Muta

Mutation Research, 229 (1990) 189–200
Elsevier

MUT 02711

Cyclophosphamide in the F_0 male rat: physical and behavioral changes in three successive adult generations

M. Auroux, E. Dulioust, J. Selva and P. Rince

Laboratoire de Biologie de la Reproduction et du Développement, CHU Bicêtre, F-94275 Kremlin Bicêtre Cedex (France)

(Received 2 June 1988)
(Accepted 14 February 1989)

Keywords: Cyclophosphamide; Progeny; Physical changes; Behaviour; (Rat)

Male-mediated anomalies of pregnancy outcome are currently well known in both humans and animals. They may result from natural conditions such as aging or from the mutagenic effects of certain environmental factors.

The first findings come from the study of aging in man: Penrose's original work revealed, in 1955, that advanced paternal age may lead to a higher frequency of dominant autosomal malformative syndromes in the progeny. Since then, it has been reported that paternal age might also be involved in fetal mortality (Selvin and Garfinke, 1976), Recklinghausen's disease (Carey et al., 1979; Riccardi et al., 1984; Kaplan and Toutain, 1987) and even in X-linked recessive mutations such as hemophilia A (Hermann, 1966) or Duchenne's muscular dystrophy (Hutton and Thompson, 1970). Finally, in a previous experimental study in rats, we were able to demonstrate that apart from other, especially malformative, anomalies, paternal aging may induce decreased learning capacity in the adult offspring (Auroux, 1983).

In animals, it is known that environmental conditions may also damage the paternal genome. Thalidomide has been shown to increase fetal and neonatal mortality rates (Lutwak-Mann et al., 1967), while methadone and morphine are known to also cause reduced birth weight and behavioral alterations in adulthood (Soyka et al., 1978). Besides, it has been shown that paternal alcohol exposure may induce decreased litter size, growth retardation, reduced levels of cerebral DNA and RNA contents in the offspring (Tanaka et al., 1982) as well as behavioral impairments (Klassen and Persaud, 1976). Moreover, male lead exposure (Brady et al., 1975) or sperm X-irradiation (Schroder, 1978, 1980) may also alter offspring behavior.

So, clinical and experimental data enable the integration of the possible consequences of various male-mediated genetic alterations into a continuum beginning with fetal death, including patent malformations, then growth retardation and metabolic disorders compatible with life, and ending with isolated behavioral deficits in apparently healthy individuals (Auroux and Dulioust, 1985).

In this context, the side effects of antimitotic drugs whose mutagenic properties are now well documented give rise to particular concern. Indeed, recent studies have revealed that in man recovery of spermatogenesis is possible after antimitotic treatments and may lead to procreation. However, this apparent normalization raises the question of the genetic quality of the produced gametes and of the child to be conceived. Surveys have been carried out on the progeny of treated men, but these are often limited and sometimes

Correspondence: Dr. M. Auroux, Laboratoire de Biologie de la Reproduction et du Développement, CHU Bicêtre, F-94275 Kremlin Bicêtre Cedex (France).

contradictory (Li and Jaffe, 1974; Russel et al., 1976; Hinkes and Plotkin, 1973; Blake et al., 1976; Etteldorf et al., 1976; Holmes and Holmes, 1978; Senturia et al., 1985). Moreover, only malformations directly discernible at birth are taken into consideration, thus leaving out the possibility of functional anomalies compatible with life, as has been shown in animals. Indeed, in both the rat and the mouse, male exposure to cyclophosphamide (CP), a commonly used cytostatic drug with mutagenic properties (Mohn and Ellenberger, 1976; Mirkes, 1985), leads not only to reduced numbers of embryos (Botta et al., 1974; Cooke et al., 1978), increased fetal loss, malformations and growth retardation (Trasler et al., 1985, 1986) but also to postnatal behavioral disturbances (Adams et al., 1981, 1982; Fabricant et al., 1983). Since those anomalies have been observed in prepubescent animals from inbred strains after postmeiotic exposure of paternal gametes, it seemed to be necessary to answer the three following questions.

(1) Can similar results be obtained with non-inbred animals, a situation that would be closer to the genetic heterogeneity of the human species?
(2) Do functional troubles still exist in adulthood?
(3) Can spermatogonial exposure induce similar effects?

Besides, the following issues appeared to be relevant too:

(4) Does the duration of spermatogenesis recovery after chemotherapy influence the occurrence and the nature of the alterations?
(5) Are these transmissible?
(6) Do non-alkylating antimitotic drugs have the same effects?
(7) Is it possible to link the functional disturbances with particular organic alterations?

We therefore carried out a series of investigations in Wistar rats (Iffa Credo, L'Arbresle, France), housed in a colony room with pulsed air maintained at $23 \pm 1°$ C, $50 \pm 10\%$ humidity and a 12–12 h light–dark cycle. Food (UAR, Epinay-sur-Orge, France) and water (supplied in bottles) were available ad libitum. The same person was in charge of the animals during the experiments. 3-month-old males were treated with either CP alone (Endoxan, Lucien Laboratory, France) or with a combination of CP plus vinblastine (V, Velbé, Eli Lilly, France), the latter substance acting not on the DNA but on the mitotic process itself by stopping the formation of the spindle microtubules (Russel et al., 1981). Matings took place either 100 days (a period of time equivalent to 2 spermatogenic cycles) or 60 days (1 spermatogenic cycle plus 10 days of epididymal transit) after the end of treatment, and offspring were investigated. Three successive generations (F_1, F_2, F_3) were studied. In all generations, deliveries were followed by litter size standardization and cross-fostering. Given the heritability in rodents of some behavioral traits and more particularly of learning capacity (Bovet et al., 1969), we verified that the treated males and their female partners were successful in the tests later to be given to their offspring.

Treatment of the male rats: methods used

CP was given intraperitoneally in a daily dose of 10 mg/kg for 15 days (54 rats). In the combined protocol, V was added to this treatment in single doses of 250 μg/kg and 150 μg/kg on days 1 and 8, respectively (20 rats). In both cases, the modalities of treatment were chosen according to previous studies (Fritz et al., 1973; Botta et al., 1974; Cooke et al., 1978) and preliminary experiments, in order to induce an alteration of spermatogenesis compatible with the recovery of normal fertility (Auroux and Dulioust, 1985; Auroux et al., 1986). The control rats received the drug vehicles.

