The Effect of Chloral Hydrate upon Mitosis in
Aspergillus nidulans

By B. MERCER and N. R. MORRIS

Department of Pharmacology, College of Medicine and Dentistry of New Jersey,
Rutgers Medical School, Piscataway, New Jersey 08854, U.S.A.

(Received 13 November 1974: revised 28 December 1974)

INTRODUCTION

Although chloral hydrate has been used as an anesthetic since 1872, its effect on cell
structures and its mechanism of action are not clearly known. Early investigators (Nemec,
1904; van Regemorter, 1916; Strasburger, 1907; Sakamura, 1916) have shown that chloral
hydrate produces uncoordinated chromosome movements and e-mitosis i.e. colchicine mitosis
(Levan, 1938). Ris (1949) reported that chloral hydrate causes the disappearance of the mitotic
spindle in grasshopper spermatocytes. Using eggs of Pleurodeles waltlii, Sentein
(1974) demonstrated that chloral hydrate alters the ultrastructure of chromosomes and arrests
mitosis by destroying spindle fibres. Mole-Bajer (1969) found that immediately after chloral
hydrate treatment in Haemanthus katherinae, the kinetochore and continuous microtubules
were absent, but microtubules soon began to re-form anew. Since most of these studies were
not quantitative, the chloral hydrate effect has mainly been described qualitatively.

The simple eukaryote Aspergillus nidulans has certain advantages for the study of mitosis,
the most important of which is that the genetics of the organism are well known. The cytology
of mitosis in A. nidulans is similar to that of the higher eukaryotes in most respects (Robinow
& Caten, 1969). During mitosis, the chromosomes condense, a mitotic spindle is formed, the
chromosomes undergo anaphase separation, the spindle then disappears, the chromosomes
relax and two daughter nuclei are formed (Robinow & Caten, 1969). As with many other
fungi, the nuclear membrane remains intact during all phases of mitosis. A set of tempera-
ture-sensitive, conditionally lethal, mitotic mutants of A. nidulans has been isolated (Morris,
unpublished observations). One of these mutants has been used as a tool to analyse quanti-
titatively the effect of chloral hydrate on mitosis.

METHODS

The wild-type haploid strain of A. nidulans, FGSC154 (Fungal Genetics Stock Center,
Humboldt State College, Arcata, California, U.S.A.), and a temperature-sentitive (ts)
diploid derivative derived in this laboratory from FGSC154, ts-706 diploid, were used for
these experiments. Aspergillus nidulans strain ts-706 is characterized by a reversible anaphase
arrest such that mitotic spindles and condensed chromatins accumulate co-ordinately with
time at temperatures above 38 °C (Morris, unpublished). A diploid strain of A. nidulans
ts-706 was constructed (Morris, unpublished) with larger, more easily identifiable spindles
than the haploid. Czapek-Dox minimal medium (Difco) was supplemented with the nutri-
tional requirements of strain 154, which were (per litre distilled water): 20 mg adenine,
0·05 mg biotin, 0·25 g methionine, 0·25 mg choline, 0·25 mg nicotine, 0·5 g sulphite and
0·5 g nitrite.
RESULTS AND DISCUSSION

Strains of *A. nidulans* 154 and ts-706 diploid were grown in streaks on a single piece of dialysis tubing overnight at 32 °C on supplemented Czapek-Dox minimal agar medium. They were placed in liquid Czapek-Dox medium at 32 °C containing 0.02 M-chloral hydrate (Fisher, No. 723811) and samples were removed, fixed and stained at intervals. The interphase nuclei stain very lightly with 'aceto-orcein', while the condensed chromatin of the mitotic figures stains darkly. Acid fuchsin stains the mitotic spindle, if present, and the nucleolus (Robinow & Caten, 1969). Chloral hydrate caused the rapid disappearance of spindles such that after 15 min of exposure, none of the hyphal tips contained mitotic spindles in both strain 154 and ts-706 diploid. In addition, chloral hydrate caused a dramatic change in the structure of the nuclei. After 20 min of exposure, more than 90% of the hyphal tips contained nuclei in which the chromatin was darkly stained and dispersed, as opposed to the well-defined structure of the chromatin of untreated nuclei. This phenomenon may be attributed to condensed chromosomes scattered about the hyphae as in c-mitosis (Levan, 1938), or represent remnants of fragmented nuclei. With lower concentrations of chloral hydrate, these effects were observed after longer exposure times; higher concentrations of chloral hydrate produced these effects more rapidly.

Samples of strain 154 were grown overnight at 32 °C on Czapek-Dox agar media on dialysis tubing. Four pieces of tubing were transferred for 30 min into Czapek-Dox medium with 0.02 m-chloral hydrate at 32 °C, while four samples were maintained in Czapek-Dox media at 32 °C as a control. All samples were washed for 5 min in Czapek-Dox medium and returned to Czapek-Dox agar medium. Since there was no difference in the number or size of the colonies of the different samples, the chloral hydrate effect is reversible.

The *A. nidulans* strains ts-706 (diploid) and 154 (as a control) were grown overnight and maintained at a restrictive temperature of 40 °C for 90 min until 51.5% of the hyphal tips of ts-706 diploid contained spindles. Each piece of dialysis tubing was divided in two such that each half contained cultures grown under identical conditions. One-half was placed in Czapek-Dox medium containing 0.02 m-chloral hydrate, at a permissive temperature of 32 °C, while the other half was placed in Czapek-Dox medium at permissive temperature as a control. The percentage of hyphal tips with mitotic spindles in ts-706 (diploid) dropped from 51.5 to 10% in 1 h at permissive temperature. However, in the presence of 0.02 M-chloral hydrate, the percentage of hyphal tips with spindles dropped from 51.5 to 1% in 15 min and to 0% in 1 h. This shows that chloral hydrate immediately causes the disappearance of accumulated mitotic spindles.

Chloral hydrate also effectively prevents the formation of mitotic spindles. Aspergillus strains 154 and ts-706 (diploid) were grown overnight at 32 °C. These cultures were transferred to Czapek-Dox media at 38 °C for 2-75 h. As described earlier, each culture was then divided in half. One-half was transferred to Czapek-Dox medium with 0.02 m-chloral hydrate at 38 °C and one-half was maintained in Czapek-Dox medium at 38 °C. While the percentage of hyphal tips with spindles increased from 25.3 to 49% in 1 h in Czapek-Dox media at 38 °C, in Czapek medium at 38 °C containing chloral hydrate, the percentage of hyphal tips with spindles fell to 0.4% in 15 min and to 0.2% in 1 h. This shows that 0.02 m-chloral hydrate effectively prevents the formation of mitotic spindles, in addition to destroying those previously formed.
REFERENCES


