

**Results.** The results are summarized in the Table. It will be seen first that when hysterectomy was performed as late as day 15 p.c. any existing corpora lutea always lasted for approximately 24 days. Second, in both intact and hysterectomized rabbits an increasing dose of oestradiol-17 $\beta$  was associated with an increase in the life span of the corpora lutea. Third, a higher dose of oestrogen was required to maintain corpora lutea in intact animals compared with hysterectomized animals.

**Discussion.** The reduced effect of oestrogens on the corpora lutea after hysterectomy could result either from lowering of the effective concentration of the hormone by uterine utilization or from reduced competition with an uterine 'lytic factor'. We know of no data which convincingly favours the hypothesis that a luteolytic factor exists in the rabbit and of data from 3 experiments which are difficult to explain on that hypothesis.

First, however late hysterectomy is performed, if any corpora lutea are still present they always then persist for a total of 24 days. Second, corpora lutea may regress asynchronously<sup>7,8</sup>. Third, by giving two injections of oestrogen (100  $\mu$ g) at 6 and 30 h p.c., the uterus is delayed by 4 days in its development into a progestational state, as judged by histology, uteroglobin production and ability to support embryos<sup>9-11</sup>. However, the corpora lutea appear unaffected and the onset of their regression is not delayed by a corresponding 4 days<sup>12</sup>. To explain this result in terms of an uterine lytic factor, it would be necessary to postulate that the time course of production of such a factor was uniquely unaffected by the delaying treatment.

These considerations lead us to favour the hypothesis that the level of oestrogen support determines luteal life span<sup>13,14</sup>. In this respect the rabbit would resemble more those species such as the mouse where luteal persistence depended on luteotrophic support rather than those species such as the guinea-pig where a luteolytic mechanism was of prime importance.

**Résumé.** L'effet de différentes doses d'oestradiol-17 $\beta$  sur la durée de vie du corpus luteum a été examiné chez la lapine intacte et pseudogravide hystérectomisée. Une relation directe entre dose et durée de vie observée dans les deux groupes de lapines, mais les animaux hystérectomisés s'avèrent les plus sensibles à l'action de l'oestradiol-17 $\beta$ .

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## Effect of Certain Alcohols on Cytology of *Datura Innoxia* Mill.

Seeds of *Datura innoxia* Mill. contain up to 0.9% total alkaloids, mainly hyoscyamine and hyoscyne (GERASEMINKO et al.<sup>2</sup>) and are medicinally quite important. These seeds from wild sources at Jammu are poor in quality because of low alkaloid content, which normally varies between 0.12 and 0.22%. In an effort to increase the active principles, SINGH and KAUL<sup>6</sup> induced polyploidy in *D. innoxia* by pre-sowing treatment to seeds with ethanol followed by temperature shock. BATIKYAN et al.<sup>1</sup> exposed the radicles of onion to 23–25°C and found that mitotic activity of their cells decreases with the increase in the duration of exposure. Consequent to the treatment with ethyl alcohol, KABARITY<sup>3</sup> observed chromosomal aberrations at meiosis in *Triticum vulgare* Vill. while REIGER and MICHAELIS<sup>4</sup> reported similar abnormalities in the mitotic cells of *Vicia faba* L. Effect of pre-treatment to seeds with normal and tertiary butyl alcohols separately, followed by temperature shock, on the alkaloid content of seeds and general morphology of the plants of *D. innoxia* have been reported earlier by SINGH<sup>7</sup> and SINGH<sup>8</sup>, but no reference to chromosomal behaviour has been found so far. The present work describes the chromosomal modifications in *D. innoxia* consequent to similar treatments given to seeds.

Mature seeds were collected at one time from a single wild clone to ensure genetic uniformity, and divided into

several lots. Each lot was separately treated with 9, 12 and 15% aqueous solutions of normal and tertiary butyl alcohols at room temperature (25°) for an hour, washed thoroughly with tap water and kept at 45°C for 30 mts. One lot of seeds was simultaneously soaked in tap water for 1 h at room temperature to serve as control. Seedlings from all the above sets were separately raised in pans under identical conditions of soil, light and irrigation, etc. The seedlings were subsequently transplanted in beds and plants raised under identical conditions. Flower buds of different sizes were collected in 3:1 Carnoy's solution from promising plants and transferred, after 24 h, to 70% ethyl alcohol. Cytological observations were made in

<sup>1</sup> G. G. BATIKYAN, E. G. SIMONYAN and G. E. SAMVELYAN, *Biol. Zaharm* 12, 13 (1967).

<sup>2</sup> I. I. GERASEMINKO, N. I. LIBIZOV, (Eds. B. S. NIKOLSKAYA and F. A. SATSIPEROV; *Durman Indiasckii*, Medgiz, Moscow 1953), p. 28.

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<sup>7</sup> P. SINGH, *Experientia* 26, 211 (1970).

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aceto-carmin 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<sup>3</sup>), 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.

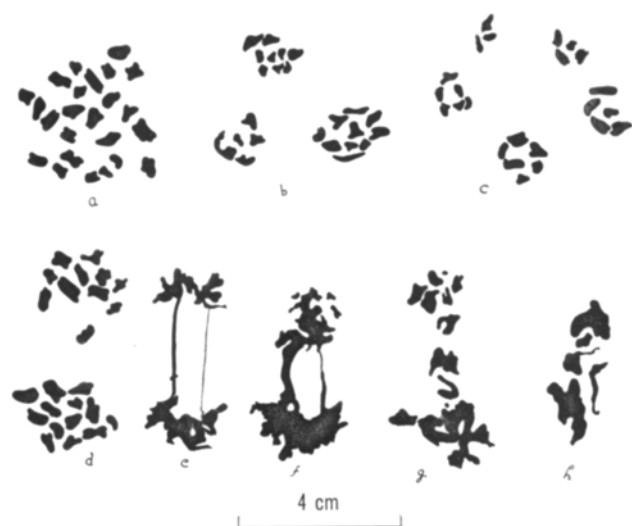
REIGER and MICHAELIS<sup>4</sup> observed that the chromosomal aberrations in *Vicia faba* were induced by ethanol in the first part of the interphase either before or during chromosomal reduplication. They attribute the action of ethanol to denaturation or structural changes of the proteins needed for DNA synthesis. RONCHI and ARCARA<sup>5</sup> 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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## 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 (Miltan®; Miltanetten®, Aneurall®) see ref.<sup>1</sup>. Using rat brain homogenates, meprobamate inhibits both oxidative phosphorylation and ATPase activity<sup>2</sup>. 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<sup>3,4</sup>. 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 (brood 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)<sup>5</sup> 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<sup>4</sup>, 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 means of an *Xple* stock (sc ec ct v g f) until now 15 of the lethals have been localized. Their distribution approximates a spontaneous

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