Effects of the treatments on the males

The males were evaluated for growth, spermatogenesis and fertility.

Effects of cyclophosphamide alone

Growth. A significant weight loss was observed, which lasted until the end of treatment and was then rapidly reversed.

Spermatogenesis (Fig. 1). Two rats were killed on days 1, 20, 40, 50, 60, 80 and 100 after the end of treatment. Stage 7 sections of seminiferous tubules (Clermont, 1967) were evaluated for the number of pachytene spermatocytes I, mature

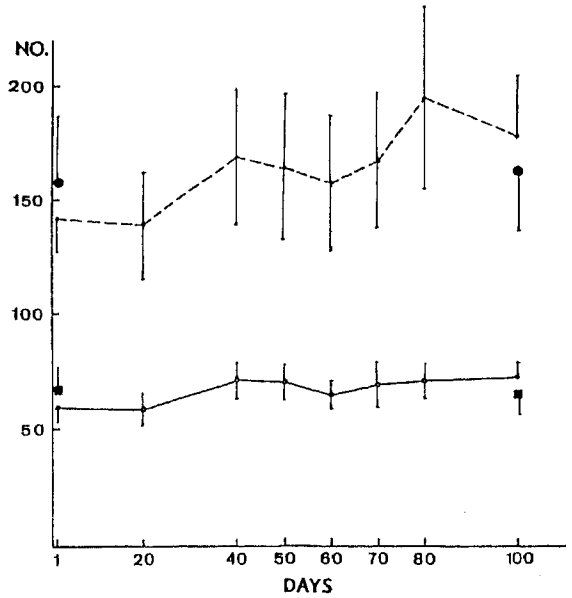

Fig. 1. Mean number (±SD) of spermatocytes I (–) and step 19 spermatids (---) in control (■, ●) and treated rats as a function of the number of days following the end of treatment. Linear regression (treated, -cytes I: $r = 0.795$, $df = 6$, $p < 0.02$, -tids: $r = 0.823$, $df = 6$, $p < 0.02$).

spermatids and Sertoli cells. Control rats were investigated on days 1 and 100 (Auroux and Dulioust, 1985).

Comparison with the control group revealed that the mean numbers of spermatocytes and spermatids were significantly decreased on days 1 and 20, suggesting that CP had a damaging effect on spermatogonia. On day 40, both cell types were as numerous as in the control population, a rebound effect being subsequently observed. However, this normalization was characterized by interindividual differences, which is in accordance with the heterogeneity observed in humans under similar conditions (Buchanan et al., 1975; Etteldorf et al., 1976; Alfiler et al., 1979). By contrast, the number of Sertoli cells remained stable, and no interindividual differences could be observed in the control group.

Fertility. Mating of the remaining treated males with untreated virgin females 100 days after the end of treatment demonstrated the recovery of a normal fertility, in accordance with the histological findings and the results of other authors (Botta et al., 1974; Cooke et al., 1978).

Effects of cyclophosphamide plus vinblastine

Growth. Despite a more dramatic weight loss than with CP alone, the rats resumed weight as rapidly when the treatment was stopped.

Spermatogenesis. Two rats were killed 1, 20, 45, 60 and 75 days after the end of treatment. Seminiferous tubules were examined as above. A significant decrease in the numbers of spermatocytes I and spermatids was observed from day 20 on. The damaging effects on spermatogenesis were more pronounced and lasted longer than with CP alone. Indeed, 75 days after the end of treatment, the values remained below those of the controls, despite a slight increase.

Fertility. In contrast with more severe histological alterations, the fertility of the treated males mated with untreated virgin females 60 days after treatment was found to be normal.

Effects on the F_1 progeny

From F_0-treated males mated with 3-month-old untreated virgin females 3 groups of F_1 progeny were produced: the CP-100 group, born of rats treated with CP alone and mated 100 days after treatment; the CP-60 group, born of rats treated with CP alone and mated 60 days after treatment; and the CP + V-60 group, born of rats treated with CP plus V and mated 60 days after treatment.

Offspring were examined for physical parameters: litter size, sex ratio, frequency of gross external malformations, growth, mortality rate. Then, between 12 and 16 weeks of age, behavioral tests were performed including an evaluation of open-field spontaneous activity and of learning ability of a conditioned avoidance response (shuttle box). All tests used automated devices.

CP-100 offspring

Litter size, sex ratio, birth weight, growth and mortality were not modified. No external malformation could be observed. The spontaneous

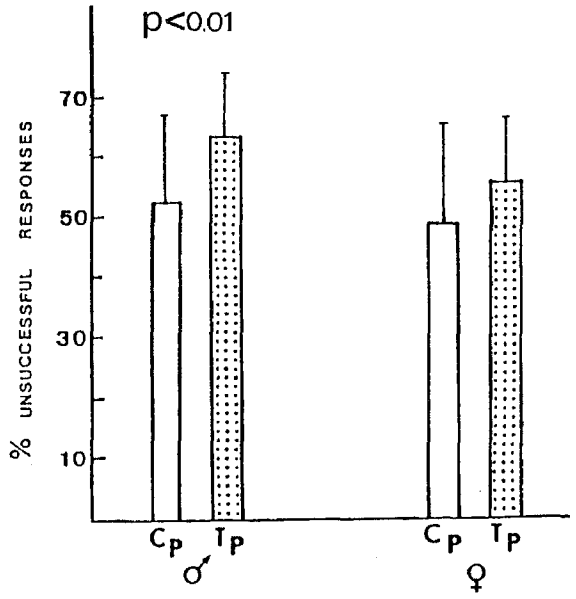

Fig. 2. First generation born of CP-treated rats. 100 days spermatogenesis recovery. Increased mean number of errors (±SD) before success in the males. C_P, T_P: progeny born of the control and treated males respectively. The difference is significant only in the males (analysis of variance: $F = 7.93$, $df = 1/48$, $p < 0.01$).

activity was not altered. In contrast, learning capacity was impaired in male offspring. Indeed, whereas the proportion of conditioned males (success rate) did not differ from the control population, the frequency of failures before conditioning was achieved was significantly increased (Fig. 2). So only a slight functional disturbance affecting apparently normal subjects was observed.

