Progeny recovered from X-irradiated st Ki p^p females mated to ri; ++ compound-males and results of testcrosses

Experiment	Recovered fly	Tested with	Theoretically expected and actually recovered genotypes of progeny 1	2	3	4
A	ri; Ki p^p	+; ++	ri; ++ (46)	+; Kip^p (54)	+; ++ (2)	ri; Ki p^p (0)
B	ri; Ki p^p	st; ++	ri; ++ (5)	st; Ki p^p (5)	st; ++ (0)	ri; Ki p^p (0)
B	st; ++	ri; + p^p	st; + p^p (13)	ri; ++ (10)	ri; + p^p (0)	st; ++ (0)
C	st; ++	ri; ++	st; ++ (20)	ri; ++ (22)	–	–
C	st; ++	ri; ++	st; ++ (34)	ri; ++ (34)	–	–

Figures in brackets refer to actually recovered genotypes of progeny.

zygotes are lethal because of aneuploidy[3]. A few offspring can be obtained if nondisjunctional maternal pronuclei come together with an appropriate type of sperm[4]. If the same experiment is repeated with X-rayed females, additional progeny will be obtained. Most of the additional offspring will contain 1 paternal compound chromosome and a newly induced compound of maternal origin[5]. Exceptionally, flies carrying 2 newly induced maternal compounds can be recovered[5].

In our experiments, females (2L *2R; 3L *3R) homozygous for the following markers on chromosome 3 were used: scarlet (st 3-44.0), Kinked (Ki 3-47.6) and pink peach ($_{p}^{p}$ 3-48.0). For a detailed description of the genetic markers see LINDSLEY and GRELL[1].

Five-day-old virgin females were irradiated with 400 R (50 keV X-rays, 520 R/min) and crossed to C(3L)RM, ri; C(3R)RM, +males (C = compound; RM = reversed metacentric; ri = radius incompletus 3-47.0). 2 flies with the phenotype ri; Ki p^p and 3 with st; ++ were recovered. All 5 proved to be fertile in crosses with compound-three-partners. The progeny is listed in the Table.

The low frequency of progeny of the 3rd and 4th type (column 3 and 4 in the Table, sometimes called parent-types[6]) can be explained by the segregation behaviour of compound chromosomes in the female meiosis[6] as it is predicted by the distributive pairing hypothesis[2].

The progeny obtained shows that the marker Kinked is located on the parental C(3R)RM chromosome, which is homozygous for pink peach. Therefore the centromere is situated left of the Kinked-locus. HOLM et al.[7] showed evidence for the position of the centromere between the markers eagle (eg 3-47.3) and deformed (Dfd 3-47.5).

Today two hypotheses on the origin of compound chromosomes are discussed: 1. Misdivision of the centromere[5,8] and 2. two-break-aberrations[9,10]. The data presented in this paper do not allow us to distinguish between these two possibilities.

Zusammenfassung. Homozygote *Drosophila-melanogaster*- Weibchen, genetisch markiert mit scarlet (st), Kinked (Ki) und pink peach (P^p) wurden bestrahlt, um Compound-3-Chromosomen herzustellen. Es wurden ausschliesslich st/st- und P^p Ki/Ki(?) P^p-Compoundchromosomen gefunden. Dies zeigt, dass das Zentromer links der Markierung Kinked liegt.

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Enhanced Survival of Germfree Mice after Infection with Irradiated Scrapie Brain

The nature of the etiological agent of scrapie is an enigma. The high resistance to irradiation, heat, and chemical disinfectants (e.g., formalin) distinguish the agent of scrapie from other known living disease-producing agents[1]. The irradiation data has been interpreted by some workers to indicate that the scrapie agent is devoid of nucleic acid[2].

An hypothesis to explain the activity of the extremely resistant material present in the brain homogenate of scrapie infected animals is that this material is an inducer of a latent virus already present in the brain of uninoculated animals, and once induced, the virus makes more inducer substance. Treatment with formalin or high irradiation levels could well be ineffective in inactivating a peptide inducer for example; yet the scrapie agent itself could be a nucleic acid directed replicating system conforming to the current dogma.

A possible way to test the above hypothesis is by the use of germfree animals. By virtue of their caesarian delivery and germfree maintenance, such animals harbour fewer viruses than do their conventional counterparts, although it has been shown that germfree mice carry the virus of latent leukemia[3].

