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# THE MODIFYING EFFECT OF SODIUM ASCORBATE ON DNA DAMAGE AND REPAIR AFTER N-METHYL-N'-NITRO-N-NITROSOGUANIDINE TREATMENT IN VIVO

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## SUMMARY

A biological reducing agent, sodium ascorbate, was used to modify both the damage induced by N-methyl-N'-nitro-N-nitrosoguanidine to mouse gastric mucosal cell DNA and the repair of that damage in vivo. Freshly-mixed carcinogen and sodium ascorbate enhanced DNA fragmentation as measured by shifts in alkaline sucrose gradient sedimentation profiles whereas incubation of the two compounds for a short period resulted in reduced DNA fragmentation. Furthermore, periodic administration of sodium ascorbate following stomach cell DNA damage with carcinogen inhibited DNA repair.

## INTRODUCTION

Chemical carcinogens may produce tumours independently or in conjunction with various modifying or promoting conditions (17,18,21). Alternatively, reactive electrophilic intermediates may be removed by trapping agents (7,10,20,23,24) or their formation prevented by inhibition of appropriate enzymes (1,4). Inhibition of mutagenesis in microbial test systems and carcinogenesis in animal test systems have been demonstrated when electron scavengers are applied concurrently with carcinogens (8,10,15,22). Ascorbic acid and L-cysteine reduce the carcinogenic effect of tobacco and marijuana smoke in cultured hamster lung (13) as well as the mutagenic action of N-hydroxy and N-acetoxy AAF, MNNG, 4NQO, aflatoxin $B_1$, MMS, AF-2, methylguanidine and methylurea nitrosation products, and nitrofurazone in Salmonella typhimurium (23).

We have previously reported an in vivo assay system for the determination of DNA fragmentation using shifts in alkaline sucrose

Abbreviations used in this paper are MNNG (N-methyl-N'-nitro-N-nitrosoguan 4NQO (4-nitroquinoline-1-oxide), MMS (methyl methanesulphonate), AAF (acety aminofluorene), and PBS (phosphate buffered saline).

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gradient sedimentation profiles of DNA from mouse gastric mucosal cells(11). In this paper we utilize this assay for DNA fragmentation to observe both the enhancing and depressing effect of sodium ascorbate on DNA damage in epithelial cells. In addition, the gradual increase in molecular weight of MNNG-damaged DNA over a 30 h period was used as a standard against which to measure the DNA repair-inhibiting effect of low doses of sodium ascorbate.

## METHODS AND MATERIALS

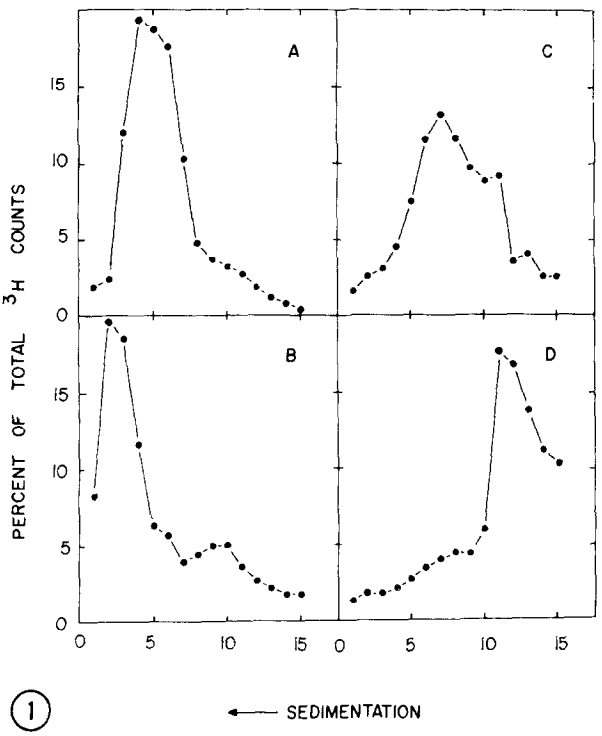
The method used in this work is a modification of our previously reported method (11). Mouse gastric mucosal cell DNA was labelled by administration of $5 \times 10^{-5}$ Ci (0.05 ml) of $^{3}$H-TdR (NEN Canada, Lachine, P.Q.)(specific activity 20 Ci/mmol) followed 24 h later by injection of an additional $5 \times 10^{-5}$ Ci. Mice were used 72 h following the second injection. Water without food was allowed ad libitum 24 h prior to chemical feeding.

