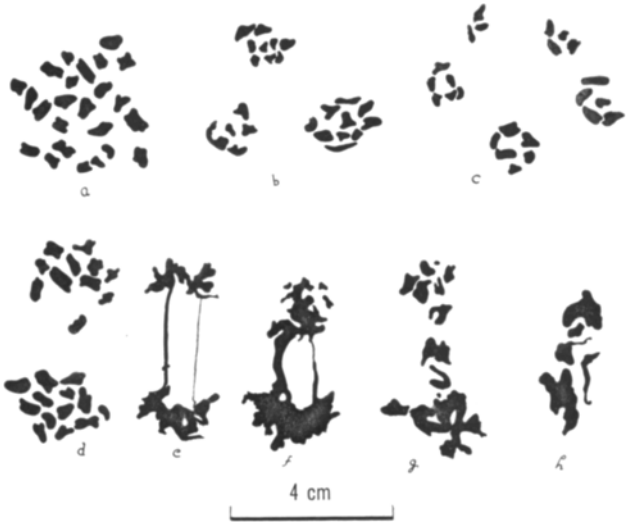
aceto-carmine squashes, and camera lucida sketches of chromosomes at different stages were drawn.

Most of the plants raised from treated seeds grew vigorously with leaves, flowers and capsules larger than those of the control. Normally *D.innoxia* has 24 univalents at metaphase 1 (Figure a), but the deviations mentioned below were observed at meiosis in plants raised from the treated seeds:

1. Chromosomes grouped separately with unequal numbers (Figures b and c). 2. Unequal numbers of chromosomes at the 2 poles with laggards at anaphase 1 (Figure d). 3. Chromatid bridges with fragments (Figure e). 4. Chromatin bridges (Figure f) and laggards at telophase 1 (Figure g). 5. Unequal chromatin masses at telophase 1 (Figure h). 6. Few polyploid cells with 48 univalents.




The chromatin bridges may be due to stickiness and tardy disjunction of chromosomes (KABARITY[3]), whereas the formation of chromatid bridges with fragments suggest that inversion has taken place. Occurrence of few polyploid cells with 48 univalents may be due to the failure of cytokinesis, suggesting that the alcohol treatment referred to, followed by temperature shock, widen the scope for induction of polyploidy.

REIGER and MICHAELIS[4] observed that the chromosomal aberrations in *Vicia faba* were induced by ethanol in the first part of the interphase either before or during chromosomal reduplication. They attribute the action of ethanol to denaturation or structural changes of the proteins needed for DNA synthesis. RONCHI and ARCARA[5] also confirm the high sensitivity of this phase to the alcoholic treatment. These arguments may offer explanations for the occurrence of chromosomal aberrations observed in the present studies.

*Zusammenfassung.* Nachweis, dass Butylalkoholeinwirkung und anschliessender Temperaturschock auf die Samen von *Datura innoxia* Polyploidie und Veränderungen im Alkaloidgehalt der Pflanze verursachen und zu charakteristischen Chromosomenaberrationen führen.

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[9] The authors are grateful to Drs. C. K. ATAL and AKHTAR HUSAIN for their interest in the work.

## Mutagenicity Experiments with the Tranquillizer Meprobamate in *Drosophila melanogaster* and in Human Leukocyte Chromosomes in vitro

Since the fifties, meprobamate (2-methyl-2n-propyl-1, 3-propanedioldicarbamate) has been used as a tranquillizer (Miltaun®, Miltaunetten®, Aneural®) see ref.[1]. Using rat brain homogenates, meprobamate inhibits both oxidative phosphorylation and ATPase activity[2]. From these findings one can speculate that this drug might exhibit mutagenic activities by inhibiting DNA synthesis and repair processes. To examine this question experimentally, the effect of meprobamate on *Drosophila melanogaster* (in vivo test) and human leukocyte chromosomes (in vitro test) was investigated.

A) *Drosophila.* In *Drosophila,* the frequencies of recessive X-chromosome lethals, partial and total chromosome loss and non-disjunction have been determined. Partial loss frequency can be taken as a certain measure of the breakage frequency produced by a mutagen, whereas recessive lethals are mainly caused by point mutations or small deletions.

