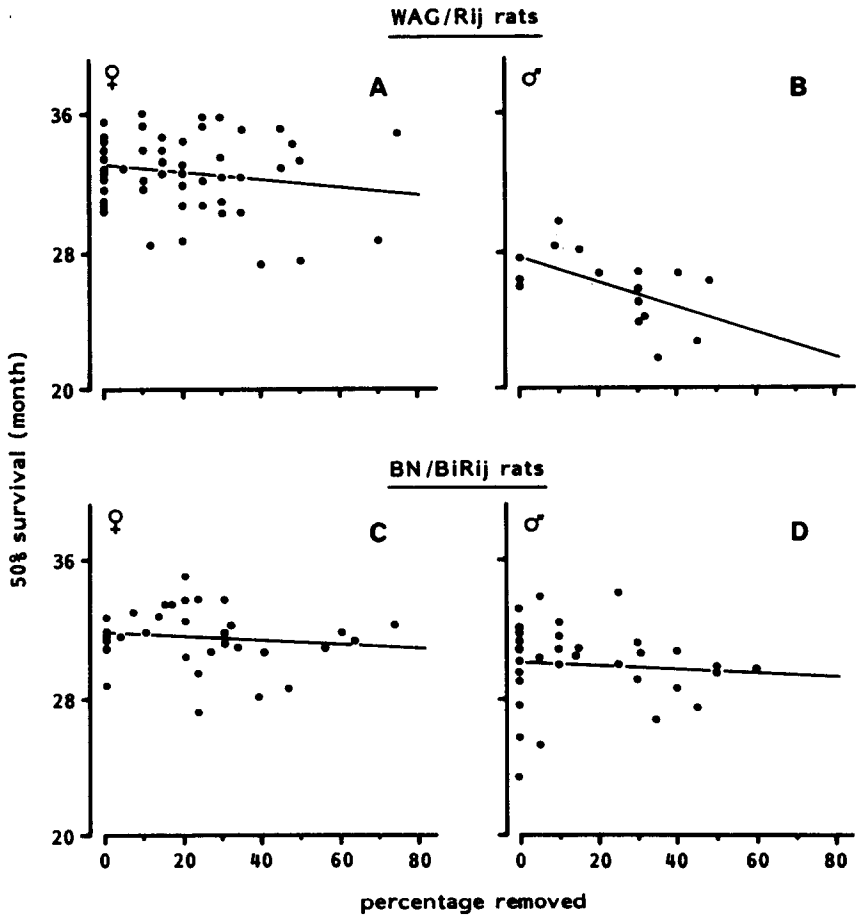

Fig. 5. The effect of removing animals from cohorts on the 50% survival of male and female WAG/Rij and BN/BiRij rats. Each point represents one intact or incomplete cohort. With the exception of the WAG/Rij males no significant correlations were observed.

the median survivals are correlated with the maximum survival of cohorts. Thus, the 50% survival has some predictive value for survival and it can be obtained by almost all investigators, assuming one does not remove too many of the animals from a cohort.

Influence of removal of animals on maximum survival

The maximum survival of cohorts decreased as more animals were removed (Fig. 6) except for the WAG/Rij males ($r = -0.38$, $P = 0.17$). Correlation coefficients for the WAG/Rij females were, $r = -0.52$, $P < 10^{-4}$; for the BN/BiRij females, $r = -0.53$, $P = 0.0002$; and for the BN/BiRij males, $r = -0.34$, $P = 0.08$. However, it is clear that the maximum survival from intact cohorts (Y-axis of each panel)

