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Antimitotic Drugs in the Male Rat
Behavioral Abnormalities in the Second Generation

MAURICE R. AUROUX,* EMMANUEL JB. DULIOUST,* NABIL NY. NAWAR†, SAMEH G. YACOUB,† MARIE J. MAYAUX,‡ DANIEL SCHWARTZ,‡ AND GEORGES DAVID*

From the Laboratoire d'Histologie-Embryologie-Cytogénétique, Cedex, France, the Laboratory of Anatomy and Embryology, Faculty of Medecine, Ain-Shams University, Cairo, Egypt,† and INSERM, Sante Publique, Epidemiologie, Reproduction Humaine, Cedex, France‡*

The second generation descended from rats treated either with cyclophosphamide alone or with both cyclophosphamide and vinblastine were investigated. As in the first generation, the offspring were evaluated for mean litter size, sex ratio, frequency of gross external malformations and, within the first 4 months of life, growth and mortality. When they reached adulthood, between 12 and 16 weeks of age, the offspring were also tested for spontaneous activity and learning capacity. At birth, the progeny of the treated grandfathers did not show malformations or any other obvious disorder. However, when compared with the control population, the experimental animals showed significantly decreased success rates in a learning task, whatever the learning performance of their parents. Furthermore, decreased spontaneous activity was observed in the male subjects from unsuccessful parents. The similarities between the anomalies found in the first and the second generations argue for the induction of mutations by antimitotic drugs. This hypothesis and the subtle differences between generations and between sexes are discussed.

Key words: cyclophosphamide, male rat, spermatogonia, mutation, second generation, deaths, behavioral anomalies.

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Reprint requests: Maurice R. Auroux, M.D., Ph.D., Laboratoire d'Histologie-Embryologie-Cytogénétique, C.H.U. Bicetre, 94275 Le Kremlin-Bicetre, Cedex, France.

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The risks concerning the progeny of men treated with antimitotic drugs are now under investigation. Some epidemiologic studies have found an apparently higher percentage of congenital malformations in such cases than in the general population (Li and Jaffe, 1974; Russel et al, 1976). According to other authors, however, no difference has been noted (Senturia et al, 1985). It has been shown experimentally that treatment with cyclophosphamide, when administered to the male, results in a diminished number of embryos in the impregnated female (Fritz et al, 1973; Botta et al, 1974; Cooke et al, 1978). Furthermore, it has been found that a postmeiotic exposure of the male rat gamete to cyclophosphamide led to behavioral abnormalities in the prepubertal progeny (Adams et al, 1981, 1982; Fabricant et al, 1983). In our work, we have observed similar results after a premeiotic exposure to cyclophosphamide. Moreover, the effects seemed more obvious when

the treated male rats were mated soon after the treatment period; when mating occurred 60 days after the end of the treatment, we observed postnatal deaths in the offspring and the survivors later exhibited decreased spontaneous activity as well as decreased learning ability (Auroux et al, 1986). In contrast, when mating occurred 100 days after the end of the treatment, only learning impairment was observed (Auroux and Dulioust, 1985). The purpose of the present work was to investigate further the hypothesis that mutations are induced by antimitotic drugs by studying the transmissibility of the observed anomalies from the first to the second generation.

Materials and Methods

First Generation (F1)

The detailed description of the methods used in producing the first generation and the results concerning that generation have been published previously (Auroux et al, 1986). A summary is given below:

Producing the First Generation. Wistar rats (Parental generation, P) were mated 60 days after the end of a treatment with either cyclophosphamide alone in a dose of 10 mg per kg daily for 15 days (15 animals) or with a combination of cyclophosphamide (same regimen) and vinblastine, 250 μg/kg on the first day and 150 μg/kg on the eighth day (six animals). Eighteen control males received 0.2 ml of the solvent (distilled water).

Results in the First Generation. The study of the first generation (F1) concerned the mean litter size, the sex ratio, the number of external malformations and, during the first 4 months, the increase in weight and the mortality rate. At adulthood, between 12 and 16 weeks, spontaneous activity (open-field), emotionality (number of fecal boluses in the open-field), and learning capacity (conditioning avoidance response) were also studied. The comparisons with the control group showed that mortality was significantly increased within the first 40 days after birth and that at an adult age, spontaneous activity was significantly decreased and learning capacity was modified in two ways. First, the proportion of animals succeeding in the learning test (success rate) was lower than in the controls. Second, in the successful animals, the percentage of failures before learning was achieved (success mode) was higher than in the controls, although this difference was significant in the males only. No differences in the mortality rate or in behavioral abnormalities were observed between animals descended from males treated with either cyclophosphamide alone or with cyclophosphamide plus vinblastine.

