MORTALITY IN SYNGENEIC RAT PARABIONTS OF DIFFERENT CHRONOLOGICAL AGE*†

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INTRODUCTION

The life span of tissue grafts from older donors to younger syngeneic recipients (heterochronic grafts) may exceed that of the donor.[5] Grafting of entire syngeneic mammals (parabiosis) of different chronological age, first suggested by Alex Comfort in 1956,[2] was attempted by Lunsford and colleagues,[6] but the number of long-term survivors was too small to obtain data on life span. The experiments reported here were designed to answer the following questions: Does the life expectancy of the older member of a heterochronic pair of parabionts differ from that of both single and parabiosed controls? What is the effect of parabiosis *per se* on life expectancy? If heterochronic or homochronic parabiosis, or both, influences life expectancy, is this influence sex-dependent?

MATERIALS AND METHODS

Basic Design

Buffalo rats were used. They were inbred for this project by the Simonsen Laboratories, Gilroy, Calif. Animals coming from the breeding colony were randomly assigned to the experimental groups shown in TABLE 1. The entire experiment involved thus 534 animals. As the table shows, parabiosis, in the animals subjected to this condition, was established only between animals of the same sex. In pairs of unequal age, surgery was performed when the age of the younger member of the pair was approximately the same as that of the pairs of equal age; this age was 60–85 days. The older member of the pair was approximately 280 days older.

Surgery

The rats were under sodium pentobarbital narcosis (1.8 mg/kg rat) and received immediately prior to surgery Bicillin-fortified-SM (15,000 units combined penicil-

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lin and 12.5 mg dihydrostreptomycin/500-g rat in the thigh muscles). We had found on the basis of earlier work with parabiosis that streptomycin affords significant protection against the development of chronic obscesses in the tissue bridge between the parabiotic pair. No antibiotic treatment was given to the single control animals. Surgery was carried out on a heated table (30° C), using ophthalmological instruments that were found suitable for work with small rodents. Skin, muscle, and fasciae of the abdominal and thoracic wall were united. The adjacent scapulae of the two animals were united. Catgut was used for all sutures, except those involving the peritoneal membranes, which were joined by sutures of No. 4 cotton. In some animals, the scapulae separated after three or four months of parabiosis. These were surgically repaired. If infection was noticeable during the first weeks after surgery, the above-mentioned antibiotic treatment was given during three consecutive days.

Maintenance

All animals were kept in plastic cages of 14 × 12 × 7 in., on sawdust and at a room temperature of 72° F. Tap water and standard rat pellets (Purina Fox) were supplied ad libitum. One parabiotic pair was kept per cage. The independent control animals were kept two per cage, sexes separated. The animals were allowed

TABLE 1
ASSIGNMENT TO GROUPS

Group I:	Single Controls	38 males 27 females
Group II:	Control Parabionts (of equal age)	65 pairs of males 61 pairs of females
Group III:	Parabionts of unequal age	58 pairs of males 51 pairs of females

to live out their natural life span. When one member of a parabiotic pair died, the other died within three or four days. At the end of the experiment, when most of the animals had died, the survivor was sacrificed when the other member was found dead.

Results and Discussion

In all animals having undergone parabiosis surgery, mortality was attributable to four factors: acute surgical trauma, the effect of sustained parabiosis on those animals surviving the acute surgical trauma, the death of the parabiotic partner, and the effect of conditions associated with old age.

As in most studies of the use of parabiosis, an appreciable number of animals were lost during or within the first three weeks after surgery. The risk of acute death is present as long as hair has not grown again in the skin areas involved in surgery, as inflammatory reactions are noticeable in the tissue bridge between the parabionts or as the skin sutures are not yet extruded; after this critical three-week period, long-term survival is assured. The numbers of parabionts lost during acute surgical trauma are: 23 (35%) of the homochronic males, 33 (54%) of the homochronic females, 19 (33%) of the heterochronic males, and 19 (37%) of the heterochronic females. No significant difference at the 10% level occur between the