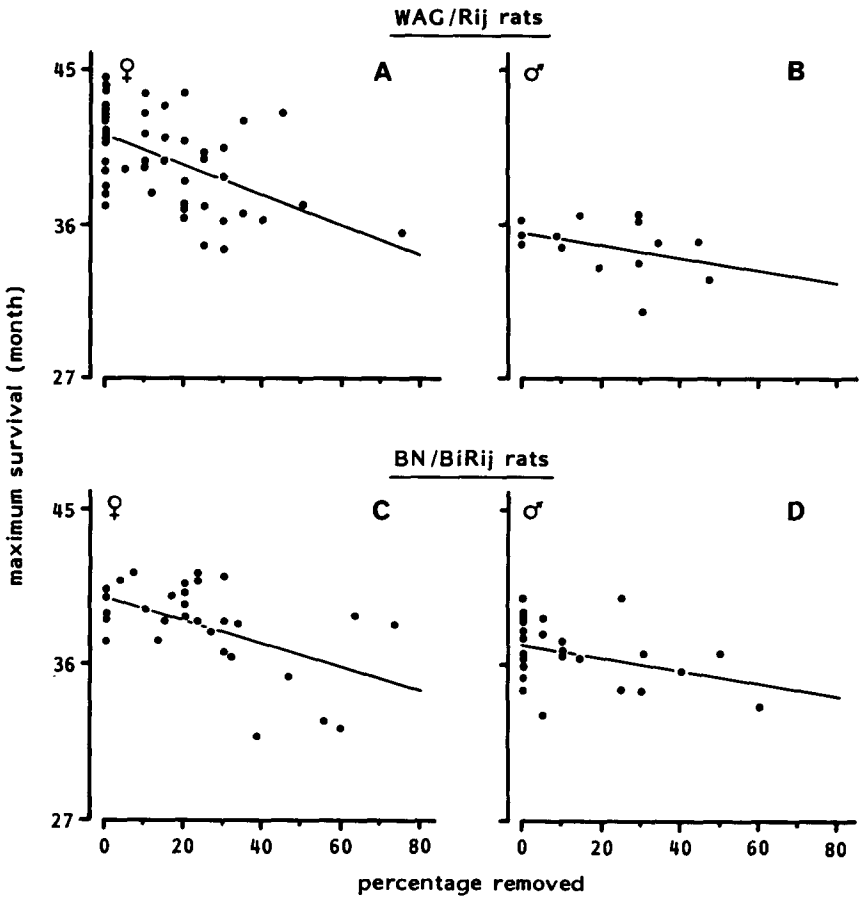

Fig. 6. The effect of removing animals from cohorts on the maximum survival of male and female WAG/Rij and BN/BiRij rats. Each point represents one intact or incomplete cohort. Significant negative correlations were observed between the percentage of animals removed and the maximum survival.

already shows considerable variation. Statistical comparison reveals that the maximum survival is not significantly different between intact cohorts and cohorts with up to 25% of the animals removed. Thus, some confidence can be given to the survival values obtained from these cohorts. The variation, however, remains so large that caution is necessary on cross-sectional studies with cohorts from which animals have been removed, and the maximum survival is then definitely not the parameter of choice.

Comparison of survival curves from combined cohorts

Survival curves were constructed for both strains and sexes by combining intact cohorts and by combining cohorts with up to 70% of the animals removed. The