Second Generation (F2)

Since no differences were found between the offspring from the two categories of treated rats, the offspring from these two categories were pooled to produce the second generation. At the age of 7 months, the future parents of the second generation were randomly chosen from among all the first generation animals. At the same time, the control parents were also chosen randomly. There were two types of matings in each group to control for a possible relationship between the learning characteristics of the parents and offspring, because earlier studies (Bovet et al, 1969) had shown a certain degree of heritability for such traits. The successful animals in each group were mated and the unsuccessful ones were mated to produce four subgroups of second generation subjects: two subgroups from control P males and two from treated P males. There were 32 couples in each subgroup, except in the unsuccessful control subjects where only 25 females were available. Two hypotheses were therefore considered: either there was a possible relationship between parent and offspring learning capacity, which would introduce a bias, and thus reduce the ability to demonstrate the transmissibility of the disorders from the first to the second generation, or there was an effect of the grandfathers' treatment. This was our main hypothesis. It should be noted that both of these hypotheses were applied to the population obtained from the treated males, while only the first one was tested in the control population.

The couples were left together for 8 days in 20 × 20 ×18-cm cages. The general conditions of breeding and the identification of the pregnant females corresponded to methods previously described (Auroux and Dulioust, 1985; Auroux et al, 1986). Each of the four subgroups and their corresponding progeny were designated with a coded letter, so that the researcher could not know the actual identity of the animals. At the end of gestation, the mothers were examined twice daily, and the newborn rats were counted. Births took place over 6 days. On the 4th day, the pups were carefully inspected, the sex ratio was determined on the living subjects and external malformations, neonatal deaths, and missing pups were recorded. At the same time, each litter was culled to four rats, two males and two females that were randomly chosen, except when the litter was made up of four rats only, whatever the sex ratio. However, when the litter was made up of less than four rats, it was completed from other litters to produce the sex ratio of two males and two females, although these rats were not siblings. Then, to control for postnatal maternal factors, each culled litter was given to a foster mother from one of the four subgroups. In this way, one litter produced by successful control parents could be adopted by an unsuccessful female produced by a treated male, etc. The mothers and their litters were then randomly distributed in the animal house.

At the age of 4 weeks, the young were weaned, the males and the females were separated and housed seven or eight per cage (40 × 40 × 18 cm). The cages were distributed randomly in the animal house. The animals were weighed at the age of 4 days, and at 5, 8, and 13 weeks. The behavioral tests were carried out between 12 and 16 weeks of age. Those tests have been described by Auroux et al (1986). They comprised a study of learning capacity in a device designed to condition avoidance responses (shuttle box) with, in each group, evaluation of the percentage of animals reaching the learning criterion (success rate) and, in the successful subjects, evaluation of the frequency of failures recorded up to and including conditioning (success mode), and a study of open field spontaneous activity.

In the present work, we used a device called Opto-varimex Columbus (Columbus Instruments International Corp., Columbus, OH). It consists of a rectangular box, 43.2 × 44.4 cm, whose adjacent perpendicular sides each have 15 infrared emitters. Each beam is 0.32 cm in diameter and is separated from the next one by a distance of 2.65 cm. The interrupted beams indicate the position of the animal. With the aid of a computerized system, the following parameters were calculated: a) the total distance covered by the animal and the time during which the animal moved, b) the time during which the animal remained still, and c) the number of stereotypic movements (movements on the spot). Moreover, another identical set of 15 beams, situated at a suitable distance above the first system, permitted the recording of the vertical movements of the animal.

The movements were recorded by the computerized system on paper. To avoid overly complex final images, the duration chosen for the test was 3 minutes. Each rat began the experiment 1 minute after being placed in the box. The device was uniformly illuminated by a 20-W neon lamp, located at 170 cm above the center of the device.

The study of emotionality by the number of fecal boluses left in the open field was not performed in this experiment because no difference was ever found in our previous studies (Auroux, 1983; Auroux and Dulioust, 1985; Auroux et al, 1986).

Statistical Tests

Comparisons were made, first, within each group (control or experimental) between the two subgroups, second, between the pooled controls (see Results) and each experimental subgroup, and finally, as a verification, between the experimental subgroups and the unpooled homologous controls. In each case, overall comparisons were made before comparing two groups separately.

