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PAUL H. HARDY
E. ELLEN NELL

Department of Microbiology,
Johns Hopkins University School of Medicine,
Baltimore, Maryland.

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Petite Mutants induced in Yeast by Dithranol (1,8,9-trihydroxyanthracene), an Important Therapeutic Agent against Psoriasis

DITHRANOL (1,8,9-trihydroxyanthracene) is one of the most important local therapeutic agents against the common skin disease, psoriasis. The mechanism of the effect of this compound is not completely known. In recent experiments, however, it has been shown that dithranol forms a complex with nucleic acids, and it was proposed that dithranol thereby acts as a cytostatic agent[1]. To verify this hypothesis the effect of dithranol on the thymidine incorporation of guinea-pig epidermis was investigated. It was shown that twelve hours application of dithranol in the form of an ointment decreases the number of labelled cells to a third of that of the untreated epidermis[2].

Some other compounds that form complexes with DNA, like acridine derivatives, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene, are mutagenic or carcinogenic[3,4]. It may therefore be suspected that dithranol also has a mutagenic effect. A very sensitive test for the mutagenic effect of acridine derivatives is the induction of respiratory deficient yeast mutants. These mutants have no cytochrome oxidase activity, and ferment glucose as if they were growing anaerobically. They are slow in growth, form small colonies, and are therefore called petite mutants.

The effect of dithranol was tested in three different experiments. In experiment No. 1 a haploid strain of *Saccharomyces cerevisiae* was used. It had a high spontaneous frequency of petite mutants. In this experiment, where relatively high concentrations of dithranol were tested, the mutagenic effect was very evident. Using lower concentrations one might expect that the high spontaneous frequency of petite mutants might conceal a possible effect of lower concentrations of dithranol. A diploid strain was therefore made by crossing two haploid auxotrophic strains of opposite mating type. Diploid wild-type cells were selected on minimal substrate. It was found that these isolates contained a considerably lower proportion of spontaneous petite mutants, and thus they were used in the experiments No. 2 and No. 3.

Cells from an overnight culture were used to inoculate 20 ml. of complete medium in a 100 ml. 'Ehrlenmeyer' flask. After 3 h growth at 30° C dithranol was added and the culture was incubated for another 12 h at 30° C on a shaker. The treatment was stopped by washing the cells twice in salt solution. After dilution the cells were spread on complete medium and inoculated for 3 days at 30° C. Colonies of petite mutants were identified using the tetrazolium (TTC) technique[3] (modified after Nagai *et al.*). Normal cells reduce TTC and turn red while respiratory deficient petite mutants remain unstained. The cell suspensions and the plates were shielded from light to avoid a possible photodynamic effect.

The solubility of dithranol in water is extremely low. To obtain proper concentrations for treatment a saturated solution of dithranol in ethyl alcohol (95 per cent) was further diluted in ethyl alcohol (95 per cent), and a small amount was then added to the cell suspensions. The low concentrations of ethanol in the cell suspensions (as a rule 1 per cent) had no effect on growth or the frequency of petite mutants. The controls contained the same concentrations of alcohol as the treated series. The concentrations of dithranol given in the tables refer to per cent by volume of the saturated dithranol solution. A preliminary test of the solubility of dithranol in ethyl alcohol (95 per cent) gave the result that about 7·5 mg of dithranol dissolves in 10 ml. of alcohol. Assuming that dithranol was dissolved in the cell suspensions, 1 per cent by volume of the original saturated solution corresponds to about 7·5 p.p.m. or $3{\cdot}30 \times 10^{-5}$ moles/l.

Table 1

Experiment No.	Concentration of dithranol (per cent of the saturated solution in ethanol (95 per cent))		Estimated concentration of dithranol in moles/l.	Per cent petite mutants
	5·0		$1{\cdot}65 \times 10^{-4}$	96·5
	0	Control	0	16·0
1	1·0		$3{\cdot}30 \times 10^{-5}$	98·9
	0	Control	0	13·7
	0·5		$1{\cdot}65 \times 10^{-5}$	97·9
	0	Control	0	11·9
	0·1		$3{\cdot}30 \times 10^{-6}$	92·0
2	0·01		$3{\cdot}30 \times 10^{-7}$	77·5
	0·001		$3{\cdot}30 \times 10^{-8}$	7·7
	0	Control	0	3·2
	0·005		$1{\cdot}65 \times 10^{-7}$	13·2
	0·002		$6{\cdot}60 \times 10^{-8}$	8·5
3	0·001		$3{\cdot}30 \times 10^{-8}$	6·4
	0	Control	0	5·9

The three experiments showed that dithranol in a concentration of 10^{-7} molar gives an appreciable increase of petite mutants. The genetic material and the molecular events during the replication of the genetic material are similar in different organisms. It may be suspected therefore that dithranol is able to induce other types of mutations, and it might be mutagenic also in human cells. Such factors as penetration and inactivation of dithranol may give different degrees of mutagenic activity in various organisms.

Dithranol has been used as a local therapeutic agent against psoriasis for about 50 yr, and to our knowledge no reports of an increased cancer frequency in treated patients have been published. In mice, however, dithranol has been shown to have a tumour promoting property[5]. Other possible results of the mutagenic effect of dithranol may be the induction of auto-immune diseases and teratogenicity. Efforts to detect such possible side effects of dithranol treatment of psoriatic patients should be increased.

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B. O. GILLBERG
Institute of Genetics,
University of Uppsala.

G. ZETTERBERG
Institute of Physiological Botany,
University of Uppsala.

G. SWANBECK
Department of Dermatology,
University Hospital,
Uppsala.

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