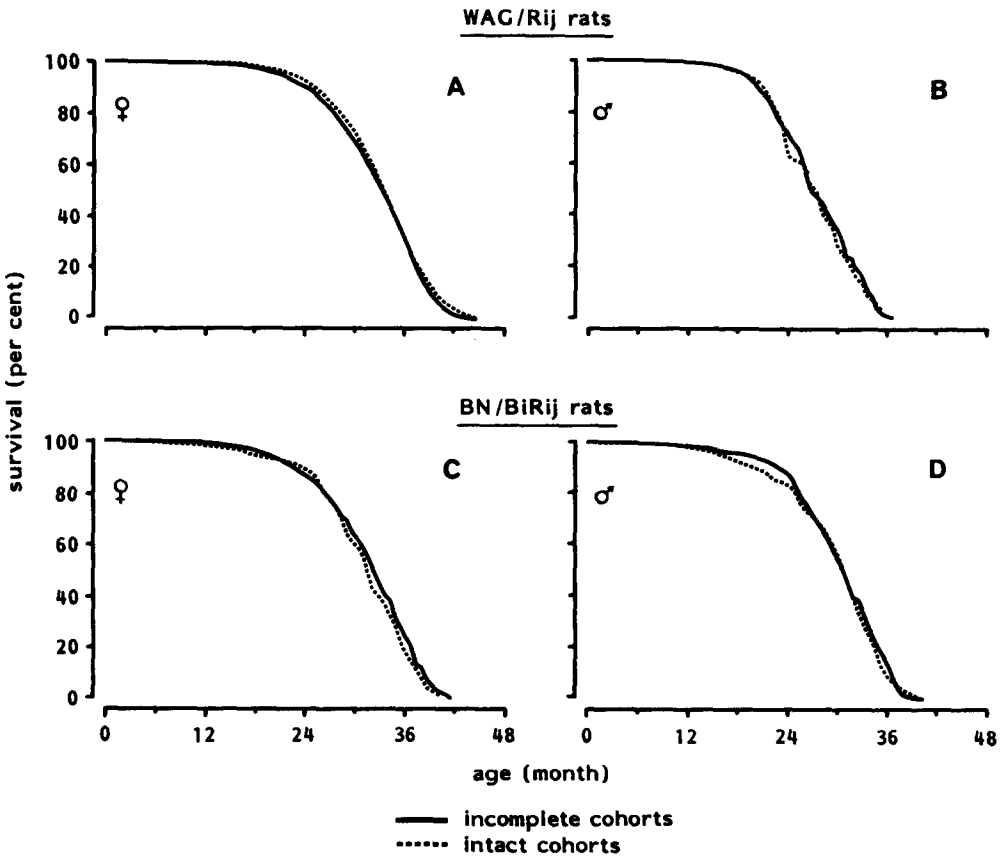

Fig. 7. Survival plots of grouped cohorts. Intact and incomplete cohorts were combined to form new groups. The resulting survival characteristics are highly similar, therefore equally reliable survival curves can be constructed when one only has data of cohorts from which animals were removed. See Table I for the number of animals used to construct the resulting survival curves.

resulting survival curves are depicted in Fig. 7. The curves are very similar in shape. Median survival times were not significantly different and the 10% survival is also quite similar.

As may be anticipated, the combination of many cohorts leads to smoother curves for both types of cohorts. The relevance of these data is that, even if one has data for incomplete cohorts, the combination of such cohorts may lead to an equally smooth and rectangular survival curve as would be the case for combined, intact cohorts.

Effects of cohort size on estimates of 90, 50 and 10% survival

Data from intact cohorts of BN/BiRij males were combined into larger cohorts by randomly adding the data from different cohorts so as to increase the total size of the group of animals under study. This permits an estimate of optimum cohort size for

the determination of several survival points. For practical purposes, the 90% survival, the median and the 10% survival were calculated together with the 95% confidence limits of these estimates. For intact cohorts, Fig. 8A shows the rapid contraction in the confidence limits of the 90% and 10% survival by increasing the cohort size. Cohort sizes larger than 80 do not substantially narrow the confidence band around the survival times.

Median survival times have the most narrow confidence limits with small cohort size. With increasing cohort size, the 10% survival confidence limits become the smallest. A similar analysis was carried out for incomplete cohorts (Fig. 8B). Again, the method of combining the cohorts into a larger group was random. The 90% survival was slightly higher than found by combining complete cohorts. At first, confidence bands (90%) are wider than for the parallel data in Fig. 8A. With increasing combined cohort size, the confidence limits decrease to lower values. Median and 10% survival are estimated with roughly equal precision, regardless of whether intact or incomplete cohorts are combined. The confidence bands for incomplete cohorts reaches a plateau at a cohort size of approximately 150 animals, which is higher than for the combined intact cohorts.

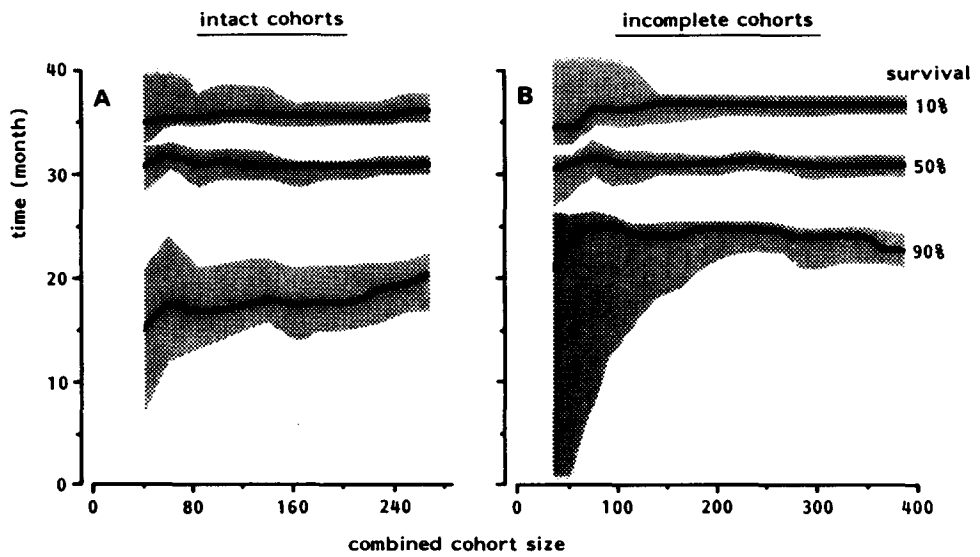

Fig. 8. A. A random permutation was made of intact BN/BiRij male cohorts. These were subsequently combined to form larger groups. From these increasing cohorts, the 90, 50 and 10% survival were calculated with the respective 95% confidence limits. As a function of the increasing cohort size, the survival points are plotted with the confidence band. Cohort sizes larger than 80 did not result in narrower confidence limits, nor in an appreciable change of 90, 50 or 10% survival. The shaded area indicates the 95% confidence band. B. A similar analysis as under 8A was made by combining a random permutation of incomplete male BN/BiRij cohorts. Fairly stable survival values and confidence limits are reached between a cohort size of 80–150 animals.

