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The induction of ouabain-resistant mutants by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in Chinese hamster cells

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SUMMARY

Clones of V79 Chinese hamster cells resistant to the steroid compound ouabain have been induced by mutagen treatment with MNNG (= *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine). The frequency of resistant colonies was increased from spontaneous value of 2×10^{-6} to a frequency of $1{\cdot}2 \times 10^{-3}$ with MNNG treatment.

The highest number of induced mutants appeared when an expression time of 40 h was allowed between MNNG treatment and addition of ouabain.

The steroid compound ouabain inhibits the growth of mammalian cells (McDonald *et al.* 1972; Mayhew & Lewinson, 1968) by the specific inhibition of the plasma membrane Na^+–K^+–Mg^{2+} activated ATPase (Wheeler & Whittam, 1962). Baker *et al.* (1974) have recently described the isolation of somatic cell mutants resistant to ouabain in both mouse L and Chinese hamster ovary (CHO) cells. Such ouabain-resistant cells were found by somatic cell hybridization to result from a single codominant mutation.

Baker *et al.* (1974) also demonstrated that the frequency of ouabain-resistant cells could be increased 40 times by ethyl methane sulphonate treatment, 10 times by UV exposure but only 2 times by MNNG. In view of the possible value of this mutational system in the study of chemical mutagens in somatic mammalian cells, we have examined the effects of MNNG treatment upon Chinese hamster lung (V79) cells in order to determine whether the low response to MNNG shown by Baker *et al.* is a generalized effect.

Chinese hamster V79 cells have been widely used in studies upon the induction of somatic cell mutants resistant to the anti-metabolite 8-azaguanine by both physical and chemical mutagens (Chu & Malling, 1968; Bridges & Huckle, 1970; Duncan & Brookes, 1973). The experimental procedures for using V79 in mutational studies have been described elsewhere (Arlett, 1972).

Actively growing monolayer cultures of V79 containing approximately 4×10^6 cells total were exposed to varying concentrations of MNNG from 0 to 1·5 μg/ml for 2 h. The chemical treatment involved the removal of growth medium followed by the addition of MNNG dissolved in phosphate-buffered saline. The treated cultures were then washed 3 times with phosphate-buffered saline to remove

residual MNNG. Samples were then plated at 10^5 cells/plate in 9 cm plates for mutant selection (5 plates per MNNG concentration) and after appropriate dilution plated into 5 cm Petri dishes for viability estimates. Filter-sterilized ouabain was added to a final concentration of 1 mM to mutation plates at various time intervals up to 66 h to allow for maximum mutant expression. Plates were then incubated at 37 °C for 10 and 6 days and then stained with methylene blue to score the frequency of mutant and viable cells respectively.

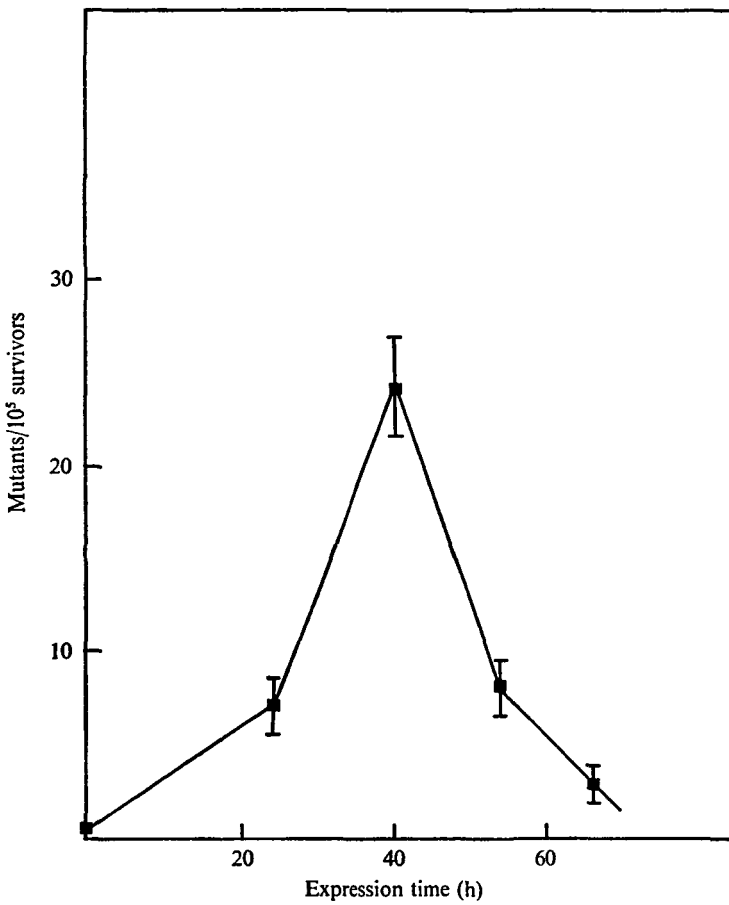

Fig. 1. The effects of varying expression times upon the frequency of ouabain-resistant mutants induced by 0·5 μg/ml of MNNG (80% survival). (Error bars represent standard deviation.)