## APPLICATION OF CHEMICALS

MNNG (Aldrich Chemical Co., Milwaukee, Wisconsin) was dissolved in DMSO (Burdick and Jackson Lab., Inc., Muskegan, Michigan) for a final solution of 0.5% DMSO. Sodium ascorbate (Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water only. MNNG and sodium ascorbate solutions were made at twice the desired concentration in distilled water alone and then mixed. All concentrations containing the desired amount of compound were delivered in a volume of 0.5 ml. Mice were force-fed by esophageal intubation under ether anaesthesia with a 1 ml tuberculin syringe equipped with a 5 cm piece of medical polyethylene tubing (internal diameter 0.015 in).

## ALKALINE SUCROSE GRADIENTS

The animal was killed by cervical dislocation and exsanguination. The stomach was excised proximal to the pyloric sphincter and distal to the exophagus, opened along its anterior aspect and rinsed twice in ice-cold phosphate-buffered saline (PBS, pH 7.4). Gastric surface mucosa was removed by scraping with a cold microscope slide. The scrapings were mixed with 0.5 ml of ice-cold PBS and a 25 microliter aliquot was layered on an alkaline lysing solution over a 5-20% alkaline sucrose gradient and a 2.3 M sucrose shelf. The gradient was spun at 77,561 X g at an average radius of 11.10 gm for 30 min and 15 sequential fractions taken from the bottom of the tube, precipitated with cold 19% trichloroacetic acid and collected on nitrocellulose membrane filters (Millipore Corp, Bedford, Mass.). Samples were counted



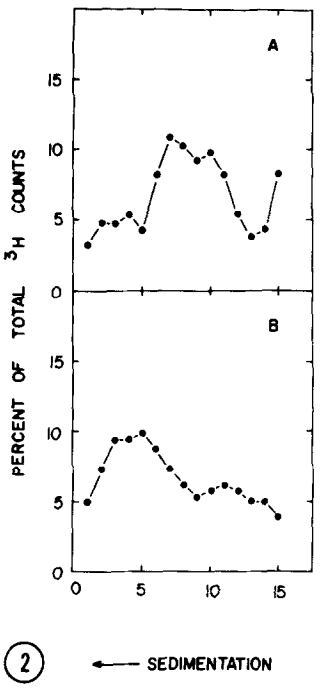




Figure 1 Alkaline sucrose gradient sedimentation profiles of mouse gastric mucosal cells A: 4 hours after force-feeding 0.5 ml distilled water, B: 4 hours after force-feeding 100 mgm sodium ascorbate/kgm mouse in a total volume of 0.5 ml., C: 4 hours after force-feeding 15 mgm MNNG/kgm mouse in a total volume of 0.5 ml., D: 4 hours after force-feeding a mixture of 100 mgm sodium ascorbate/kgm mouse plus 15 mgm MNNG/kgm mouse in a total volume of 0.5 ml.

Figure 2 Alkaline sucrose gradient sedimentation profiles of mouse gastric mucosal cells 4 hours after force-feeding a mixture of 20 mgm MNNG/kgm mouse plus 100 mgm sodium ascorbate/kgm mouse in a total volume of 0.5 ml. A: MNNG plus sodium ascorbate force-fed immediately after mixing, B: MNNG plus sodium ascorbate force-fed after a 30 minute incubation *in vitro* at 37° C.

by liquid scintillation fluorimetry using a Searle Delta 300 liquid scintillation counter.

## RESULTS AND DISCUSSION

DNA from radioactively labelled animals force-fed 0.5 ml distilled water containing 0.5% DMSO normally sediments in fractions 2-4 (Fig. 1A). When sodium ascorbate was force-fed to mice at a

concentration of 100 mgm/kgm body weight, no shift in sedimentation profile was observed (Fig. 1B). If the carcinogen MNNG was administered at a concentration of 15 mgm/kgm body weight, a moderate level of damage was observed (Fig. 1C). However, if MNNG and sodium ascorbate were mixed and force-fed immediately, an enhanced amount of DNA fragmentation was generated (Fig. 1D). That this damage is not a direct DNA-fragmenting effect of sodium ascorbate is supported by the observation that use of even twice the enhancing concentration will not, alone, fragment DNA (Fig. 1B).

On the other hand, a contrary effect of sodium ascorbate on carcinogen action was observed if the two compounds were incubated at 37° C for 30 min before force-feeding. In this case the mixture showed less DNA-fragmenting ability than MNNG and sodium ascorbate administered immediately on mixing (Fig. 2).

This inhibiting effect may be due to the scavenging of active electrophilic carcinogenic species that has already been observed when reducing agents such as cysteine are incubated with MNNG (12). The enhancing effect has not been observed in conjunction with biological oxidizers, except in the case of propyl gallate increasing the mutagenicity of some carcinogens used in bacterial mutagenesis assays (24). Other compounds, such as the carboline derivatives, harmane and norharmane, have been observed to both enhance and inhibit mutagenic activity of carcinogens (4,14,16,19). The differential inhibition of separate enzyme systems that can increase or decrease the size of the ultimate carcinogen "pool" may be important (2,3,19).