Figures 8A and B are based only on one random permutation of cohorts and therefore no far reaching conclusions can be drawn. However, this exercise does give some indication of the influence of cohort size on the reliability of survival curve characteristics. This point will be dealt with later.

Influence of season of birth on longevity

The time of birth of cohorts was categorized in four classes, viz. summer, autumn, winter and spring. Analysis of variance revealed no consistent effects of the season of birth on median or maximum survival, nor for strains nor for sexes.

DISCUSSION AND PRACTICAL CONSIDERATIONS

The present evaluation of cohort survival characteristics over a 5-year period reveals a number of important facts:

1. The variability in median survival and maximum survival is quite large. Ninety-five percent confidence limits for the mean value of the 50% survival of the cohorts is between 0.6 and 1.1 months. Males and females do not differ much in this respect. The 95% confidence limits for the maximum survival of the cohorts varies between 0.7 and 0.9 months.
2. There is no systematic trend over the years in the survival of the two strains studied. This is a confirmation of the stability of the maximum survival that seems to have reached its plateau under present conditions of housing and feeding. Earlier reports on prolongation of survival over the years in C57BL mice [7] and Sprague–Dawley rats [3] are not applicable to our aging rat colonies. They are in agreement, however, with data presented by Schlettwein-Gsell [4] and Jones and Kimeldorf [5] on Wistar and Sprague–Dawley rats. Due to the variation between successive cohorts caution is needed to conclude that systematic changes occur in longevity.
3. Maximum survival and median survival are correlated within a cohort. Thus, establishing the median survival has some predictive value for the maximum survival, although the variability is appreciable.
4. Removing animals from a cohort has hardly any effect on the estimation of the median survival. This is therefore the parameter of choice in defining an aging cohort and to defining old rats [2]. However, removing animals from the cohort does result in lower values for maximum survival. Retrospective analysis refutes the possibility that this is due to more animals being removed during the last part of their natural survival.
5. Combining intact and incomplete cohorts into large groups results in very comparable survival characteristics. Any investigator might therefore estimate the median survival more accurately by adding data from different cohorts, even though cohorts are not allowed to complete their natural survival.
6. An attempt was made to estimate the optimum cohort size with regard to the

confidence limits of the 90, 50 and 10% survival. Cohort sizes larger than 80 animals (BN/BiRij males) does not lead to a marked improvement in confidence bands. This value is close to the number of animals Storer used in 1966 to describe longevity in 22 inbred mouse strains [8]. Combining incomplete cohorts requires 80–150 animals to reach approximately the same level of accuracy.

Practical considerations:

a. The wide variation in the median and maximum survival of successive cohorts cautions against cross-sectional studies using small sized cohorts. One can better combine animals from different cohorts within a single study with less attention being paid to the absolute age in months since the selection of a particular cohort may prove to be unlucky (either an extremely long living cohort or a short living one). The planning of experiments and the reservation of cohorts should take into account this variation between cohorts.

b. If the study design is cross-sectional, at least some indication of the previous variability of median and maximum survival should be given as background data. In this case the removal of animals does not change median survival time, but it does lead to a negative bias in the maximum survival. Median survival is generally the parameter of choice to characterize the survival curves of both intact and incomplete cohorts.

c. Estimates of the cohort size necessary ensure that a certain number of animals remain alive over a given time period do not become more accurate with cohort sizes greater than 80 animals in intact cohorts or greater than 150 in incomplete ones. Examination of the references cited by Walford [2] reveals that cohort sizes as small as 12 to 14 are sometimes used to *define* survival characteristics.

Although most studies employed higher numbers of experimental animals, several studies used only a marginal number of animals as compared to the indications we found that approximately 80 animals are needed.