*X-chromosome lethals.* For the determination of recessive *X*-chromosome lethals, the Basc-technique was used. Berlin wild males were fed or injected with $2.3 \times 10^{-2}$ *M* solution of the drug. Feeding of the test substance was carried out according to the technique already described in detail[3,4]. After 3 days feeding, or 24 h after injection, each male was mated individually to 1 virgin female of the Basc-stock. At intervals of 3 days they were remated to new females up to ten successive broods. Thus germ cell stages of different age of spermatogenesis including early spermatogonia could be tested separately. The results can be seen in Table I. In both injection and feeding experiments the mutation rates ranged from 0.10% to 0.55% with only one exception (broad IX, experiment 4) showing a frequency of 1.14%. Out of 20,551 chromosomes totally tested, 25 recessive lethals corresponding to 0.12% were scored. The spontaneous rate for recessive lethals in the *Drosophila* stock used ranges from 0.08 to 0.56% (20,000 chromosomes tested)[5] being on average 0.18%. Our results with the Basc-technique do not show any elevation of the recessive lethal frequency over the baseline after application of meprobamate per os or by injection. Analogous to the megaphene results[4], the rates of semilethals and of visible mutations did not show any difference to the control level. Further, except for male No. 11 in experiment 4 which carried 2 lethals (one in brood III, the other in brood VI), no clusters of mutations were found. By menas of an *Xple* stock (sc ec ct v g f) until now 15 of the lethals have been localized. Their distribution approximates a spontaneous

[1] K. OPITZ, Arzneimittel-Forsch. (Drug Res.) *12*, 333 (1962).
[2] L. DECSI and J. MÉHES, Experientia *14*, 145 (1958).
[3] H. LÜERS, Atompraxis *5*, 288 (1959).
[4] H. LÜERS, Ärztl. Forsch. *14*, 127 (1960).
[5] H. LÜERS and G. OBE, Mutation Res. *15*, 77 (1972).

Table I. The frequency of sex-linked lethals in male germ cells of *Drosophila melanogaster* after treatment with $2.3 \times 10^{-2} M$ meprobamate by adult feeding for 3 days or by injection

| Experiment | Brood No. | Chromosomes tested $N$ | Recessive $N$ | Lethals % |
|---|---|---|---|---|
| Adult feeding | | | | |
| 1 | I | 1177 | 3 | 0.25 |
| 2; 6; 7 | I | 3778 | 3 | 0.08 |
| | II | 2574 | 1 | 0.04 |
| | III | 2972 | 4 | 0.13 |
| | I-III | 9324 | 8 | 0.09 |
| 4 | I | 1246 | – | – |
| | II | 568 | – | – |
| | III | 1038 | 2 | 0.19 |
| | IV | 890 | 1 | 0.11 |
| | V | 569 | 2 | 0.35 |
| | VI | 651 | 1 | 0.16 |
| | VII | 933 | 1 | 0.11 |
| | VIII | 563 | – | – |
| | IX | 263 | 3 | 1.14 |
| | X | 183 | 1 | 0.55 |
| | I-X | 6904 | 11 | 0.16 |
| Injection | | | | |
| 3; 5 | I | 1119 | – | – |
| | II | 1052 | 2 | 0.19 |
| | III | 975 | 1 | 0.10 |
| | I-III | 3146 | 3 | 0.10 |
| Total | | 20551 | 25 | 0.12 |

Mean spontaneous rate approximately 0,18%

distribution as had been described by BELITZ[6]. That means that there are 2 peaks, one at the left end, the other at the right end of the *X*-chromosome.