CP-60 and CP + V-60 progenies

There was no difference between CP-60 and CP + V-60 offspring in any parameter. Therefore, although the testicular effects appeared to be increased by the addition of vinblastine, this did not induce more anomalies in the progeny. In contrast, both offspring exhibited 2 types of anomalies compared to the controls (Fig. 3): increased postnatal lethality from day 1 to day 40, and behavioral deficits including altered spontaneous activity and impaired learning capacity in males as well as in females. As noted above for the CP-100 group, those males that succeeded in the learning test committed more errors before conditioning

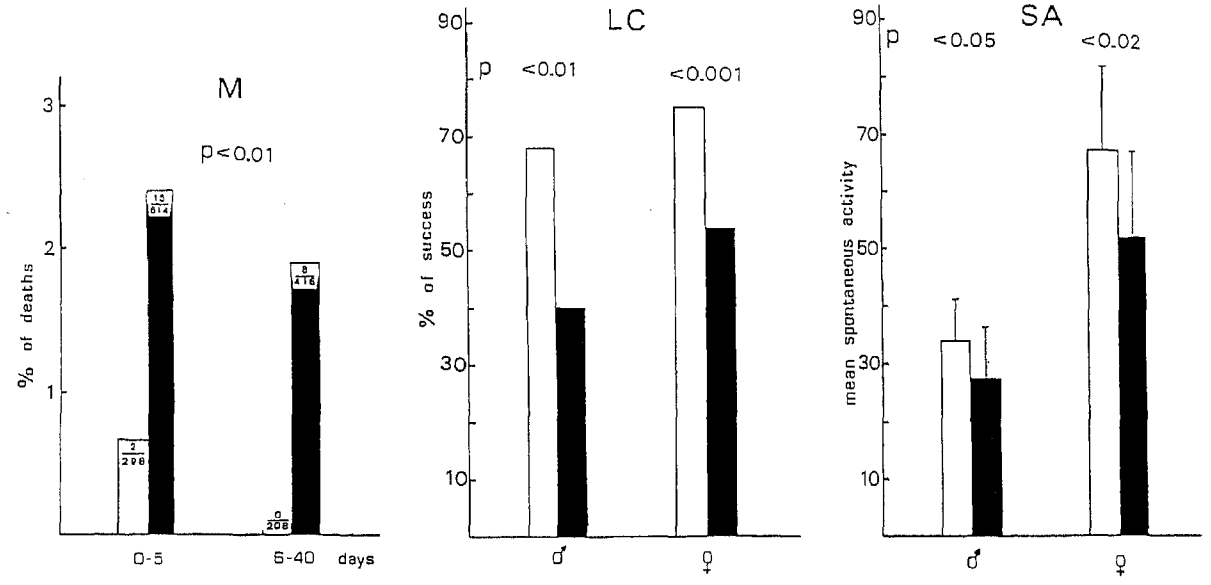

Fig. 3. First generation born of rats treated with CP or CP+V (no difference was found between the 2 treatments). 60 days spermatogenesis recovery. Increased mortality rate (M, ♂ + ♀, log-rank test: $p < 0.01$). Decreased learning capacity (LC; ♂: $\chi^2 = 8.04$, $df = 1$, $p < 0.01$; ♀: $\chi^2 = 12.45$, $df = 1$, $p < 0.001$) and decreased spontaneous activity (SA; mean number of displacements ± SD; analysis of variance, ♂: $F_{1/138} = 4.13$, $p < 0.05$; ♀: $F_{1/138} = 6.30$, $p < 0.02$) in both sexes. White and black columns: control and experimental animals respectively.

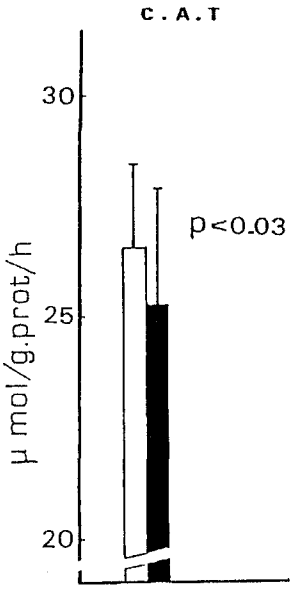

Fig. 4. First generation born of rats treated with CP or CP+V. 60 days spermatogenesis recovery. Reduced activity of the hippocampal choline acetyltransferase (C.A.T., mean μmole synthesized acetylcholine/g protein/h ± SD. Analysis of variance: $F_{1/46} = 5.39$, $p < 0.03$).

than the control males (Auroux et al., 1986).

Vinblastine being apparently without effect, cyclophosphamide may be considered to be responsible for the observed anomalies, which would be in accordance with the observation that CP acts on the DNA (Mohn and Ellenberger, 1976; Mirkes, 1985), whereas the cellular target of V is the microtubular system (Russel et al., 1981). Besides, the observed anomalies were more diversified and severe than in the CP-100 progeny. This seems to indicate the importance of the time interval between the end of treatment and mating. In agreement with this, Trasler et al. (1985, 1986) have reported a high proportion of embryonic deaths and malformations when mating takes place during treatment.

The question was raised as to the causes of such anomalies. Postnatal deaths were preceded by no perceptible signs and remained so far unexplained. In this respect, it is interesting to mention the possible genetic origin of postnatal mortality in the mouse (Guenet, 1984).

Concerning the behavioral deficits, especially the impairment of learning capacity, we examined whether neurochemical modifications might be implicated. The activity of an enzyme involved in memory processes, the hippocampal choline acetyltransferase (CAT), was therefore evaluated in the males. Indeed, previous studies (Ebel et al., 1976; Durkin et al., 1977) have revealed that the differences in learning capacity observed among certain strains of mice are related to variations in the activity of this enzyme which controls the synthesis of acetylcholine. Assays were made in randomly chosen CP-60 and CP + V-60 subjects 12 months after the period of learning assessment in order to eliminate possible effects of learning on the biochemical results (Auroux et al., 1987). CAT activity was significantly decreased in 24 experimental subjects as compared to 24 controls (Fig. 4). So this association observed in our experiment between decreased CAT activity and impaired learning capacity is in accordance with genetically determined variations occurring spontaneously in the mouse.

