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Chromosome Aberrations Induced by Methanol in Germinal Cells of Grasshopper, *Oxya velox* Fabricius

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Three lots of grasshoppers were injected with 0.5% and 0.3% methanol and distilled water respectively. In the first two treated lots, analysis of the data evinced the influence of methanol in aberration induction. Qualitatively, dicentric bridge, gaps and constriction, extra elements, breaks, end to end fusion of elements etc. were found in the treated lots. Quantitative result showed 3.52 and 2.6% aberrations in 0.3 and 0.5% treated lots respectively. But in the third controlled lot, aberration was absent. The result of the study has been discussed in the light of oxidation process, or antimetabolite formation or stickiness of chromosomes as is advanced in case of other alcohols studied on plant and animal chromosomes. The probable mode of action of methanol might be the oxidation of it into formaldehyde which is itself mutagenic in nature.

THE effect of alcohols in inducing chromosome aberrations has not been so much investigated in animals as inp lants[1-4]. The action of glycol had been studied by D'Amato[5]. The efficiency of alcohols in decreasing the X-ray induced aberrations had been studied[6]. Manna and Mazumder[4] produced X-chromosome aberrations by 0.5% ethanol in the grasshopper, *Phloeba antennata*. Studies on the effects of alcohols on grasshopper chromosomes were not done in details before Manna and Mazumder[4] and Mazumder and Manna[7,8].

Materials and Methods

Adult males of the grasshopper, *Oxya velox* Fabricius were initially injected with 0.5% methanol in close vicinity of testes at the rate of 0.02-0.03 ml/individual by a hypodermic syringe. But the rate of mortality being high, they were later injected with 0.3% methanol and kept in insect-rearing cages. These two lots served as the treated series. A third lot, injected with distilled water, served as the controlled series.

After 30 hr, the testes were dissected out in normal saline (0.67%), fixed in aceto-alcohol (1:3) and finally stored in 70% alcohol. Permanent squash preparation of the material was made following Smith[9] and then stained in Heidenhain's haematoxylin (1%).

Results and Discussion

In the controlled series among 1150 cells, no chromosome abnormality was found, but the following quantitative result was got in the treated series.

In 0.5% treated series, 151 cells showed 4 aberrations, thus giving a frequency of 2.6%. Further study in this series was abandoned due to high mortality. But in 0.3% treated series, most of the study was done, and 38 aberrations were obtained among 1080 cells, thus showing 3.52% aberration. The result has been summarised in Table 1, wherefrom it is sure that, as only a limited number of cells were examined in 0.5% treated series, aberration percentage could not be taken as an indication of aberration induction. But the aberration percentage in 0.3% treated series could be taken as an indication of aberration production. So, it could be said that methanol has an effect at 0.3% level in genesis of aberration. The distributing pattern of aberrations has been shown in Table 2.

In the treated series, the following types of aberrations were seen qualitatively :

(i) Gaps and constrictions—Seen in both the series but more in 0.3% series (Plate 1, Figs 1, 2, and 3 and Plate II, Fig. 8). Of them, constrictions were more frequent. Sometimes, they co-existed in the same bivalent.

(ii) Extra fragments and elements—Found in 0.3% series in a great number. But the origin of them could not be definitely ascertained, and so they might be of unknown origin (Plate I, Fig. 4).

(iii) Dicentric bridge — Observed in Anaphase I in both the series (Plate II, Fig. 5). It was considered to be formed as a result of breakage and fusion of one of the homologues of a bivalent during interphase, consequent formation of a chiasma between the centromere and breakage point, and subsequent separation of the homologues during Anaphase I.

(iv) Chromosome and chromatid breaks — Mainly noticed in 0.3% series and once in 0.5% series. They were moderately frequent types of aberrations (Plate II, Fig. 6).

(v) End to end fusion of the elements — Seen only once in 0.3% series in Diakinesis (Plate II, Fig. 7), and so it was a rare type of abnormality. It might be due to the general stickiness of chromosome at the terminal ends of the elements.

(vi) Heteropycnosity — Extreme negative heteropycnosity resembling achromatic segment in one bivalent was seen in Metaphase I in 0.5% series (Plate II, Fig. 9).