*Chromosome aberrations.* In a second series the ability of meprobamate to produce single breaks, chromosome loss and gain was investigated. Virgin females of the genetic constitution *y w spl sn*$^3$ (genetic symbols see ref.[7]) were crossed to $y/B^s\ Y\ y^+$ males treated with $4.6 \times 10^{-3} M$ meprobamate by adult feeding for 2 days. The P males derived from a cross of virgin *y*-♀♀ to $y/B^s\ Y\ y^+$ ♂♂. In the $F_1$ offspring the following classes (without the markers *spl* and *sn*$^3$) were checked for:

| | |
|---|---|
| 1. yellow females | expected class |
| 2. white-Bar males | expected class |
| 3. yellow-white males | loss of *X* or *Y*, sterile |
| 4. white males | loss of $Y^L$ |
| 5. yellow-white Bar males | loss of $Y^s$ |
| 6. Bar females | non-disjunction in males |

Further details on the test procedure see ref.[8]. Exclusively mature sperm were tested in these experiments by setting up 1 brood of 24 h duration. In Table II the results of 3 single experiments including mean values are listed. None of them suggest an increase in the aberration frequencies. Thus meprobamate also remained ineffective with respect to the induction of chromosome aberrations in mature sperm.

B) *Human chromosomes.* Microcultures were set up (slightly modified from ref.[9]) with the blood of a normal healthy man. 24 h before fixation (whole culture time 96 h) water solutions of meprobamate were added to the cultures to final concentrations ranging from $10^{-7} M$ up to $10^{-4} M$. 2 cultures were set up for each concentration.

[6] H. J. BELITZ, Z. Abst. Vererbungslehre *88*, 434 (1957).
[7] D. L. LINDSLEY and E. H. GRELL, Carnegie Inst. Washington. Publ. No. 627 (1968).
[8] E. VOGEL, Mutation Res. *20*, 339 (1973).
[9] D. T. ARAKAKI and R. S. SPARKES, Cytogenetics *2*, 57 (1963).

Table II. The frequency of sex-chromosome complete or partial loss and non-disjunction (NDJ) in mature sperm of *Drosophila melanogaster* treated with $4.6 \times 10^{-3} M$ meprobamate for 17 h

| Experiment | Treatment | Expected progeny | | Progeny | Loss of *X* or *Y* | | Loss of $Y^L$ | | Loss of *Y*s | | NDJ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ♀ | ♂ | (♀ + ♂) | $N$ | % | $N$ | % | $N$ | % | $N$ | % |
| 1 | control | 1805 | 1817 | 3622 | 2 | | 0 | | 0 | | 0 | |
| | meprobamate | 5580 | 5592 | 11172 | 7 | | 3 | | 0 | | 3 | |
| 2 | control | 608 | 593 | 1201 | 1 | | 0 | | 0 | | 1 | |
| | meprobamate | 855 | 867 | 1722 | 2 | | 0 | | 0 | | 1 | |
| 3 | control | 2077 | 2133 | 4210 | 2 | | 1 | | 0 | | 0 | |
| | meprobamate | 2827 | 2644 | 5471 | 2 | | 1 | | 0 | | 0 | |
| 1–3 | control | 4440 | 4543 | 9033 | 5 | 0.05 | 1 | 0.01 | 0 | – | 1 | 0.01 |
| | meprobamate | 9262 | 9103 | 18365 | 11 | 0.06 | 4 | 0.02 | 0 | – | 4 | 0.02 |

Table III. Chromatid abberrations produced by meprobamate in human leukocyte chromosomes in vitro

| Meprobamate concentration (*M*) | No. of cells analysed | Achromatic lesions (AL) | Chromatid breaks (B') | Sum total of all breaking events per cell |
|---|---|---|---|---|
| $10^{-7}$ | 200 | 6 | 6 | 0.030 |
| $10^{-6}$ | 200 | 3 | 8 | 0.040 |
| $10^{-5}$ | 200 | 8 | 12 | 0.060 |
| $10^{-4}$ | 200 | 3 | 7 | 0.035 |
| Total | 800 | 20 | 33 | 0.041 |

Only achromatic lesions (AL) and chromatid breaks (B') were seen (Table III). The sum totals of all breaking events per cell, representing the sum total of all B' per cell in these experiments, ranges from 0.030 to 0.060. With all mitoses tested (800) this value is 0.041. Control data from our laboratory range from 0.0250 to 0.1025 with a middle of 0.0633, ref.[10]. Our data show no chromosome breaking ability of meprobamate on human leukocytes in vitro.