Finally, in another experiment, we examined 4-day-old CP-60 embryos, 253 of which produced by 15 untreated males mated with 30 untreated females and 258 produced by 13 CP-treated males mated with 26 untreated females. The mean number of cells per embryo in the controls was 7.17 ± 2.48 vs. 7.36 ± 2.25 in the experimentals and the difference was not significant. Given that chromosomal aberrations are mainly transmitted from postmeiotic stages (Albanese, 1982), such anomalies were not examined in our experiment, in which only premeiotic stages were considered. However, a cytogenetic study was performed on a sample of the embryos. No anomaly was found (unpublished data) but one may wonder whether more sensitive techniques such as evaluating the frequency of sister-chromatid exchanges (Vogel and Spielmann, 1987) might have led to other conclusions.

Transmissibility of the anomalies to the second generation

The hypothesis that male cyclophosphamide exposure may induce genetic disorders in the progeny had to be supported by experiments demonstrating the transmissibility of the observed

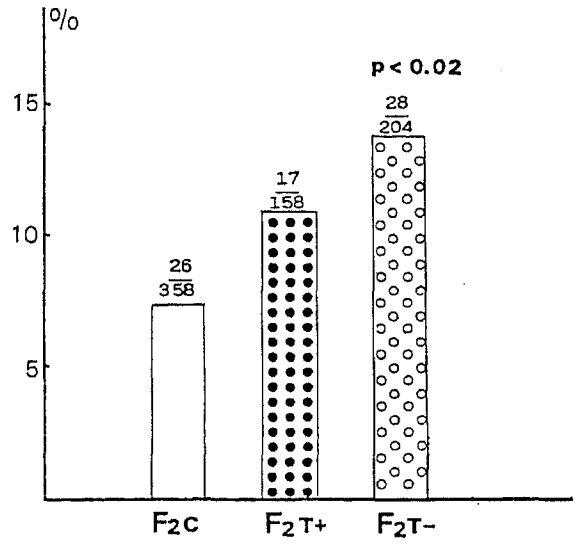

Fig. 5. Second generation. Increased neonatal mortality rate (days 0–4) in experimental subjects born of unsuccessful parents (F_2T- ♂ + ♀, χ^2 $F_2C/F_2T- = 6.25$, $p < 0.02$). F_2C: controls; F_2T+: experimental subjects born of successful parents.

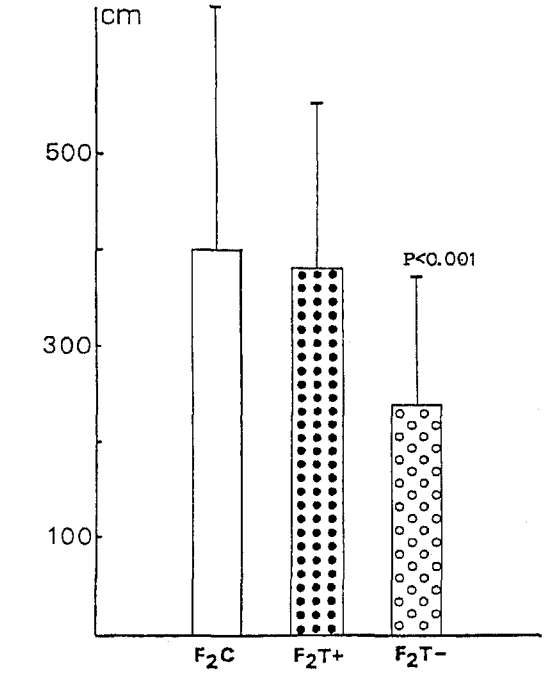

Fig. 6. Second generation. Decreased mean spontaneous activity (covered distance, ± SD) in the experimental males born of unsuccessful parents (F_2T-, Student's t test: $t = 3.41$, $df = 107$, $p < 0.001$). The other males (F_2T+) and the females were not affected.

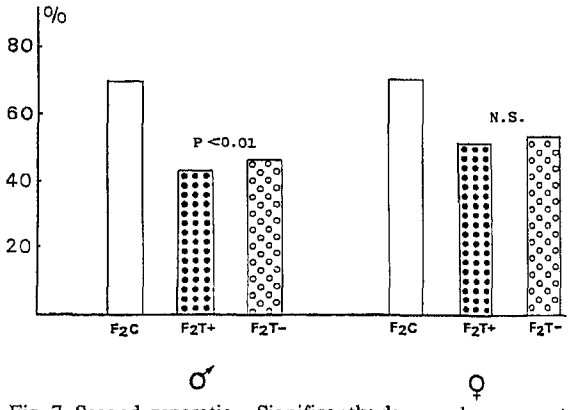

Fig. 7. Second generation. Significantly decreased success rate in the learning test in the males only. F_2C, F_2T+, F_2T-: see Fig. 5. Learning performance was impaired in both experimental subgroups (χ^2 $F_2C/F_2T+/F_2T- = 9.71$, $df = 2$, $p < 0.01$).

anomalies. For this purpose, CP-60 and CP + V-60 F_1 offspring being pooled (due to their homogeneity), couples were randomly constituted according to the learning performances of the partners. So, in the second generation, 2 groups were studied: controls (F_2C) and experimentals (F_2T), each group including 2 subgroups (F_2C+ and F_2C-; F_2T+ and F_2T-) depending on whether the rats were born of F_1 parents that were both successful (+) or unsuccessful (−) in the learning test. The aim

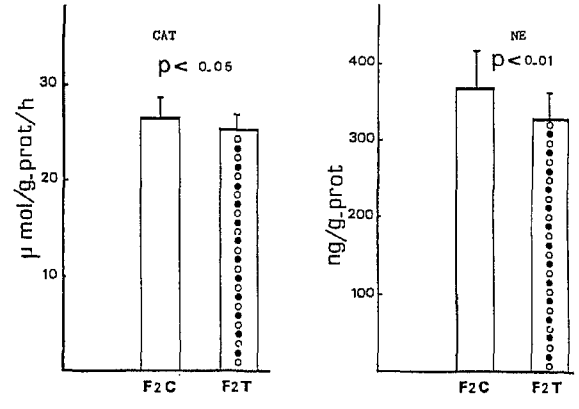

Fig. 8. Second generation. Significant decrease in both the activity of hippocampal choline acetyltransferase (CAT) ± SD (analysis of variance: $F_{1/39} = 4.86$, $p < 0.05$) and the level of norepinephrine (NE) ± SD in the frontoparietal cortex (analysis of variance: $F_{1/39} = 9.03$, $p < 0.01$). F_2C: control males; F_2T: experimental males. Females were not investigated due to the biochemical variations caused by the estrous cycle.

of this procedure was to eliminate as much as possible the possibility that differences would be fixed in the F_1 and F_2 groups by sampling from genetically variable material rather than due to the treatment. In this context, it should be remembered that all the F_0-treated males and their female partners had been successful in the learning test which was later given to their offspring. The second generation was investigated according to the methods used for the first generation.