That sodium ascorbate could have a cellular effect as well as an extracellular interaction with carcinogen to modify it's action is demonstrated in Fig. 3. When mice were force-fed MNNG and then allowed to repair for 30 h, DNA sedimentation profiles shift from those indicating damage to those indicating close to control DNA (Fig. 3A,B). However, when sodium ascorbate is force-fed to the mice every 4 h beginning 4 h following MNNG treatment, the shift of the DNA sedimentation profile indicating repair does not take place by 30 h (Fig. 3C,D). It is delayed until, at most, 72 h following carcinogen treatment (Fig. 3E). Treatment of mucosal cells with chemical vehicle or 4-hourly treatments of sodium ascorbate alone does not result in a damage profile of stomach cell DNA (Fig. 3F,G).

Ascorbic acid, like some other biological reducing agents, may then have either an enhancing or a reducing effect on the DNA-

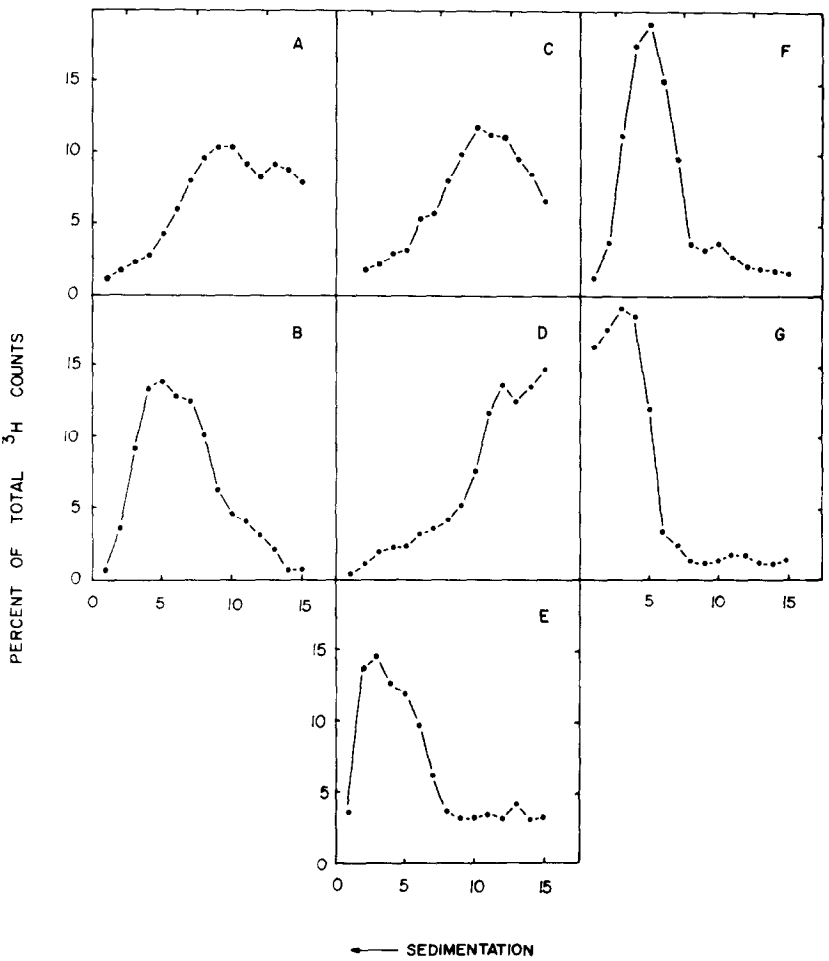



Figure 3 Alkaline sucrose gradient sedimentation profiles of mouse gastric mucosal cells A,C: 4 hours after force-feeding 40 mgm MNNG/ kgm mouse in a total volume of 0.5 ml B: 30 hours after force-feeding 40 mgm MNNG/ kgm mouse in a total volume of 0.5 ml. D: 30 hours after force-feeding 40 mgm MNNG/kgm mouse. 6 intermediate force-feedings of 20 mgm sodium ascorbate/kgm mouse administered every 4 hours were made, E: 72 hours after force-feeding 40 mgm MNNG/kgm mouse followed by 6 force-feedings of 20 mgm sodium ascorbate/kgm mouse administered every 4 hours following MNNG treatment. F: 4 hours after force-feeding 0.5 ml distilled water. G: 30 hours after the first of 6 doses of 20 mgm sodium ascorbate/kgm mouse, which were administered every 4 hours.

damaging capacity of carcinogens administered *in vivo*. In addition, chronic "low" doses of ascorbic acid may have a depressing effect on the DNA repair capacity of cells, causing a situation analogous to

β-decarboxylase (13) and other enzymes by cyanoalanine. However, several mechanisms, including some not involving covalent binding to the apo-enzyme, are also conceivable so that further work is underway in order to clarify this phenomenon.

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