C) *Therapeutical doses.* The therapeutical doses of meprobamate are 0.40 to 1.20 g per day in adults. Assuming a body weight of 75 kg we have a dose of 0.005 to 0.016 g per kg or roughly per litre (l) body fluid. The doses used in our test are in *Drosophila* 5.02 g/l (*X*-chromosome lethals) and 1.00 g/l (chromosome aberrations) and in human chromosomes in vitro up to 0.022 g/l ($10^{-4}$ *M*). These calculations should be taken with caution but they show that the doses used in our test are well above the range of the doses used therapeutically.

*Zusammenfassung.* Mutagenitätsuntersuchungen mit Meprobamat an *Drosophila melanogaster* (*X*-chromosomale rezessive Letalmutationen, Chromosomenaberrationen in reifen Spermien) und an menschlichen Leukozytenchromosomen in vitro ergaben keine Anhaltspunkte für eine genetische Wirksamkeit dieser Substanz.

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[10] G. OBE and J. HERHA, Fortschr. Med. *91*, 533 (1973).

## Developmental Studies in *Drosophila nasuta* III. Developmental Obstinacy of the Eggs Laid after Starvation

The function of the female reproductive system is to produce eggs and provide for their fertilization and deposition[1]. The time spent by each egg in the uterus is stable[2]. The egg retention in *Drosophila* may occur under 2 circumstances: 1. When the females are virgins and 2. when the mated females are not offered a suitable site for egg laying[2]. In the latter case, the embryonic development begins within the female's reproductive system[3].

Delcour has given a procedure for rapid egg collection in *Drosophila*[4]. This has been employed by many investigators for competition and developmental studies[4,5,6]. The procedure is, after starving a stock of well-fed flies for 5 h, they are exposed to media for egg laying (100 $cm^3$ distilled water, 3 g Agar Agar, 1.5 $cm^3$ acetic acid, 2.5 $cm^3$ ethyl alcohol and a few drops of yeast, DELCOUR[4] has further suggested discarding the first batch of eggs obtained during 'First Hour' after starvation, reasoning that they may contain eggs of variable developmental stages.

[1] D. BODENSTEIN, in: *Biology of Drosophila* (Ed. M. DEMEREC; John Wiley and Sons, Inc., New York 1950, p. 275–367.
[2] J. DAVID and J. BOULETREAU-MERLE, Drosoph. Inf. Serv. *46*, 63 (1971).
[3] R. C. KING, Drosoph. Inf. Serv. *38*, 96 (1963).
[4] J. DELCOUR, Drosoph. Inf. Serv. *44*, 133–134 (1969).
[5] M. BUDNIK, D. BRNCIC and S. KOREF-SANTIBANEZ, Evolution *25*, 410 (1971).
[6] H. A. RANGANATH and N. B. KRISHNAMURTHY, Sci. J. Mys. Univ., in press (1973).

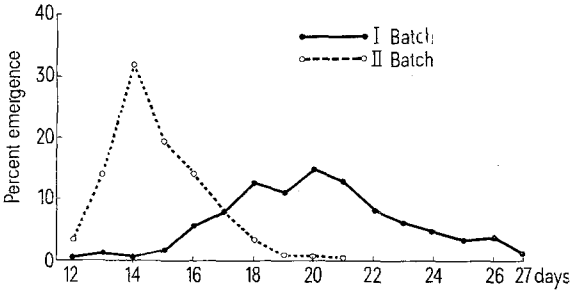


Fig. 1. Pattern of development at $T^0$ (21 °C).

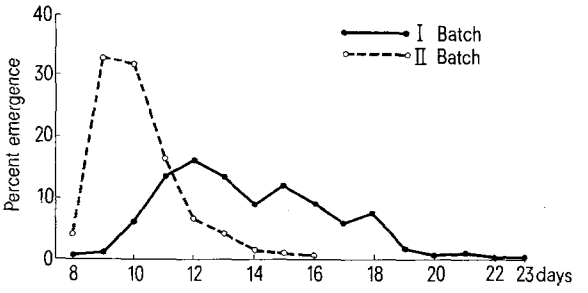


Fig. 2. Pattern of development at $T'$ (23–28 °C).