No difference was found between the control subgroups. By contrast, the experimental subgroups were heterogeneous. Indeed, increased postnatal mortality (Fig. 5) and reduced spontaneous activity (Fig. 6) were observed exclusively in the F_2T- subgroup, which would confirm that the unsuccessful experimental parents were more severely affected than the successful experimental ones. Moreover, in both F_2T+ and F_2T- subgroups, the learning ability of the males was significantly impaired compared to that of the controls (Fig. 7), whereas no difference was noted between experimental and control females (Auroux et al., 1988). Finally, biochemical assays (unpublished data) showed, as for the first generation, a decrease in CAT activity as well as in the level of another memory support, norepinephrine of the frontoparietal cortex (Kempf et al., 1978) (Fig. 8). In conclusion, the anomalies observed in the second generation were similar to those found in the first generation.

Study of the third generation

Data obtained so far strongly suggested the existence of mutations induced by the exposure of the F_0 males to CP. One of the questions raised by those apparently dominant mutations concerned their stability. In an attempt to answer this question, we carried out, within the second generation, 3 series of matings: (1) between control subjects; (2) between experimental subjects; (3) between control and experimental subjects. Three groups of F_3 progeny were thus constituted: a control group (F_3C) and two experimental groups, 'treated' (F_3T) and hybrid (F_3H). This last group comprised 2 subgroups depending on whether the father or the mother was an experimental subject. The third

♀ \ ♂	F2 C	F2 T
F2 C	9.5 ± 2.7 a 21*	11.1 ± 1.4 c 11*
F2 T	6.7 ± 1.2 b 8*	8.3 ± 2.6 d 23*

Fig. 9. Third generation. Reduced litter size (±SD) in the experimental females of the second generation. (F_2C: controls, F_2T: experimental subjects; Wilcoxon's test: a–b, c–d: $p < 0.01$). * Number of litters.

generation was investigated as previously described.

A new anomaly was directly discernible: the litter size of the F_2T females was significantly reduced, whatever the origin of the partner (Fig. 9). In addition, both experimental groups (F_3T and F_3H) showed anomalies. These affected either the 2 groups or unexpectedly the F_3H group alone. The anomaly affecting the 2 groups consisted of a highly significant weight excess appearing in

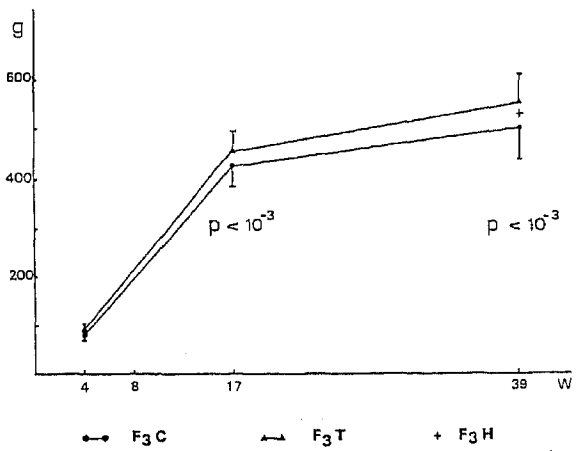

Fig. 10. Third generation. Increased weight (g±SD) in the males of both experimental groups. F_3C: controls; F_3T ('treated'): born of experimental parents; F_3H (hybrid): born of control and experimental parents (Student's t test 17 weeks, F_3C/F_3T: $t = 3.36$, $df = 75$, $p < 0.001$; 39 weeks, F_3C/F_3T: $t = 3.78$, $df = 75$, $p < 0.001$; F_3C/F_3H: Wilcoxon's test: $p < 0.04$).

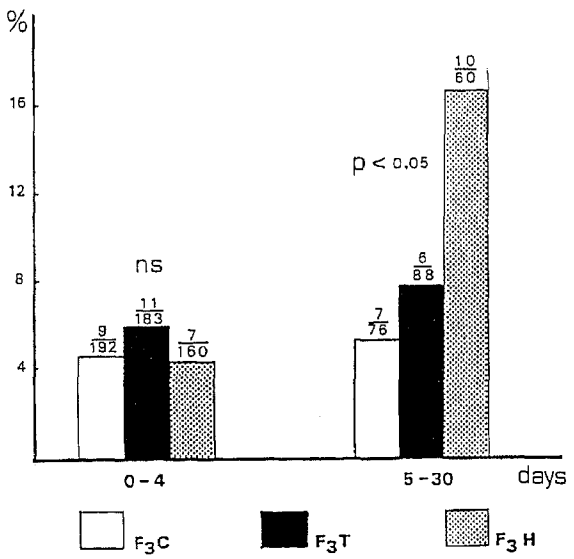

Fig. 11. Third generation. Increased mortality rate in the F_3H subjects. F_3C, F_3T, F_3H: see Fig. 10 (χ^2 $F_3C/F_3H = 4.7$, $p < 0.05$).

adulthood in males only and accentuating with age. Increased weight appeared at an earlier age in the F_3T group. Moreover, at 9 months the weight excess in the F_3T was about twice that observed in the F_3H (Fig. 10). Besides, increased postnatal mortality rate was noted in the F_3H group alone (Fig. 11). In addition, the F_3H males exhibited a slight learning deficit: while their success rate did not differ from that of the controls, the overall stimulation time was on average longer (Fig. 12). Interestingly, no difference could be found between the two F_3H subgroups in any parameter. The neurochemical assays revealed no anomalies, which seems to be in accordance with the slightness of the observed behavioral disorders (Dulioust et al., 1988).