The result indicates that methanol has effect on grasshopper chromosomes in aberration induction. The effect can be interpreted in terms of the effects obtained by previous workers working with other primary alcohols. It might be noted in this connection that the first step in the oxidation of primary alcohol is aldehyde formation. Methanol, a primary alcohol, on oxidation first forms formaldehyde, then

TABLE 1 — INDUCTION OF CHROMOSOME ABERRATIONS BY METHANOL

[No chromosome abnormality was found in controlled series]

No. of cells (Diplo-Ana. I & Meta. II-Ana. II.)	Breaks	Gaps	Constrictions	Extra elements & fragments	Dicentric bridges	Total aberration	Aberration, %
				Methanol, 0.5%			
151	1	1	—	—	2	4	2.6
				Methanol, 0.3%			
1080	5	5	14	8	5	37+1 (EEF)	3.52
				Distilled water			
1150	—	—	—	— —	—	—	—

EEF — End to end fusion of two bivalents.

TABLE 2 — DISTRIBUTION OF ALLOSOMAL AND AUTOSOMAL ABERRATIONS IN DIFFERENT STAGES

Diplo.	Aberr. Auto X-chr.	Diak.	Aberr. Auto X-chr.	Met. I	Aberr. Auto X-chr.	Ana. I	Aberr. Auto X-chr.	Met. II	Aberr. Auto X-chr.	Ana. II	Aberr. Auto X-chr.	Total cells	Total Aberr.
						Methanol 0.5%							
7	— —	74	1 —	35	2 —	4	1 —	11	— —	9	— —	151	4
						Methanol, 0.3%							
29	— —	439	16 1	307	15 4	50	1 —	232	1 —	23	— —	1080	38

