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Testing mutagenicity in mice by scoring agonistic behaviour of males

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Mutagenitätstest bei Mäusen durch numerische Erfassung des agonistischen Verhaltens der Männchen

S y n o p s i s : Männliche F_1-Nachkommen von bestrahlten NMRI-Albinomäusen (Gammabestrahlung der Spermatozoen mit 600 R bei einer Dosisleistung von 30 R/min) wurden mit männlichen NMRI-Mäusen unbestrahlter Väter für die Dauer von 24 Stunden zu 34 Paaren zusammengesetzt. Dabei hatte jeweils ein F_1-Männchen der bestrahlten Serie (I) mit einem gleichschweren, gleichaltrigen und unbekannten F_1-Männchen der unbestrahlten Serie (C) zu kämpfen. Die Auszählung der Läsionen am Körper und am Schwanz ergab eine signifikant höhere Zahl von C-Männchen mit verwundeten Schwänzen und eine statistisch gesicherte größere Zahl von Bissen bei den C-Männchen als bei den I-Männchen, was auf eine höhere Kampffähigkeit der F_1-Männchen der bestrahlten Serie hinweist. In einem weiteren Versuch wurde die Kampffähigkeit von 37 Männchenpaaren einer Kontrollgruppe gemessen, die aus unbestrahlten NMRI-Zuchten stammten. Die Kampffähigkeit dieser Männchen entsprach der Kampffähigkeit der C-Männchen des ersten Versuches. Der Einfluß der geringeren Wurfgröße in der Nachkommenschaft bestrahlter Männchen auf das spätere agonistische Verhalten der Tiere als auch eine mögliche Abhängigkeit der Kampffähigkeit der F_1-Männchen der bestrahlten Serie von einer durch die paternale Bestrahlung induzierten Translokationsheterozygotie werden diskutiert.

Introduction:

All conventional methods hitherto available to test mutagenicity of environmental chemicals or of ionizing radiation in vertebrates require many animals and are therefore expensive in money and time. Also, they are mostly concerned with the measurements of gross chromosomal rearrangements. Therefore, new methods are extremely desirable. One possibility concerns characters depending on polygenic sy-

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stems. Among these, the introduction of behavioural methods into mutation research seems to be one promising possibility. Significant differences between offspring of mutagenically treated and sham-treated teleost fish already have been demonstrated (HOLZBERG and SCHRÖDER, 1972, 1975; SPIESER and SCHRÖDER, 1975). Relevant data on mammals are of particular interest to estimate the genetic risk with respect to behavioural features and possibly other quantitative characters. For this purpose a series of experiments dealing with the learning ability and agonistic behaviour of laboratory mice *(Mus musculus)* has been performed at the Neuherberg Institute. Since the basic research of LAGERSPETZ (1969) and LEVINE et al. (1965) the genetic cause of differential male aggressiveness in different mouse strains is known. The present report presents the first data concerning agonistic behaviour.

Material and Methods:

The present preliminary report compares data for the agonistic behaviour of NMRI males derived either from irradiated (I) or from untreated (C) fathers which were mated to untreated females of the same origin (Neuherberg NMRI strain). All the mice were bred and kept in Macrolon boxes and were 10 - 12 weeks old when mated. These boxes contained the animals which were collected for further breeding tests as well as the couples or dams with their young. The stable rooms were completely air - conditioned, at a temperature of 24 ± 2°C and atmospheric humidity of 55 - 60%. The artificial illumination was set automatically to a 12-hour rhythm. By this means, the seasonally-caused variation in average litter-size did not reach a significant level.

The Neuherberg NMRI strain is kept as a specific-pathogen free mouse strain. To obtain this strain, pregnant NMRI females were sacrified a few hours before animal delivery. The young mice were taken by hysterectomy and were then nursed by germ-free foster mother in isolators equipped with bacterial filtration. After weaning, these founder animals were transferred into specific-pathogen free stable rooms which are protected against bacterial infiltration by a barrier system. Besides this, the specific-pathogen free mice were under regular bacteriological and virological control performed by Dr. V. Erfle. Under these conditions, the animals were free of all common pathogenic bacteria and viruses. They were fed by ALTROMIN mouse pellets and watered automatically.