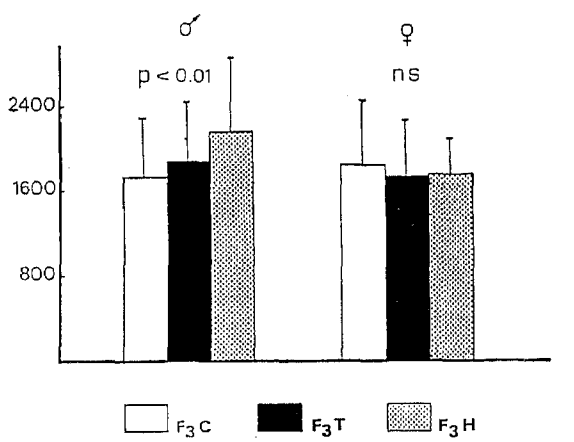

Fig. 12. Third generation. Learning performance: increased stimulation duration (sum of the stimulation durations of the 50 attempts of the test, expressed in arbitrary units ± SD) in the F_3H males. F_3C, F_3T, F_3H: see Fig. 10 (Student's t test, F_3C/F_3H: $t = 2.71$, $df = 60$, $p < 0.01$).

In summary, investigation of the third generation revealed 2 important findings. First, both experimental groups exhibited anomalies and one of these, i.e., the weight excess, showed characteristics suggesting cumulative paternal and maternal effects. Second, the presence of new anomalies as well as the heterogeneity between experimental groups provides evidence of the complexity of the underlying processes.

Conclusions and perspectives

The observation of anomalies in all treatment groups strongly suggests that CP has induced transmissible mutations. Given that only 3 generations were studied, and also that the treated and control F_0 parents were selected on the basis of a successful learning test, it would seem unlikely that the differences observed were due to a 'founder effect' rather than to the treatment. Our findings, which agree with those of other authors (Adams et al., 1982, 1984; Fabricant et al., 1983), raise several questions, more especially as the dosages used are considerably below those administered to man.

Methodology

These experiments clearly demonstrate the usefulness of long-term follow-up studies of the progeny up to adulthood and even senescence. In future studies, such investigation would make it possible: (1) to eliminate the instability factor inherent in prepubescence and responsible for imprecision; (2) to identify those disorders persisting after possible palliative processes developed in the young, as may occur in the nervous system, and thus to better evaluate the severity of the observed anomalies (such an approach might be more generally envisaged in teratogenesis studies); (3) to take in account the sometimes late expression of

some genetic disorders such as, in our experiments, the body weight increase observed in the third generation.

Post-treatment recovery: DNA repair or elimination of damaged cells?

Concerning the first generation our results, in accordance with those of Trasler et al. (1985, 1986), clearly indicate that the severity of the disorders in offspring decreases when the time interval between treatment and mating increases. This argues for the existence of palliative processes. Among different mechanisms, the existence of DNA repair systems has been demonstrated in premeiotic male germ cells (Lee, 1983). Another possibility might be a gradual elimination of damaged cells at various steps of spermatogenesis (Roosen-Runge, 1977). It would therefore be useful to determine whether increasing post-treatment recovery may achieve total elimination of the anomalies. From this viewpoint, one may wonder whether the very slight learning deficiencies, which still affected offspring even after the longest paternal recovery, do not reflect a particular fragility of certain genic systems that have appeared recently in evolution.

Mutation frequency and subtlety of the effects

The question of the frequency of induced mutations is of fundamental importance. Indeed, the quantitative differences observed between experimental and control groups did not result from major anomalies in a few individuals but rather from slight deviations affecting a great proportion of the subjects. This suggests much higher rates of induced mutations than those generally calculated from point mutations. Interestingly, studies on either spontaneous or induced variations of quantitative traits in Drosophila have led to similar conclusions (Ramel, 1983). Moreover, given that the continuous renewal of spermatogonia may facilitate the occurrence of mutations * (Vogel and Rathenberg, 1975), one may wonder whether this spontaneous situation would not represent an additional risk factor with regard to mutagenic agents.

Clearly, numerous mutations may have moderate phenotypic effects. At the molecular level, several studies have shown in Drosophila that slight modifications of a protein quantity or properties, resulting in various phenotypic effects, could be induced by different changes of the gene or its flanking sequences (Scott, 1987). In rats, results similar to ours have been reported on the effects of neonatal exposure to neurohormones (Campbell, 1982): not only did the animals exhibit hormonal and behavioral anomalies in adulthood, but those were transmissible to the progeny by either males or females. The hypothesis raised was that of abnormal adjustments in DNA structure in the not yet stabilized genome of germ cells. Such data suggest that complex genomes might show a greater lability than usually expected. Therefore, one may question the real frequency of alterations that would only modify some physiological balances within viable limits. Apart from the consequences of cytostatic treatments, such a question may also concern the recent accumulation in the environment of numerous compounds considered so far to have no or weak mutagenic properties.

Damaging effects at premeiotic stages

As shown in this study, it is possible to induce transmissible mutations in spermatogonia with low doses of CP. This new finding gives evidence of the sensitivity of the method used. Indeed, only relatively high doses of CP have been reported as yet to have genotoxic effects on spermatogonia (Trasler et al., 1985). Besides, few substances have shown mutagenic properties in those cells (Lyon, 1981; Ehling et al., 1985; Goldstein, 1987). Genetic damage at this stage raises concern about possibly long-lasting risk. However, as discussed above, our results suggest that the risk might decrease with time.

Susceptibility of the nervous system

Our results, in agreement with other works (Kalter, 1971), suggests that the nervous system might show a great susceptibility to the effects of mutations. Such a possibility would need further investigation. As regards genotoxic effects, an ex-

* The frequency of mutations appears to be higher in spermatozoa than in ova (Vogel and Rathenberg, 1975), the latter being rather responsible for aneuploidies (Kram and Schneider, 1978).

planation might be found in the high number of genes which seem to be expressed in nervous tissues, as appears from the great variety of mRNAs in brain (Van Ness et al., 1979).

Differences between the sexes

In all cases, the disorders were more severe and more diversified in males than in females. Similar discrepancies have been observed in the progeny of senescent male rats (Auroux, 1983) and, in man, in populations suffering from mental deficiency (D'Anthenaise et al., 1979). Such differences remain unexplained. However, at least in our experiments, they seem to reflect a higher sensitivity of the male organism to the effect of certain mutations rather than more severe genetic damage in males since, in the third generation, similar alterations were found whether the experimental parent was male or female.