Quantitative results were compared by analysis of variance and student's t test when the variances were not significantly different, or when the number of subjects exceeded 30. Wilcoxon's test was used in the other cases. Qualitative results were compared using the χ^2 test.

Results

The second generation of rats descended from control grandfathers were named F2C, while those from treated grandfathers were named F2T. The corresponding parents were F1C and F1T. F2C+ and F2C− subgroups were sired by the control F1 which were, respectively, successful and unsuccessful in the learning test. In the same way, F2T+ and F2T− subgroups were the offspring of, respectively, successful and unsuccessful F1T animals.

Comparisons Between Subgroups

Whatever the parameter, no significant difference was found between the control subgroups (Tables 1, 2, 3). Therefore, under our experimental conditions, the heritability for learning performance or possibly related characteristics seemed unlikely to distort comparisons with the experimental groups (in the control males, the success rate actually was slightly higher in the subgroup from the unsuccessful parents). The two control subgroups were then pooled. It must be noted that pooling the controls did not modify the results of the comparisons with the experimental subjects, except for postnatal mortality. On the other hand, a significant difference between the F2T subgroups was observed in the spontaneous activity of the males (F2T+/F2T−: $P < 0.05$; Table 3). As discussed below, this difference possibly resulted from induced genetic differences, and this led us to separate the F2T subgroups in all the comparisons with the controls, whether pooled or not.

Comparisons Between Control and Experimental Groups

Fertility of the First Generation Couples (Table 1). No difference was observed between the different groups.

General Characteristics in the Second Generation (Table 1). The incidence of mortality was the only significant difference between the groups. Deaths were recorded by counting carcasses (53%), the remains of bodies where the sex was not identifiable, and by counting the number of remaining living young. Litters in which all the pups died were not considered. The number of sporadic postnatal deaths between birth and 4 days of age was significantly increased in the F2T− when this group was compared with the pooled controls. The difference, however, was not significant if the controls were not pooled. Mortality was not increased in the F2T+.

Behavioral Study

Learning Capacity (Table 2). The results of the learning test were unrelated to the performance of the parents in both control and experimental subjects. The success rate was significantly decreased in the F2T+ and in the F2T− males, whether compared with pooled or unpooled controls. In both the F2T+ and the F2T− females, the success rate was lower than in the control females, but the differences were not significant. No significant difference was found in the success mode, either between subgroups or between each F2T subgroup and the controls.

Spontaneous Activity (Table 3). To simplify the protocol, this experiment was carried out on 30 males and 30 females (randomly chosen) in each of the four

TABLE 1. General Characteristics*

	F_2C			F_2T		Statistical Tests	
	C+	C-	Cp	T+	T-		
Pregnancies: No%†	27/32 84.4	19/25 76	46/57 80.7	21/32† 65.6	23/32 71.9	$Cp/T^+/T^-$: $X^2 = 2.58$ NS	$C^+/C^-/T^+/T^-$: $X^2 = 3.10$ NS
No per litter at birth (±SD)‡	9.5 ± 2.6	8.8 ± 3.4	9.2 ± 2.9	8.6 ± 3.2	10.1 ± 4.1	$Cp/T^+/T^-$: $F^2_{86} = 1.1$ NS	$C^+/C^-/T^+/T^-$: $F^3_{85} = 0.91$ NS
Sex ratio							
M	119	59	178	71	83	$Cp/T^+/T^-$: $X^2 = 1.41$ NS	$C^+/C^-/T^+/T^-$: $X^2 = 2.32$ NS
F	99	61	160	70	93		
Malformations	-	-	-	-	-		
Deaths (M + F)							
No, %						0–4 days	
0–4 days	14/225 6.2	12/133 9.0	26/358 7.3	17/158 10.8	28/204 13.7	$Cp/T^+/T^-$: $X^2 = 6.3$, $P < 0.05$ Cp/T^+: $X^2 = 1.75$, NS	$C^+/C^-/T^+/T^-$: $X^2 = 7.03$ NS
5–40 days	1/88 1.1	0/60 -	1/148 0.7	0/70 -	1/88 1.1	Cp/T^-: $X^2 = 6.25$, $P < 0.02$	C^-/T^-: $X^2 = 1.7$ NS
Mean weight per litter ± SD							
at 4 days	9.9 ± 1.2	9.6 ± 1.4	9.8 ± 1.2	9.9 ± 1.1	10.5 ± 1.6	$Cp/T^+/T^-$: $F^2_{69} = 2.54$ NS	$C^+/C^-/T^+/T^-$: $F^3_{68} = 1.75$ NS
Individual mean weight ± SD§							
at 13 weeks							
M	337.2 ± 31.0	337.1 ± 26.4	337.1 ± 29.0	332.7 ± 28.6	337.9 ± 35.9	$Cp/T^+/T^-$: $F^2_{148} = 0.31$ NS	$C^+/C^-/T^+/T^-$: $F^3_{147} = 0.2$ NS
F	167.4 ± 14.1	166.5 ± 15.1	167 ± 14.5	167.1 ± 14.7	168.6 ± 14.4	$Cp/T^+/T^-$: $F^2_{150} = 0.18$ NS	$C^+/C^-/T^+/T^-$: $F^3_{149} = 0.4$ NS