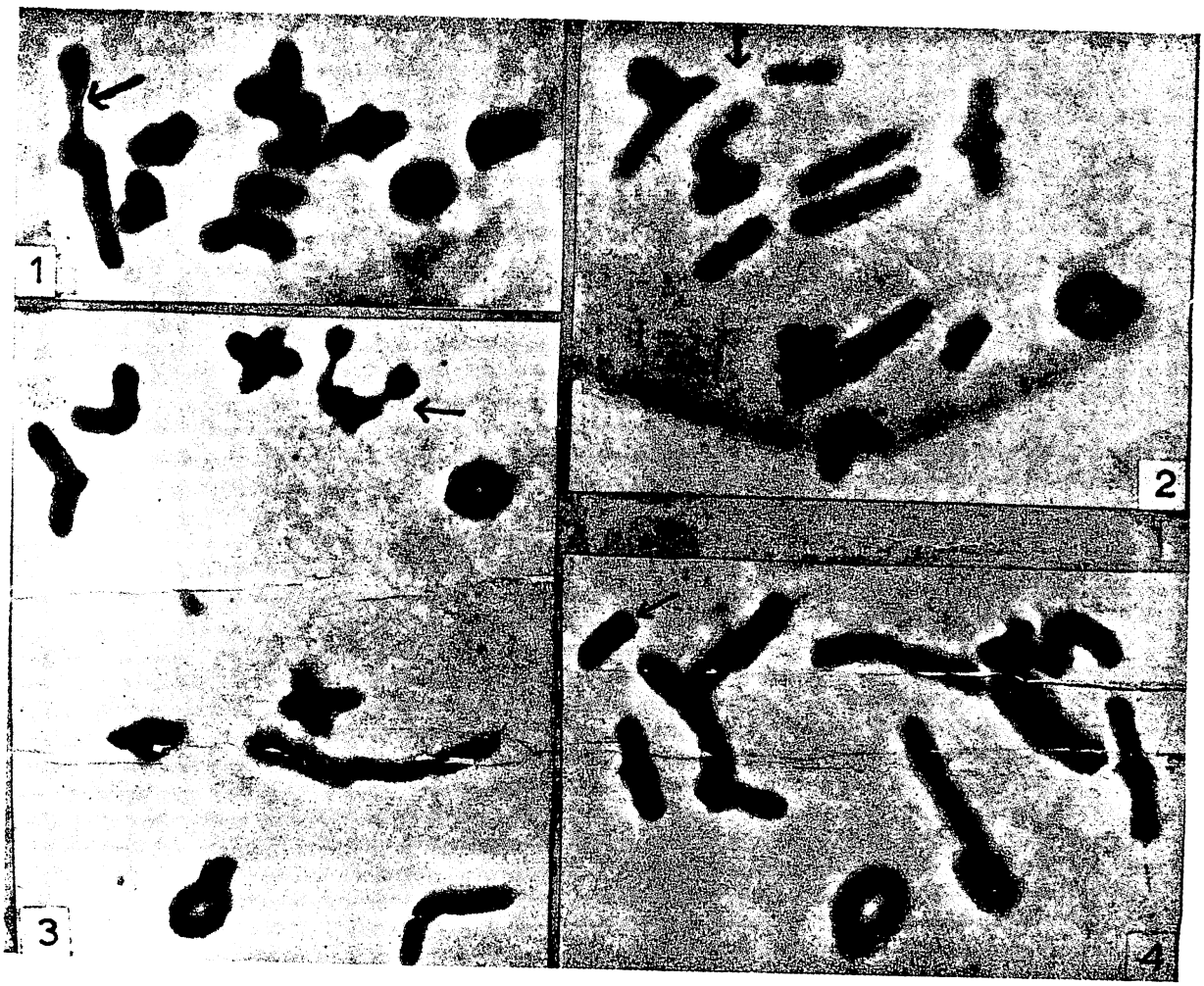

Plate I — Spermatocytic chromosomes of *O. velox* treated with 0.3% methanol × 1625 [Fig. 1 — A distinct metaphase I stage exhibiting a constriction in X-choromosome. Fig. 2 — Late diakinesis stage showing gap and constriction in the same bivalent Fig. 3 — A diakinetic stage showing gaps connected by thread in both the homologues. Fig. 4 — Metaphase I with an extra element]