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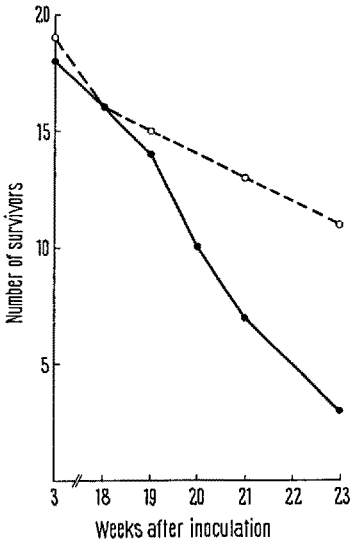
Comparison of clinical appearance with degree of astrogliosis in germfree and conventional mice

Mice		Clinical	Astrogliosis
Germfree	1	—	—
	2	—	+++
	3	—	+—
	4	+—	+
	5	+	+
	6	+++	++
	7	++	+++
	8	+—	+++
	9	+++	++
	10	+++	+++
	11	+++	+++
Conventional	1	+++	+++
	2	+++	++
	3	+++	+++

—, negligible; +—, suspect; +, mild; ++, obvious; +++, severe.



Survival curves for the germfree and conventional groups of mice.

In current experiments, mouse-adapted scrapie material obtained from the A.R.C. Animal Disease Institute, Compton, England, was passed once through the mouse strain CD1 used in this study. A $^1/_{10}$ brain homogenate of scrapie-infected CD1 mice was lyophilized and irradiated at 6*M* rad using a linear accelerator at a level of 8 Mev in order to kill contaminating bacteria and viruses. This level of irradiation has been shown to reduce the infectivity of mouse-adapted scrapie by approximately one half[4]. The irradiated material was then introduced into a germfree plastic isolator, reconstituted with sterile pyrogen-free distilled water to give $^1/_{10}$ dilution of the original mouse brain, and 0.05 ml was injected intracerebrally into 25 weanling male germfree mice lightly anesthetized with halothane. The remaining material was similarly injected into 25 conventional mice of the same age, sex and strain as the germfree group (all mice were obtained from the Charles River Breeding Laboratories, Wilmington, Mass., USA), also housed in a plastic isolator. 6 mice of the germfree and 7 of the conventional group died within 3 weeks, presumably as a result of the intracerebral injection, and were eliminated from the experiment.

The mice were observed for a period of several months, the survivors killed, their brains removed, fixed in 10% buffered formalin and stained by the Cajal gold sublimate method. At the termination of the experiment the germfree animals were found to be bacteria and fungi free by a series of standard tests[5].

The survival curves for the germfree and conventional groups are shown in the Figure. The germfree group survived to a much greater extent ($^{11}/_{19}$) than did the conventional mice ($^3/_{18}$) for the 23 weeks of experiment ($P > 0.05$). In addition, at termination 5 germfree mice were normal in appearance, while the remaining 6 showed varying degrees of the hunched, poor groomed appearance indicative of advanced scrapie in mice. The 3 conventional survivors all showed these symptom of advanced scrapie (Table). Histological analysis of the brains of the surviving animals revealed characteristic hypertrophy of astroglia[6]. The degree of astrogliosis in general paralleled the clinical appearance of the animals, i.e., the greater hunched stanced and poor grooming, the greater the degree of astrogliosis, although there was an occasional instance of disparity, e.g., animal GF 2.

In summary we have found a difference in the response of germfree and conventional mice to infection by irradiated scrapie material. While these results must be regarded as preliminary, it would appear that microbial agent(s) can intensify the disease process of scrapie.

Further experimentation using germfree animals is needed to delineate the microbial factor(s) which can enhance scrapie and to determine whether they have a direct or indirect effect. In our experiment 6*M* rad was chosen to 'sterilize' the infected brain materia, and many germfree mice developed scrapie. This may mean that 6*M* rad was not sufficient to kill contaminating 'virus' and that higher irradiation levels may be necessary. An alternative approach may be to use germfree rats in which the presence of viruses has not been detected. The delineation of the microbial factors involved in the disease process of scrapie may lead to the elucidation of the agent itself.

Zusammenfassung. Nachweis, dass nach Injektion mit β-bestrahltem «Scrapie»-Hirn keimfrei aufgezogene Mäuse länger als normale überleben. Das Ausmass der Astrogliose war im allgemeinen der Schwere der klinischen Symptome proportional. Die Theorie wird gestützt, dass das hochgradig hitze-, strahlungs- und enzymbeständige Material «Scrapie»-infizierter Mäuse die Induktion eines latenten Viruses bewirkt.

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