*Separate tests are presented only when overall comparisons revealed significant differences. C^+, C^-, CP = control parent successful, control parent unsuccessful, pooled controls, respectively. T^+, T^- = treated grandfather, and parents successful or unsuccessful in the learning test.

†1 pregnant female died before delivery; ‡Living and dead pups; §Animals were weighed individually at 5, 8 and 13 weeks of age. No differences were observed at 5 and 8 weeks.

TABLE 2. Learning Capacity

	F_2C			F_2T		Statistical Tests	
	C+	C-	Cp*	T+	T-		
Male							
Success rate (No, %)	29/43 67.4	22/30 73.3	51/73 69.9	15/35 42.9	20/43‡ 46.5	$Cp/T^+/T^-$: $X^2 = 9.71$, $P < 0.01$ Cp/T^+: $X^2 = 7.26$, $P < 0.01$ Cp/T^-: $X^2 = 6.21$, $P < 0.02$	$C^+/C^-/T^+/T^-$: $X^2 = 9.96$, $P < 0.01$ C^+/T^+: $X^2 = 4.74$, $P < 0.05$ C^-/T^-: $X^2 = 5.2$, $P < 0.05$
Success mode (± SD)	64.6 ± 8.6	59.1 ± 14.3	62.2 ± 11.6†	56.3 ± 13.3	56.1 ± 13.9	$Cp/T^+/T^-$: $F^2_{82} = 2.35$, NS	$C^+/T^+/C^-/T^-$: $F^3_{81} = 2.38$, NS
Female							
Success rate No, %)	33/44 75	19/30 63.3	52/74 70.3	18/35 51.4	23/43‡ 53.5	$Cp/T^+/T^-$: $X^2 = 5.04$, NS	$C^+/C^-/T^+/T^-$: $X^2 = 6.06$, NS
Success mode (± SD)	56.9 ± 14.9	56.5 ± 13.4	56.7 ± 14.3	56.7 ± 14.1	62 ± 12.3	$Cp/T^+/T^-$: $F^2_{89} = 1.28$, NS	$C^+/T^+/C^-/T^-$: $F^3_{88} = 0.85$, NS

*Pooled controls; whatever the parameter, no difference was found between C^+ and C^-, which then were pooled.

†C+ and C- males were pooled, though the variances were different, because the mean values were not different (Wilcoxon's test).

‡One animal was excluded because of a technical problem.