TABLE 2
CUMULATIVE PROPORTION OF POPULATION SURVIVING TO END OF PERIOD

Period	Single Control Rats		Homochronic Parabionts		Heterochronic Parabionts (Older Member of Pair)	
(Age in Days)	Males	Females	Males	Females	Males	Females
250–300	1.00	1.00	1.00	1.00	1.00	1.00
300–350	1.00	1.00	1.00	0.96	1.00	1.00
350–400	1.00	1.00	0.95	0.86	1.00	1.00
400–450	1.00	1.00	0.93	0.86	1.00	1.00
450–500	1.00	1.00	0.88	0.82	1.00	1.00
500–550	0.97	0.85	0.79	0.75	0.97	1.00
550–600	0.87	0.81	0.69	0.64	0.85	0.97
600–650	0.71	0.78	0.43	0.57	0.77	0.94
650–700	0.58	0.44	0.33	0.54	0.69	0.91
700–750	0.51	0.37	0.19	0.39	0.51	0.50
750–800	0.36	0.26	0.17	0.25	0.31	0.41
800–850	0.23	0.22	0.12	0.18	0.31	0.41
850–900	0.18	0.19	0.07	0.11	0.26	0.41
900–950	0.13	0.15	0.05	0.11	0.21	0.19
950–1000	0.10	0.11	0.00	0.04	0.15	0.19
1000–1050	0.05	0.04	0.00	0.00	0.13	0.16
1050–1100	0.03	0.04	0.00	0.00	0.05	0.09
1100–1150	0.03	0.04	0.00	0.00	0.00	0.03
1150–1200	0.00	0.00	0.00	0.00	0.00	0.03

various groups, using either χ^2 corrected for continuity or not. Since there is no sham parabiosis possible, the comparison between parabionts and single control animals in terms of survival rests on the assumption that the parabionts lost during the intial three-week period are a random sample of all animals assigned with respect to mortality. Two considerations justify this assumption. First, the animals used in this experiment are highly inbred and disclose therefore little biological variation. Second, the most variable and least controllable feature of the experiment is the quality of parabiosis surgery; thus, surgical deaths are mainly due to factors independent of the animals.

It is also assumed that the antibiotic treatment given to the animals undergoing surgery has no effect on survival other than the protection afforded during the postoperation period.

The long-term survival data of the various experimental groups are set out in TABLE 2. These data provide the basis of the actuarial comparisons. These comparisons were done in two distinct but closely related ways that illustrate different

TABLE 3
LIFE EXPECTANCIES

Group	Sex	Estimated Expected Length of Life	S.E.
I. Single Controls	M	767	±25.3
	F	740	±33.1
II. Control Parabionts (homochronic)	M	665	±22.6
	F	678	±36.4
III. Heterochronic Parabionts	M	785	±27.6
	F	835	±30.3

aspects of the data. The first is the estimated expected length of life, the second percent cumulative mortality.

The estimated expected length of life was computed together with its standard error following methods described by Kaplan and Meier.[4] The results are shown in TABLE 3. Parabiosis *per se* was distinctly life-shortening: the animals of Group II have a decreased life expectancy when compared with the single controls ($p < 0.05$). The expected length of life is nearly the same in the single control males and in the older member of the male heterochronic pairs, which suggests that, in the male rats, the parabiotic union with a younger animal cancels out the life-shortening effect of parabiosis. In contrast, the older members of the female heterochronic pair significantly outlive both the single female controls and the homochronic female control parabionts ($p < 0.05$).

The second approach to the data uses directly the percent cumulative mortality curves (FIGURES 1 and 2) and is based upon Smirnov's two sample test.[1] The results