Influence of interactions

The results obtained in the third generation show that transmission of the anomalies may not be simple. Indeed, the weight anomaly was not observed before. As regards the discrepancies between the F_3T, which exhibited only abnormal weight, and the F_3H, which in addition showed increased mortality and a learning deficit, one might hypothesize that in the F_3T, interactions between paternally and maternally inherited mutations have corrected some of the consequences. Similar balancing effects have already been described in Drosophila (Scott, 1987). One may wonder whether such mechanisms might be relevant to the adaptative capacities of complex organisms.

References

Adams, P.M., J.D. Fabricant and M.S. Legator (1981) Cyclophosphamide induced spermatogenetic effects detected in the F1 generation by behavioral testing, Science, 211, 80–83.

Adams, P.M., J.D. Fabricant and M.S. Legator (1982) Active avoidance behavior in the F1 progeny of male rats exposed to cyclophosphamide prior to fertilization, Neurobehav. Toxicol. Teratol., 4, 531–534.

Albanese, R. (1982) The use of fertilized eggs in detecting potential clastogens, Mutation Res., 97, 315–326.

Alfiler, C. (1979) Prepubertal cyclophosphamide therapy and gonadal dysfunction: a case report and a review of the literature, Aust. Paediatr. J., 15, 120–122.

Auroux, M. (1983) Decrease of learning capacity in offspring with increasing paternal age in the rat, Teratology, 27, 141–148.

Auroux, M., and E. Dulioust (1985) Cyclophosphamide in the male rat: behavioral effects in the adult offspring, Behav. Brain Res., 16, 25–36.

Auroux, M., E. Dulioust, N.Y. Nawar and S.G. Yacoub (1986) Antimitotic drugs (cyclophosphamide and vinblastine) in the male rat: deaths and behavioral abnormalities in the offspring, J. Androl., 7, 378–386.

Auroux, M., E. Dulioust, S.G. Yacoub and A. Ebel (1987) Cyclophosphamide in the F0 male rat: behavioral and biochemical cerebral troubles in F1 adult progeny, 15th International Summer School of Brain Research: Neurochemistry of Functional Neuroteratology, Amsterdam, Aug. 31–Sept. 4, p. 72.

Auroux, M., E. Dulioust, N.Y. Nawar, S.G. Yacoub, M.J. Mayaux, D. Schwartz and G. David (1988) Antimitotic drugs in the male rat: behavioral abnormalities in the second generation, J. Androl., 9, 153–159.

Blake, D.A., R.H. Heller, S.H. Hsu and B.Z. Shacter (1976) Return of fertility in a patient with cyclophosphamide induced azoospermia, Johns Hopkins Med. J., 139, 20–22.

Botta, J.A. Jr., H.C. Hawkins and J.H. Weickel Jr. (1974) Effects of cyclophosphamide on fertility and general reproductive performance of rats, Toxicol. Appl. Pharmacol., 27, 602–611.

Bovet, D., F. Bovet-Nitti and A. Oliverio (1969) Genetic aspects of learning and memory in mice, Science, 163, 139–169.

Brady, K., Y. Herrera and H. Zenick (1975) Influence of paternal lead exposure on subsequent learning ability of offspring, Pharm. Biochem. Behav., 3, 561–565.

Buchanan, J.D., K.F. Fairley and J.U. Barrie (1975) Return of spermatogenesis after stopping cyclophosphamide therapy, Lancet, i, 156–157.

Campbell, J.H. (1987) Automodulation of genes: explanation for lasting effects seen in functional neuroteratology?, 15th International Summer School of Brain Research: Neurochemistry of Functional Neuroteratology, Amsterdam, Aug. 31–Sept. 4, p. 33.

Carey, J.C., J.M. Laub and B.D. Hall (1979) Penetrance and variability in neurofibromatosis: a genetic study of 60 families, Birth Defects, XV, 271–281.

Clermont, Y., and S.C. Harvey (1967) Effects of hormones on spermatogenesis in the rat, Ciba Found. Colloq. Endocr., 16, 173–196.

Cooke, R.A., A. Nikles and H.P. Roeser (1978) A comparison of the antifertility effects of alkylating agents and vinca alkaloids in male rats, Br. J. Pharmacol., 63, 677–681.

D'Anthenaise, M., and R. Salbreux (1979) Prévalence de la déficience mentale profonde chez l'enfant, Neuropsych. Enfance Adolesc., 27, 45–58.

Dulioust, E., N.Y. Nawar, S. Yacoub, E. Kempf, A. Ebel and M. Auroux (1989) Cyclophosphamide in male rat: new pattern of anomalies in the third generation, J. Androl., 10, 296–303.

Durkin, T., G. Ayad, E. Ebel and P. Mandel (1977) Regional

acetylcholine turnover rate in the brain of three inbred strains of mice: correlation with some interstrain behavioral differences, Brain Res., 136, 475–486.

Ebel, A., J.C. Hermetet and P. Mandel (1973) Comparative study of acetylcholine esterase and choline acetyltransferase enzyme activity in brain of DBA and C57 mice, Nature New Biol., 242, 56–58.

Ehling, U.H. (1974) Differential spermatogenetic response of mice to the induction of mutation by antineoplastic drugs, Mutation Res., 26, 285–295.

Etteldorf, J.N., C.D. West, J.A. Pitcock and D.L. Williams (1976) Gonadal function, testicular histology and meiosis following cyclophosphamide therapy in patients with nephrotic syndrome, J. Pediatr., 88, 206–212.

Fabricant, J.D., M.S. Legator and P.M. Adams (1983) Post meiotic cell mediation of behavior in progeny of male rats treated with cyclophosphamide, Mutation Res., 119, 185–190.

Fritz, H., D. Muller and R. Hess (1973) Embryolethality in the mouse following treatment of males with cyclophosphamide at specific germ cell stages, Agents Actions, 3, 35–41.

Goldstein, L.S. (1987) Dominant lethal mutations induced in mouse spermatogonia by mechlorethamine, procarbazine and vincristine administered in 2-drug and 3-drug combinations, Mutation Res., 191, 171–176.

