flected differences in modal values of durations of pauses after reinforcement between conditions, rather than differences in rate when responding, or differences in the incidence of long pauses after reinforcement.

Thus the injection procedure itself seems to have a slight but significant potentiating effect on performance under short fixed ratio schedules of reinforcement, which lasts for at least 40 min after the injection. Some part of this effect may be attributed to the handling and some part to other features of the injection procedure. Although placebo controls are usually used in behavioural studies with animals, this practice is by no means universal. When control procedures are used which do not involve injection, small departures from base-line after drug administration should be treated with caution, even if they are consistent. In this study the potentiating effect of the pre-treatment conditions was positively related to the amount of apparent disturbance of the rat, which serves as a reminder that these conditions should be kept as constant as possible.

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## BIOCHEMISTRY

# Single Strand Scissions of DNA caused by a Carcinogen, 4-Hydroxylaminoquinoline 1-Oxide

4-HYDROXYLAMINOQUINOLINE 1-oxide (4-HAQO) is a reductive product of a carcinogen, 4-nitroquinoline 1-oxide (4-NQO), and is thought to be a more procimate carcinogen[1–3]. Enzyme conversion of 4-NQO to 4-HAQO is known to occur in mammalian cells[4]. When 4-HAQO or 4-NQO was injected intraperitoneally into rats bearing ascites hepatoma *AH*-130, DNA isolated from *AH*-130 cells showed the presence of fluorescent compound which has emission maxima at 470 mμ and excitation maxima at 360 mμ. This fluorescent compound seemed to be covalently bound to the DNA molecule[5,6]. This paper deals with another action of 4-HAQO on DNA, namely that of making single strand scissions of DNA.

4-HAQO was prepared by reducing 4-NQO with phenylhydrazine[7]. 4-HAQO and DNA were incubated without shaking under air gas phase in light-tight conditions. The incubation mixture was as follows: in 3·0 ml. of 10 mM *tris* 1 mM EDTA (*p*H 7·0) were 1 mg/ml. of DNA and 2·5 mmoles/ml. of 4-HAQO. After incubation for 1 h at 37° C the mixture was shaken three times with 3 ml. of chloroform to extract excess 4-HAQO. The aqueous layer (0·3 ml.), from which chloroform was evaporated, was layered on 4·6 ml. of sucrose density gradient solution (5 25 per cent, in 10 mM *tris*–1 mM EDTA–1 M NaCl, *p*H 7·0, and centrifuged with a swinging bucket type rotor, Hitachi *RPS* 40. Alkaline sucrose density gradient solution (5–25 per cent, in 10 mM *tris*–1 mM EDTA–0·3 M NaOH –0·7 M NaCl, *p*H 12, was used for sedimentation of alkaline denatured DNA. After centrifugation at 37,500 r.p.m. for 6 h at 24° C, each sample of ten drop fractions was collected from the bottom of the tube. Each fraction was diluted by adding 1·0 ml. of water and the optical density at 260 mμ was read using a Cary recording spectrophotometer, model 15.

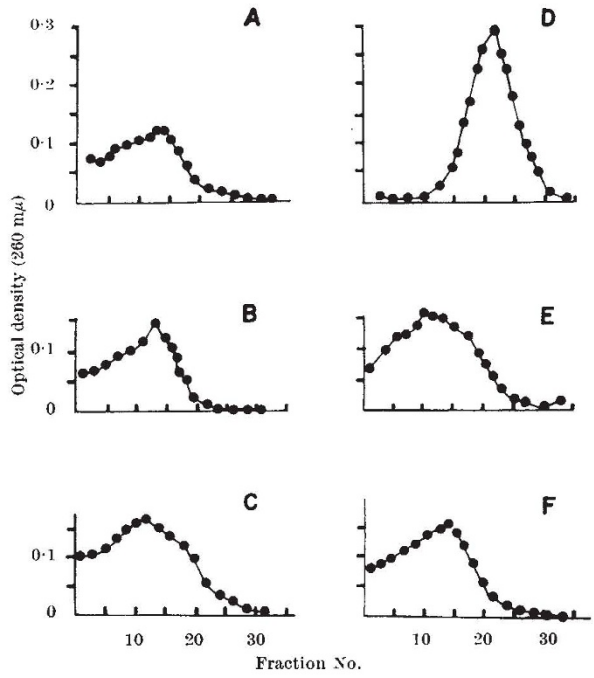


Fig. 1. Sedimentation patterns of DNA with sucrose density gradient centrifugation. *A*, Untreated DNA, *p*H 7·0; *B*, untreated DNA, *p*H 12·0; *C*, DNA treated with 4-HAQO, *p*H 7·0; *D*, DNA treated with 4-HAQO, *p*H 12·0; *E*, DNA treated with 4-NQO, *p*H 12·0; *F*, DNA treated with 4-AQO, *p*H 12·0.

The results presented in Fig. 1 show that DNA treated with 4-HAQO sedimented more slowly at *p*H 12 than at *p*H 7, while untreated DNA shows almost the same sedimentation pattern at *p*H 7 and 12. This suggests that single strand scissions were made by 4-HAQO. 4-NQO or 4-aminoquinoline 1-oxide (4-AQO), the non-carcinogenic reduction product of 4-HAQO, did not cause such a change in DNA although the same quantity of 4-NQO or 4-AQO was incubated with the DNA.

Reports have recently indicated that a reducing agent such as dithiothreitol can make single strand scissions on DNA (refs. 8 and 9). 4-HAQO can reduce cytochrome *c* (ref. 10) and this property might be important in producing scissions on DNA. 4-HAQO bound to DNA (ref. 5) might make the molecule of DNA sensitive at the site of binding so that the strand breaks[8] in alkaline conditions.

The possible relationship of such an effect on DNA to the carcinogenicity of 4-HAQO remains to be seen. The full details of experimental results, including also the effect of 4-HAQO on λ phage DNA, will be published later.

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