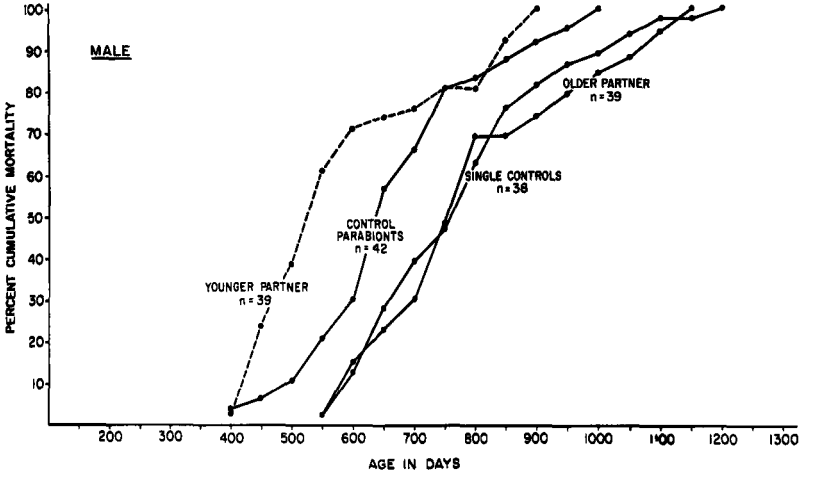

FIGURE 1. Percent cumulative mortality of male single controls, homochronic parabionts, and heterochronic parabionts.

are analogous to those obtained with the estimated expected length of life. The older member of the male heterochronic pairs did not differ significantly from the single control groups (p 0.15). However, the older member of the female heterochronic pair follows a survival curve that is significantly at the right side of the single females as was the parabiosed controls ($p < 0.05$). Furthermore, the curve of the homochronic male control parabionts is significantly at the left side of that of the single male controls.

These observations are consistent with the assumption that heterochronic parabiosis in this rat strain may increase the life expectancy of the older member of the pair. In the female animals, this phenomenon can be observed directly; in the males it is masked by deleterious side effects of the parabiotic state. It should be noted, however, that increase of life expectancy is not equivalent to decelerated aging: the younger member of the heterochronic pair may enhance survival of the older by protecting it against disease not directly attributable to senescence. For

instance, defense against infectious disease is to a large extent insured by relatively stable soluble or cell-bound agents, or both, which circulate in the peripheral blood and may reach equilibrium between a pair of parabionts within a few minutes. If aging entails qualitative or quantitative changes of such circulating agents, the older member of the heterochronic pair will benefit by the greater resistance of the younger animal to infection.

On the other hand, the possibility that actual age-related conditions may be attenuated by parabiosis with a younger syngeneic organism is suggested by recent work of Hruza,[3] who showed that heterochronic parabiosis with inbred Fisher rats increases cholesterol turnover in the older animal and reduces deposition of this compound in blood vessels and muscles to rates comparable to those of the younger.

Clearly, before any attempt to identify the effect of the younger on the older organism, one must ascertain that the older member of the heterochronic pair shows indeed less degenerative change than one would expect on the basis of its chronological age. This cannot be done by means of mortality studies but requires measurement of nonactuarial endpoints of aging in heterochronic parabionts and their controls sacrificed at preselected time intervals. Studies of this nature are in progress in our laboratory.

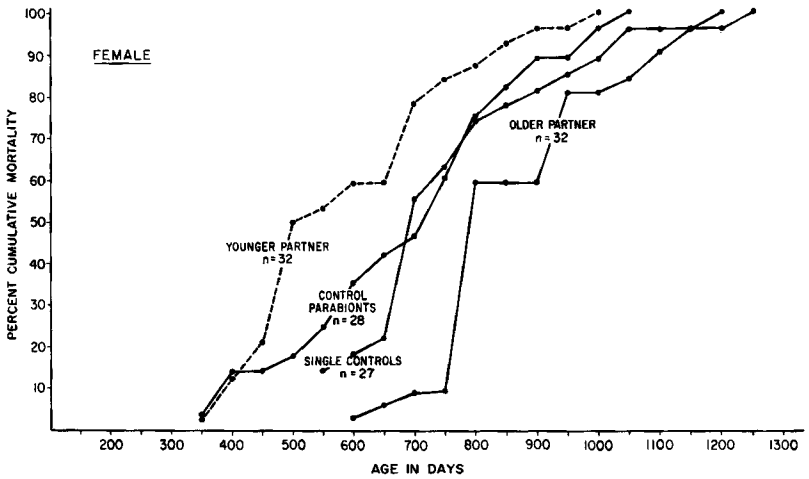

FIGURE 2. Percent cumulative mortality of female single controls, homochronic parabionts, and heterochronic parabionts.

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