Relatively few studies have focussed attention on the variation in survival characteristics of aging rat cohorts [1,3–5]. Several publications have focussed on comparing survival characteristics of various strains in different laboratories [9,10]. Although very useful, the reliability of conclusions drawn from these data depends to a great extent on the variability of survival characteristics during repeated cohort formation at the same institute or laboratory. The present study has revealed some serious possibilities for erroneous results of studies involving either cross-sectional analysis of aging animals or the determination of varying (environmental) factors on survival characteristics. Interpretation of some exciting data on life span, genetics and environmental changes (e.g. refs. 11–14) should be done with caution since group sizes are too small to definitely exclude important contributions of cohort effects.

Our results can be used to adapt experimental strategies so as to circumvent these pitfalls and thus increase the validity and impact of studies on aging. To stress the

50% survival as the parameter of choice in operationally defining old rats [1,2], does not imply that this value is of magical biological value. The point in time where most interesting changes occur related to aging may well be ahead of the 50% survival value (see also ref. 15). Biomedical studies in gerontology, whether in humans or experimental animals have sofar failed to connect definite and informative biological markers of aging to chronological age. The present study demonstrates that survival is difficult to compare between inbred cohorts and shows appreciable variation, despite similar environmental factors. However, careful experimental designs and critical interpretation of single cohort experiments may eventually help to overcome these problems.

ACKNOWLEDGEMENTS

Many people have directly or indirectly enabled a study of this kind to be made: gathering data over many years with good reliability. Our gratitude goes to the biotechnicians whose excellence in caring for the animals is greatly appreciated. Moreover, we want to thank W. Klapwijk and M. van Zwieten for support with the computer programmes. D.L. Knook and C. Zurcher initiated and standardized procedures for cohort formation and maintenance of aging rat colonies. E. van der Reijden carefully prepared the figures. M. Horan and A. Brouwer corrected the manuscript and gave valuable suggestions and comments.

REFERENCES

1 C.F. Hollander, H.A. Solleveld, C. Zurcher, A.L. Nooteboom and M.J. van Zwieten, Biological and clinical consequences of longitudinal studies in rodents: their possibilities and limitations. An overview. *Mech. Ageing Dev.*, *28* (1984) 249–260.
2 R.L. Walford, When is a mouse "old"? (Letters to the Editor). *J. Immunol.*, *117* (1976) 352–353.
3 C.A. Curcio, N.A. McNelly and J.W. Hinds, Variation in longevity of rats: evidence for a systematic increase in lifespan over time. *Exp. Aging Res.*, *10* (1984) 137–140.
4 D. Schlettwein-Gsell, Survival curves of an old age rat colony. *Gerontologia*, *16* (1970) 111–115.
5 D.C. Jones and D.W. Kimeldorf, Lifespan measurements in the male rat. *J. Gerontol.*, *18* (1963) 316–321.
6 J.D. Burek, *Pathology of Aging Rats. A Morphological and Experimental Study of the Age-Associated Lesions in Aging BN/Bi, WAG/Rij and (WAGxBN) F1 Rats*, CRC Press, West Palm Beach, Florida, 1978.
7 I. Kunstyr and H.W. Leuenberger, Gerontological data of C57BL/6J mice. I. Sex differences in survival curves. *J. Gerontol.*, *30* (1975) 157–162.
8 J.B. Storer, Longevity and gross pathology at death in 22 inbred mouse strains. *J. Gerontol.*, *21* (1966) 404–409.
9 E.J. Masoro, Mortality and growth characteristics of rat strains commonly used in aging research. *Exp. Aging Res.*, *6* (1980) 219–231.
10 G.L. Coleman, S.W. Barthold, G.W. Osbaldistan, S.J. Foster and A.M. Jonas, Pathological changes during aging in barrier-reared Fischer 344 rats. *J. Gerontol.*, *32* (1977) 258–278.
11 R.S. Menich and A. Baron, Social housing of rats: Life-span effects on reaction time, exploration, weight and longevity. *Exp. Aging Res.*, *10* (1984) 95–100.

12 D.K. Ingram and M.A. Reynolds, The relationship of genotype, sex, body weight and growth parameters to lifespan in inbred and hybrid mice. *Mech. Ageing Dev.*, *20* (1982) 253–266.
13 C.L. Goodrick, Life-span and the inheritance of longevity of inbred mice. *J. Gerontol.*, *30* (1975) 257 263.
14 M. Skalicky, H. Bubna-Littitz and G. Hofecker, The influence of persistent crowding on the age changes of behavioral parameters and survival characteristics of rats. *Mech. Ageing Dev.*, *28* (1984) 325–336.
15 C. Zurcher, M.J. van Zwieten, H.A. Solleveld and C.F. Hollander, Aging research. In: *The Mouse in Biomedical Research*, Vol. IV. Academic Press, Inc., 1982, pp. 11–35.