Fig. 1 demonstrates the effect of varying expression times upon the frequency of ouabain-resistant cells after treatment with 0·5 μg/ml of MNNG which resulted in 80% survival. The results show that the frequency of ouabain-resistant cells is raised from a control value of 2×10^{-6} to a maximum of $2{\cdot}4 \times 10^{-4}$ at an expression time of 40 h. At higher concentrations of MNNG the optimum time for maximum mutant induction was further delayed. For example, at a mutagen dose resulting

in 10% survival the maximum number of mutants appeared at an expression time of 66 h compared to an optimum expression time of 40 h after a mutagen dose resulting in 80% survival (Davies, unpublished results). The expression time of 40 h was utilized in all further experiments, although this may result in an underestimation of mutation frequency at higher mutagen doses which caused greater lethality.

The effect of 0–1·5 μg/ml MNNG upon the frequency of ouabain-resistant mutants are shown in Fig. 2 and are expressed on the basis of the mutant frequency

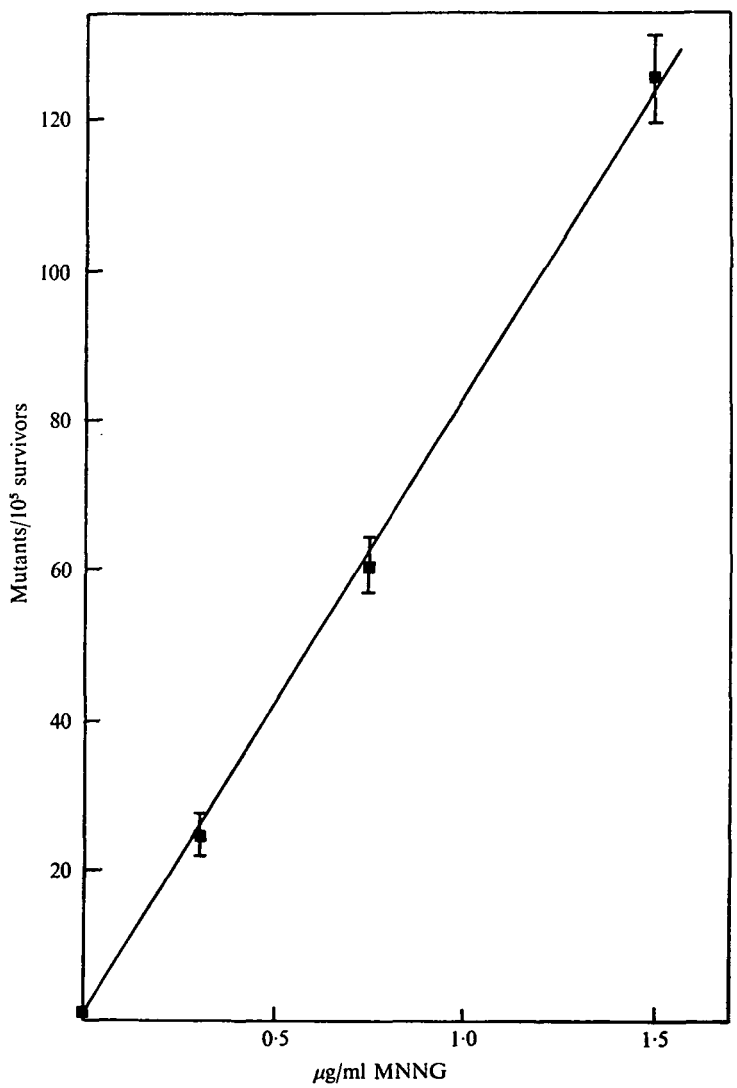

Fig. 2. Effect of MNNG upon the induction of ouabain-resistant mutants at doses of 0·3 μg/ml, 0·75 μg/ml and 1·5 μg/ml. The percentage cell survivals after the above treatments were 80%, 39% and 10% respectively. (Expression time, 40 h; bars represent standard deviation.)

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per 10^5 surviving cells. The results clearly demonstrate that MNNG is an extremely effective mutagen in V79 cells producing a 600 times increase in mutation frequency at 10% survival.

In order to show whether the ouabain-resistant phenotype was due to a stable, heritable change, 5 ouabain-resistant clones were isolated at random from mutagenized V79 cultures and grown for periods of up to 2 months in the absence of the selecting drug ouabain. Subsequent retesting of the cultures with 1 mM ouabain showed that all 5 clones had retained their ouabain-resistant phenotype over these prolonged periods of serial subculture. Thus, in diploid V79 cells, ouabain-resistant clones can be readily obtained by single-step selections similar to the ouabain-resistant mutants obtained by Baker *et al.* (1974) using Chinese hamster ovary cells.

Our results together with the demonstration (Baker *et al.* 1974) that ouabain resistance was found by somatic cell hybridization to result from a single codominant mutation suggest that such a system may be used in conjunction with, or as an alternative to, 8-azaguanine resistance in the study of the response of somatic mammalian cells to chemical mutagens.

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