NMRI males were irradiated with 600 R of Cs-gamma rays at a dose-rate of 30 R/min, measured by a Victoreen dosimeter. During the exposure, only the pelvic region was irradiated directly while the head and the front part of the trunk were shielded with lead 10 - 12 mm thick. To use irradiated spermatozoa for fertilization of the ova, irradiated and sham-treated NMRI males were mated to untreated NMRI females during the first week after exposure (OAKBERG, 1957). Thirty-four 10 to 12 weeks old male F_1 offspring of either series were put together for 24 hours (from 10 a.m. to 10 a.m. next day), each box (25x20x14 cm) containing one pair of males, i.e. one I and one C male. Because the males had been kept isolated since weaning, the two males of a given pair were unknown to each other and unexperienced in fighting before being tested. After separation of the paired males, the number of wounded sites («sores») at the tail and at other parts of the body was counted according to a proposal of LEVINE et al. (1965). In order to prevent home-cage effects, both males were put together into a new Macrolon box at the same moment. The weight differences between the two males of a pair varied between 0 and 3 g, the absolute values of weights of the 64 male mice ranging from 37 to 47 g.

A second experimental series of thirty-seven pairs of NMRI males both without any parental treatment served as controls. These 74 individuals were 14 weeks old when put together for scoring their agonistic behaviour. The weight differences between the males of a pair varied between 0 and 1.2 g, the absolute values of weights of these 74 control males ranging from 32 to 54 g.

Results:

(I). *F_1 males:*

The number of wounded mice with any lesion was significantly higher in the C than in the I series. This holds also true for the number of males with wounded tails as well as for the number of wounded sites («sores») at the tails revealing that the untreated F_1 males (C) received more wounds than the 600 R F_1 males (I), or, expressed the other way around, the I males were more successful in delivering bites than the C ones (Table 1). No significant differences between the two treatment groups were found as to the number of males with other lesions than wounded tails and to the number of males with both types of lesions, i.e. wounded tails plus any additional lesion. Though weight differences between the pairs of males were minimized as far as possible, males with more sores at their tails than their partners were found to have the same (1 case), higher (5 cases) or lower (25 cases) weight than their less wounded companion. Thus, this result indicates a possible influence ($p<0.05$ by use of the sign test) of weight on the success of fighting in this experiment. To estimate this influence, 15 males of the I series were once more put together with 15 other males of the C series, the males of each pair again being unknown to each other. In this second experiment which was carried out four weeks after the first one, all winners of the first experiment had to fight against heavier partners whereas the losers of the first experiment were put together with lighter companions. Under these conditions no correlation was found between the weight of the animals and their success in fighting. Males with less sores than their partners were found to be heavier in 4 male pairs, lighter in 7 pairs, and of equal weight in 1 pair ($\hat{T}=3$; $2\sqrt{n}=6.62$). No dif-

Table 1: Agonistic behaviour of NMRI F_1 male mice derived from spermatozoal gamma irradiation with 600 R (I) directed to untreated NMRI males (C).

CRITERION OF AGONISTIC BEHAVIOUR	Series	Number*	Mean ± S.E.**	95%-Confidence limits of the mean	χ^2 P
Males with wounded tails	I	5	0.15 ± 0.06	0.02 – 0.28	12.45 0.0004
	C	24	0.71 ± 0.08	0.54 – 0.87	
Wounded sites (»sores«) at the tails	I	21***	0.62 ± 0.44	0.00 – 1.54	284.85 $< 10^{-10}$
	C	343***	10.09 ± 2.25	5.36 – 14.81	
Males with lesions other than wounded tails	I	5	0.15 ± 0.06	0.02 – 0.27	0.09 0.76
	C	6	0.18 ± 0.07	0.03 – 0.32	
Males with both types of lesions (wounded tails plus any additional lesion)	I	2	0.06 ± 0.04	0.00 – 0.15	0.20 0.65
	C	3	0.09 ± 0.05	0.00 – 0.19	
Wounded males with any lesion	I	8	0.24 ± 0.07	0.08 – 0.39	10.31 0.0015
	C	27	0.79 ± 0.07	0.65 – 0.94	
Males without any detectable lesion	I	26	0.76 ± 0.07	0.62 – 0.90	10.94 0.001
	C	7	0.21 ± 0.07	0.07 – 0.35	