TABLE 3. Behavioral Study: Spontaneous Activity

	F_2C			F_2T			
	C+	C−	Cp*	T+	T−	Statistical Tests†	
Distance ± SD							
M‡	404 ± 276	397 ± 232	400 ± 25‡	384 ± 176	239 ± 129	$Cp/T^+/T^-$: $F^2_{107} = 6.19$, $P < 0.01$ Cp/T^+: $t_{107} = 0.32$, NS Cp/T^-: $t_{107} = 3.41$, $P < 0.001$ T^+/T^-: $t_{107} = 2.57$, $P < 0.01$	$C^+/C^-/T^+/T^-$: $F^3_{106} = 4.09$, $P < 0.01$ C^+/T^+§: $\epsilon = 0.09$, NS C^-/T^-: $t_{106} = 2.92$, $P < 0.01$
F	740 ± 228	648 ± 241	694 ± 237	650 ± 242	699 ± 263	$Cp/T^+/T^-$: $F^2_{116} = 0.4$, NS	$C^+/C^-/T^+/T^-$: $F^3_{115} = 1.22$, NS
Time resting ± SD							
M‡	103 ± 36	101 ± 32	101 ± 34	99 ± 29	120 ± 25	$Cp/T^+/T^-$: $F^2_{107} = 4.35$, $P < 0.025$ Cp/T^+: $t^{107} = 0.37$, NS Cp/T^-: $t^{107} = 2.63$, $P < 0.01$ T^+/T^-: $t^{107} = 2.54$, $P < 0.02$	$C^+/T^+/C^-/T^-$: $F^3_{106} = 2.89$, $P < 0.05$ C^+/T^+: $t^{106} = 0.44$, NS C^-/T^-: $t^{106} = 2.42$, $P < 0.02$
F	59 ± 22	68 ± 24	63 ± 23	74 ± 33	65 ± 25	$Cp/T^+/T^-$: $F^2_{116} = 1.7$, NS	$C^+/C^-/T^+/T^-$: $F^3_{115} = 2.48$, NS
Vertical movements ± SD							
M‡	9.4 ± 8.5	9 ± 6.9	9.1 ± 7.6	9.5 ± 5.5	5.6 ± 4.2	$Cp/T^+/T^-$: $F^2_{107} = 3.58$, $P < 0.05$ Cp/T^+: $t_{107} = 0.24$, NS Cp/T^-: $t_{107} = 2.43$, $P < 0.02$ T^+/T^-: $t_{107} = 2.25$, $P < 0.05$	$C^+/T^+/C^-/T^-$: $F^3_{106} = 2.38$, NS C^+/T^+§: $\epsilon = 0.84$, NS C^-/T^-: $t_{106} = 2.01$, $P < 0.05$
F	16.5 ± 7.4	14.7 ± 6.6	15.6 ± 7	13.7 ± 5.3	17.6 ± 6.7	$Cp/T^+/T^-$: $F^2_{116} = 2.5$, NS	$C^+/C^-/T^+/T^-$: $F^3_{115} = 2.47$, NS

*Whatever the parameter, no difference was found between C^+ and C^-, which then were pooled (Cp.).
†Separate tests in the females are not presented, as none of the overall tests reached the 5% confidence level.
‡A technical problem forced us to exclude five males in C^+ and T^+ subgroups.
§Wilcoxon's test.

subgroups. No difference was observed between the pooled controls and the F2T+ animals (males or females). By contrast, the three activity parameters showed significant differences between the F2T− males and either the control or the F2T+ males. The F2T− females did not differ from all the others.

Additionally, all results were checked for possible effects related to the origin of the foster mother. No significant variations were observed. As an example, the success rates in subjects fostered by C+, C−, T+ or T− females were, respectively:

Pooled controls: 10/19; 16/21; 7/8; 18/25. $\chi^2 = 4.31$, df = 3, NS.

F2T+: 5/13; 2/4; 4/12; 4/6. $\chi^2 = 2.01$, df = 3, NS.

F2T−: 6/14; 4/5; 3/12; 7/12. $\chi^2 = 5.23$, df = 3, NS.

As in the first generation, a preliminary analysis compared the progeny from the males treated by cyclophosphamide alone with the progeny from the males treated by cyclophosphamide and vinblastine. No difference was found.

Discussion

The changes observed in the experimental second generation, that is, behavioral anomalies and, possibly, increased postnatal mortality, and the lack of significant changes in other considered characteristics, were quite similar to the pattern of disorders observed in the first generation.

Postnatal Mortality

Postnatal mortality was significantly increased between birth and 4 days of age in the F2T− compared with the pooled controls, but the difference was not significant compared with the unpooled F2C−. Therefore, this anomaly deserves further investigation. However, since a parental bias seemed excluded, we felt it was better to consider the controls as a whole when comparing them with the experimental subjects.

As in the first generation, the animals died without showing any obvious disease, and the precise causes of death remained unknown. It can also be noted that, as in the first generation, embryonic lethality probably was not significantly increased, since the mean litter size was not reduced. The deaths occurred earlier and more frequently than in the first generation. However, these changes also affected the control group and suggested the influence of some deleterious factors in the environment.

Behavioral Abnormalities

Learning Capacity. No relationship between the learning performance of the parents and offspring was detected in the control population. This does not conflict with the heritability observed in other studies (Bovet et al, 1969) since more than one generation is needed to produce obvious effects. Actually, our results implied that the learning impairments in the experimental groups could not be explained by the natural inheritance of learning ability. Furthermore, the success rate was decreased not only in the F2T−, but also in the F2T+, whose parents were successful in the learning test. Different environmental conditions or foster mother influence seemed irrelevant in the present study. On the other hand, our results were consistent with the hypothesis of induced genetic differences between the experimental and control population.