Guenet, J.L. (1984) La génétique de la souris, Recherche, 155, 602–615.

Hinkes, E., and D. Plotkin (1973) Reversible drug induced sterility in a patient with acute leukemia, J. Am. Med. Ass., 223, 1490–1491.

Hermann, J. (1966) Der Einfluss des Zeugungsalters auf die Mutationen zu Hämophilie A, Humangenetik, 3, 1–16.

Holmes, G.F., and F.F. Holmes (1978) Pregnancy outcome of patients treated for Hodgkin disease, Cancer, 41, 1317–1322.

Hutton, E.M., and M.W. Thompson (1970) Parental age and mutation rate in Duchenne's muscular dystrophy, Am. J. Hum. Genet., 22, 26a (Abstract).

Kalter, H. (1971) Correlation between teratogenic and mutagenic effects of chemicals in mammals, in: A. Hollaender (Ed.), Chemical Mutagens, Plenum Press, New York, pp. 57–69.

Kaplan, J., and A. Toutain (1987) La maladie de Recklinghausen, in: M.L. Briard, J. Kaplan, M. le Merrer and J. Frezal (Eds.), Affections Dominantes à Expression Variable et Conseil Génétique, INSERM U 12. Département de Pédiatrie, Hôpital des Enfants Malades, Paris, pp. 42–52.

Kempf, E., M. Gill, G. Mack and P. Mandel (1978) Renouvellement de la noradrenaline dans diverses zones du système nerveux central de souches consanguines de souris et de leurs recombinants, C.R. Acad. Sci. Paris, 286 D, 1156–1164.

Klassen, R.W., and T. Persaud (1976) Experimental studies on the influence of male alcoholism on pregnancy and progeny, Exp. Path., 12, 38–45.

Kram, D., and E.L. Schneider (1978) An effect of reproductive aging: increased risk of genetically abnormal offspring, in: E.L. Schneider (Ed.), The Aging Reproductive System, Aging, Vol. 4, Raven Press, New York, pp. 237–270.

Lee, I.P. (1983) Adaptative biochemical repair responses toward germ cell DNA damage, Am. J. Ind. Med., 4, 135–147.

Li, F.P., and N. Jaffe (1974) Progeny of childhood cancer survivors, Lancet, ii, 704–714.

Lutwak-Mann, C., K. Schmid and H. Keberle (1967) Thalidomide in rabbit semen, Nature (London), 214, 1018–1020.

Lyon, M.F. (1981) Sensitivity of various germ cell stages to environmental mutagens, Mutation Res., 87, 323–345.

Mirkes, P.E. (1985) Cyclophosphamide teratogenesis: a review, Teratogen. Carcinogen. Mutagen., 5, 75–88.

Mohn, G.R., and J. Ellenberger (1976) Genetic effects of cyclophosphamide, ifosfamide and trophosphamide, Mutation Res., 32, 331–360.

Penrose, L.S. (1955) Parental age and mutation, Lancet, ii, 312–313.

Ramel, C. (1983) Polygenic effects and genetic changes affecting quantitative traits, Mutation Res., 114, 107–116.

Riccardi, V.M., C.E. Dobson, R. Chakraborty and C. Bontke (1984) The pathophysiology of neurofibromatosis. IX. Paternal age as a factor in the origin of new mutations, Am. J. Med. Genet., 18, 169–176.

Roosen-Runge, E.C. (1977) The process of spermatogenesis in animals, in: M. Abercrombie, D.R. Newth and J.G. Torrey (Eds.), Developmental and Cell Biology Series, Cambridge University Press, London, 214 pp.

Russel, J.A., R.L. Powles and R.T.D. Olivier (1976) Conception and congenital abnormalities after chemotherapy of acute myelogenous leukemia in two men, Br. Med. J., 1, 1508.

Russel, L.D., J.P. Malone and D.S. MacCurdy (1981) Effect of the microtubule disrupting agents, colchicine and vinblastine, on seminiferous tubule structure in the rat, Tissue Cell, 13, 349–367.

Schroder, J.H. (1978) Water escape performance and water escape learning ability in laboratory mice (*Mus musculus*) as dependent on different gonosomal constitution and paternal irradiation of spermatozoa with 600 R of gamma rays, Ber. Nat. Med. Ver. Innsbruck, 65, 163–188.

Schroder, J.H. (1980) Increase in agressiveness of male mice after irradiation of paternal spermatozoa with 600 R of gamma-rays as dependent on fertility, Behav. Genet., 10, 387–400.

Scott, M.P. (1987) Complex loci of Drosophila, Annu. Rev. Biochem., 56, 195–227.

Selvin, S., and G. Garfinke (1976) Paternal age, maternal age and birth order and risk of a fetal loss, Hum. Biol., 48, 223–230.

Senturia, Y.D., C.S. Peckham and M.J. Peckham (1985) Children fathered by men treated for testicular cancer, Lancet, 5, 766–769.

Soyka, L.F., J.M. Joffe, J.M. Peterson and S.M. Smith (1978) Chronic methadone administration to male rats: tolerance to adverse effects on sires and their progeny, Pharmacol. Biochem. Behav., 9, 405–409.

Tanaka, H., N. Suzuki and M. Arima (1982) Experimental studies on the influence of male alcoholism on fetal development, Brain Dev., 4, 1–6.

Trasler, J.M., B.F. Hales and B. Robaire (1985) Paternal

cyclophosphamide treatment of rats causes foetal loss and malformations without affecting male fertility, Nature (London), 316, 144–146.

Trasler, J.M., B.F. Hales and B. Robaire (1986) Chronic low dose cyclophosphamide treatment of adult male rats: effects on fertility, pregnancy outcome and progeny, Biol. Reprod., 34, 275–283.

Van Ness, J., I. Maxwell and W. Hahn (1979) Complex population of non polyadenylated mRNA in mouse brain, Cell, 18, 1341–1349.

Vogel, F., and R. Rathenberg (1975) Spontaneous mutation in man, Adv. Hum. Genet., 5, 223–318.

Vogel, R., and H. Spielmann (1987) Spontaneous and cyclophosphamide induced sister-chromatid exchanges in diploid and endoreduplicated metaphases of preimplantation mouse embryos, Mutation Res., 192, 137–140.