*) 34 NMRI males of each series tested.
**) The mean was calculated by the formula:

$$\frac{\text{Number of scores}}{\text{34 males tested}}$$

***) Number of scores at the tails, compared by the Mann-Whitney U test:

$$U = 69;\ n_I = 34;\ n_C = 34;\ z = -6.24;\ p < 0.00003.$$

Table 2: Agonistic behaviour of NMRI control males directed to untreated NMRI males.

CRITERION OF AGONISTIC BEHAVIOUR	Number *	Mean ± S.E. **	95%-Confidence limits of the mean
Males with wounded tails	21	0.28 ± 0.05	0.18 – 0.38
Wounded sites (»sores«) at the tails	77	1.04 ± 0.37	0.30 – 1.78
Males with lesions other than wounded tails	4	0.05 ± 0.03	0.00 – 0.11
Males with both types of lesions (wounded tails plus any additional lesion)	4	0.05 ± 0.03	0.00 – 0.11
Wounded males with any lesion	25	0.34 ± 0.06	0.22 – 0.46
Males without any detectable lesion	49	0.66 ± 0.06	0.54 – 0.78

*) 74 NMRI control males (37 pairs of males) tested.
**) The mean was calculated by the formula:

$$\frac{\text{Number of scores}}{\text{74 males tested}}$$

ference between the number of sores of the two males were determined in 3 pairs. There was also no significant correlation between defeats (more sores than the partner) or victories (less sores than the partner) in the second experiment with defeats or victories in the first experiment as shown by use of the sign test. Concordance between the two experiments was found in 7 cases, discordance in 8 cases. This indicates that a victory in the preceding fight did not increase the chance to be successful in the second one. The number of wounded sites at the tails amounted to 45 (I) and 79 (C) for the 15 males of either series, corresponding to mean values of 3.00 ± 1.32 for I males and 5.27 ± 2.41 for C males. This difference is statistically significant ($X^2=9.32$, $P=0.0027$), again demonstrating the higher fighting ability of I males in spite of their lower average weight. In this second experiment, all the further criteria for agonistic behaviour did not reach significance.

(II). *Control males:*

There was no effect of weight on the success of fighting in the 37 pairs of control males. The male which delivered more bites than its companion («winner») was heavier in 7, lighter in 9 and of the same weight in 6 out of 22 cases. No wounded sites at the body and tail could be observed in 15 out of 37 paired control males.

The comparison of the numerical values for the agonistic behaviour of the control males (Table 2) with those of the untreated C males of the first experiment (Table 1) revealed significant differences, though these males were expected to exert similar fighting ability. These differences may be explained in terms of the different aggressiveness of the corresponding partners, i.e. NMRI male mice are more aggressive when fighting against a companion of similar aggressiveness (control males to each other, cf. Table 2) than against more aggressive partners (C versus I males, cf. Table 1).

Discussion:

Because the 600 R acute gamma-ray irradiation of spermatozoa induces a high degree of dominant lethality (cf. SCHRÖDER and HUG, 1971), F_1 males of the irradiated series were raised from smaller litters than males derived from sham-treated (C) or control fathers. This difference in juvenile environment might be an important factor in determing later male aggressiveness. Accordingly, the litter-size of all further experimental series should be equalized to the same level.*) However, the getic nature of the different aggressiveness of males derived from irradiated fathers could be proved by selective breeding of low and highly aggressive males, as it has already been shown to be the case for offspring of irradiated cichlid fish (HOLZBERG and SCHRÖDER, 1972, 1975). Cytological examination of low and highly

*) Added in proof: The repetition of these experiments with equalized litter-sizes did not give any significant deviation from the present results.

aggressive males now in progress should provide further evidence whether chromosomal rearrangements are associated with the increased fighting ability of F_1 males after irradiation of sperms with 600 R of acute gamma rays.

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