The present results agree with the findings of Adams et al (1984) in prepubertal consanguinous rats. Additionally, our study showed that male-transmitted genotoxic effects could also concern non-consanguinous animals and, since adult subjects were considered, that long lasting behavioral impairments could result from such effects. Unlike in the first generation, the second generation females showed no significant behavioral impairments and the success mode was not modified. These differences between sexes and, possibly, between generations will be discussed below.

Spontaneous Activity. Decreased spontaneous activity was observed in the F2T−, while this parameter was not affected in the F2T+. Such a decrease was not likely to result from a parental effect alone, unrelated to the treatment of the grandfather, since it was not observed in the control population. On the contrary, it was consistent with the logical hypothesis that, in the experimental first generation, the unsuccessful subjects were affected by more deleterious or more numerous mutations than the successful animals. Confirmation of increased mortality in the F2T− would support this hypothesis.

Only the males were affected in the second generation, while in the first generation, both sexes had reduced spontaneous activity. Since the device used in this trial was different from the open field used with the first generation, and the test period was slightly shorter, one may question whether the present method was sufficiently sensitive. However, the three parameters studied were significantly modified in the males, but not in the females. Therefore, real differences between the sexes in the second generation appear likely.

A final remark should be made about the behavioral assessment used. Our main purpose in these experiments was to detect slight genetic effects, and we therefore chose two classic and rather simple behavioral tests. More elaborate tests are available to describe actual learning ability or general activity, and it would be very interesting if our studies are confirmed in experiments using different tests. Adams et al have already obtained similar results with different tests than ours.

Genetic Considerations

The presence of abnormalities in both subgroups of the second generation provide new support for the hypothesis that induced mutations can be inherited from the spermatogonia of treated males. Another argument is the fact that the most significant pattern of disorders was observed precisely in the subgroup obtained from the unsuccessful experimental subjects. However, the abnormalities were not exactly the same as in the first generation. The impaired spontaneous activity in the F2T− subgroup affected the males only, while both males and females were affected in the F1. Also, the success mode was not changed. Such complex alterations raise numerous problems, and several hypotheses can be considered.

Some maternal disorders affecting the pregnancies in the F1T females cannot be excluded. However, such disorders would not explain the alterations observed in the first generation, since the parental females were not exposed to cyclophosphamide. Such maternal factors could be further investigated by intercrossing the control and the experimental populations. Another explanation may be a spontaneous genetic shift. However, in the parental generation, all the subjects (males and females) had been tested for learning performance, and the unsuccessful animals had been discarded (Auroux et al, 1986). In the first generation, the frequency of sibling matings could not be known exactly, but it was estimated to be less than 3.8%. Moreover, the two populations (treated and control) were similar in size. Therefore, a spontaneous genetic divergence and/or consanguinity effects seem unlikely to explain the differences that we have observed.

Other hypotheses concern the possible transmission of cyclophosphamide-induced genetic lesions from the first to the second generation. Chromosomal rearrangements seem to be efficiently eliminated at meiosis and appear to be infrequently

inherited from the spermatogonial stages after a treatment like cyclophosphamide (Mohn and Ellenberger, 1976). However, a cytogenetic screening would be necessary in another study to rule out chromosomal abnormalities.

Gene mutations represent another possibility. In the second generation, either dominant or recessive mutations could have been involved (though sibling matings appeared to be rare), but the alterations found in the first generation suggest the possibility of dominant effects. This also should be investigated by intercrossing the control and experimental populations. The nervous system might be particularly susceptible to mutations because a great number of different mRNAs are found in the brain (Van Ness et al, 1979). Such an hypothesis might help to understand why deaths and behavioral impairments were observed together in two subsequent generations.

It was not possible to test the differences between generations because the experimental conditions were not the same. Overall, the disorders seemed to be attenuated in the second generation. However, the present data are not sufficient to conclude that such a decrease really occurred. These subtle changes need more extensive investigation. The differences between males and females are another puzzling aspect of these experiments, and were also observed in our previous trials with cyclophosphamide (Auroux and Dulioust 1985; Auroux et al, 1986) as well as in an experiment on the effects of paternal aging (Auroux, 1983). Such differences might result from a differential inheritance of mutations, as induced for instance by a selection against gametes bearing a damaged X chromosome in the testis of the treated males. However, they also might involve other more general differences between the male and female organism.

In conclusion, we wish to emphasize that the main anomalies appear to be transmissible from the first to the second generation with, perhaps, a particular genetic susceptibility of the male. Also, an interesting question is raised as to the use of behavioral assessments in detecting slight genotoxic effects.

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