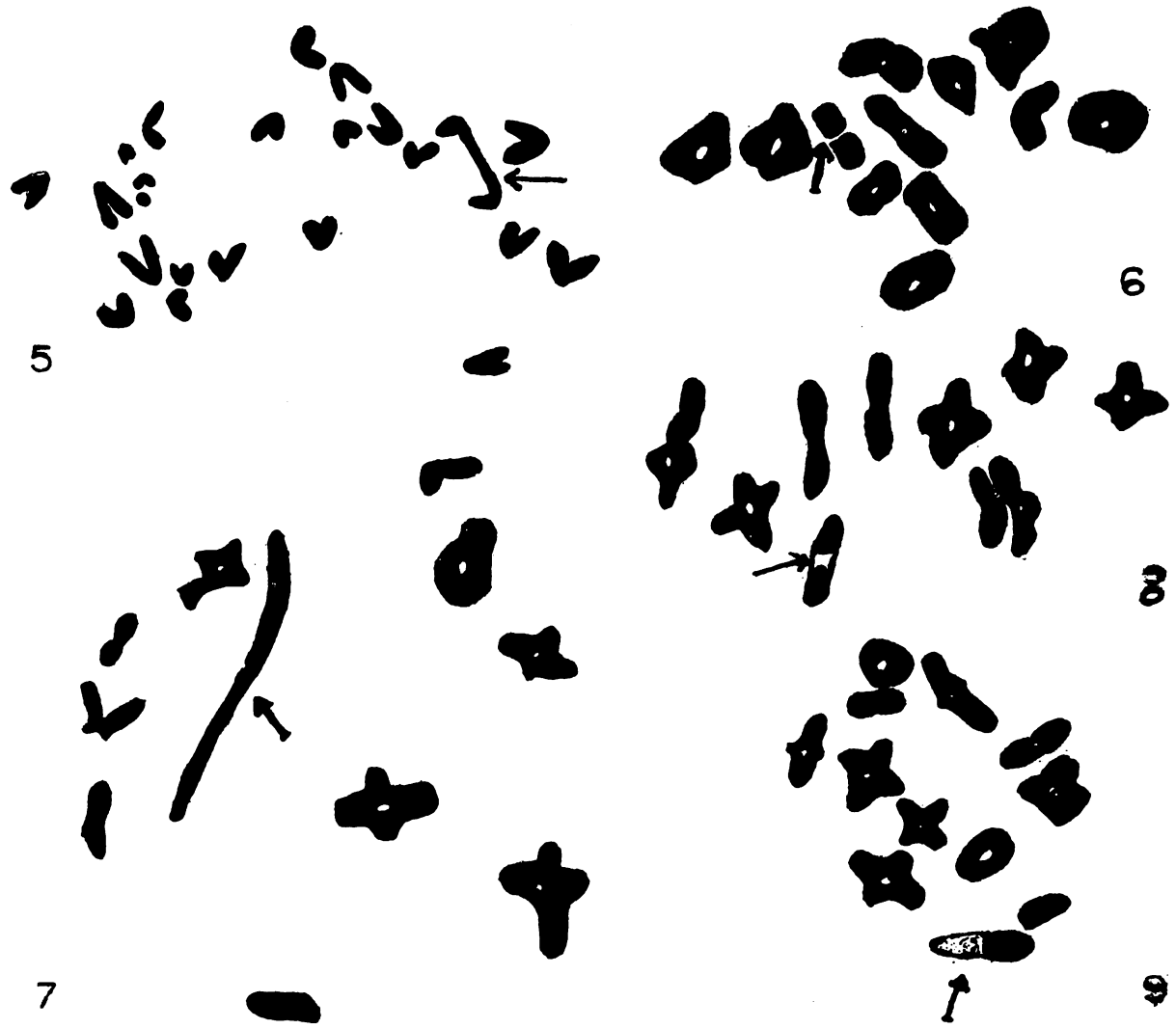

Plate II — Spermatocytic chromosomes of *O. velox* treated with 0.5 and 0.3% methanol × 1500. [Fig. 5 — An anaphase I cell with a dicentric bridge and an acentric fragment in 0.5% series. Fig. 6 — Metaphase I showing a break in X chromosome in 0.3% series. Fig. 7 — Diakinesis exhibiting end to end fusion of two bivalents in 0.3% series. Fig. 8 — Metaphase I with an achromatic gap in X chromosome in 0.3% series. Fig. 9 — Metaphase I with extreme negative heteropycnosity of a bivalent resembling an achromatic segment in 0.5% series.]

formic acid and lastly carbondioxide. Of these, the first has already been known to be mutagenic (*Vide infra*).

According to Rieger and Michaelis[1] ethanol proved to be effective under aerobic condition in high concentrations (10^{-3} to 5×10^{-1}M) and after long periods of treatment. The maximum aberration was obtained in between 18-24 hr after treatment. So, it was a delayed type of effect. The aberrations, too, were mostly localised in the heterochromatic region. In their studies, ethanol was also shown to be inactive under anaerobic condition, and the effect was suppressed by 2, 4,-Dinitrophenol, suggesting thereby that oxidative phosphorylation was somehow involved in the production of chromosome aberrations. Oxidation of ethanol, of course, produces first acetaldehyde and then acetic acid.

Alcohol and formalin may also act by producing antimetabolite to cause chromosome breakage[10]. Pre- and post-treatment of ethanol have been tested on the percentage of X-ray-induced X-chromosome aberrations in *Phloeba antennata*. The difference of breaks in X-rayed and post-treated series indicated an additive effect, while the difference of breaks in X-rayed and pre-treated series might be due to protective action[7].

Ethanol and ethylene glycol had also been reported to produce stickiness of chromosomes[11]. This might also be one of the reasons in the case of methanol treatment as is evident from end to end fusion of two elements.

The effect of methanol might be either due to the oxidative phosphorylation[1] or due to the oxidation of methanol first into formaldehyde andt hen into formic acid, the first of these known to be mutagenic in action[10] or due to the stickiness of chromosomes[11]. Among these, oxidation seems to be a more logical mechanism of aberration genesis. So methanol-induced aberration seems to be an oxygen-dependent type of aberration.

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