DNA and its precursors might interact with the food preservatives, sodium sulphite and sodium benzoate

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Summary. The interaction of sodium sulphite and sodium benzoate with nucleosides and DNA has been studied in vitro. Reduction in UV-absorbance was consistently noticed. However, no new products result from such interaction. It is likely that our previous observations of the effects of the 2 food preservatives on DNA synthesis and mitotis in *Vicia faba* root meristems is not due to direct action of the chemicals at the level of genetic material.

Sodium benzoate and sodium sulphite are used as preservatives in canned fruits, fruit juices, dried fruit pulp, etc. Toxicological data on these preservatives are available from several studies[4–10]. Sulphite has been shown to express genetic effects in T_4 phage[11], lambda phage[12], *Escherichia coli*[9,13] and yeast[13,14]. Sulphite is known to inhibit DNA synthesis in *Vicia faba* root meristems[5,15]. Both benzoate and sulphite cause mitotic inhibition, pycnosis, premature chromosome condensation and anaphase bridge and micronucleus formation in *Vicia faba* root meristems[5,6]. Hyatsu and Miura[11] demonstrated the conversion of cytosine to uracil by sulphite. However, adenine and guanine did not react similarly[6]. Also, bisulphite has been reported to lower the activity coefficient of the bases[7]. We have noted a variety of chromosome and mitotic abnormalities induced by the 2 food preservatives[5,6]. Because of their reported interaction with bases[11,17] and inhibition of DNA replication[5,15], we examined the interaction of benzoate and sulphite with DNA and its precursors (nucleosides) in vitro. Our results indicate that they might interact with DNA and nucleosides; however, no new compounds result from such an interaction, suggesting that the effects on chromosomes attributed to sulphite and benzoate are not likely to be directly at the level of DNA or nucleosides.

Material and methods. The following concentrations of the chemicals were used: 5.0 ppm adenosine, 50 ppm guanosine, 37.5 ppm cytidine, 50 ppm uridine, 37.5 ppm sodium benzoate, 50 ppm sodium sulphite and 5.0 ppm calf thymus DNA (Calbiochem). These concentrations showed maximum absorption at their specific wavelengths. All dilutions were made with plain demineralized water. Sodium benzoate and sodium sulphite were mixed separately with each of the nucleosides or calf thymus DNA in dry-acid-cleaned test tubes. Plain demineralized water was used for the blank. After incubating the mixtures for 15 min, 1 h, and 12 h at 37 °C, spectra were run in both the visible and the UV range (0.5 mm slit width for 5 min), and compared with the standard curves of Dunn and Hall[18]. Spectographs for similar mixtures were superimposed. A few representative spectra are shown in figures 1 and 2. All nucleosides were obtained from E. Merck (FRG).

Results. Small shifts in the wavelength for maximum absorption peaks occur in the UV-spectra on incubation of benzoate with adenosine, guanosine, uridine or calf thymus DNA (figure 1); and of sulphite with cytidine or calf thymus DNA. Reduction in absorbance is evident in all the mixtures of benzoate and sulphite with nucleosides and calf thymus DNA (figure 2). It is evident that some interaction is likely to have occurred between benzoate and sulphite, and the nucleosides and DNA.

Discussion. The observed reduction in UV-absorbance is a relatively persistent phenomenon. Changes in absorbance might arise as a result of ionization of the solute, or fluorescence[19]. However, in the present study, the role of ionization has to be excluded since only the mixtures, and not the controls, exhibit a reduction in absorbance. Overall shifts in wavelength at maximum peaks of absorption have been minimal in all cases except with DNA and food preservative mixtures. DNA fragments do not exhibit such shifts[18], thus the DNA must have remained intact during the course of incubation (up to 12 h). Hence, benzoate and sulphite are unlikely to affect the DNA molecule.

Both benzoate and sulphite are toxic to *Salmonella* at higher concentrations and neither of them induces point mutations in *Salmonella* histidine auxotrophs with or without metabolic activation in the Ames test[5,21]. They also do not induce sex-linked recessive lethal mutations in *Drosophila*, but cause reduced fecundity and delay in metamorphosis[5]. However, both induce widespread chromosome abnormalities including anaphase bridges and micronuclei formation, and arrest the progression of mitosis in *Vicia faba*[22]. Sulphite, and to a lesser extent ben-

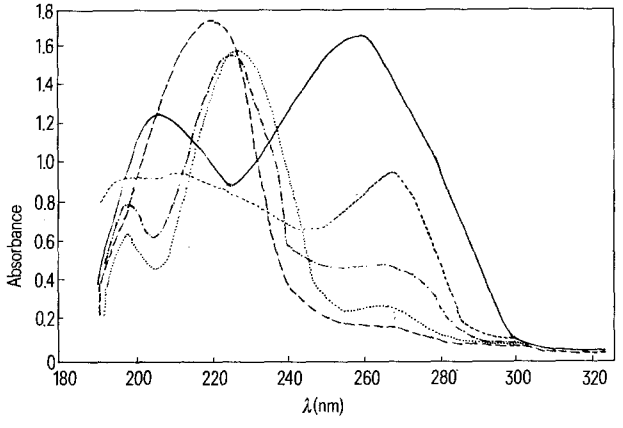

Fig. 1. Absorption spectra of 37.5 ppm benzoate and 5 ppm calf thymus DNA. – – – benzoate, ····· 1h incubation, ——— DNA, -------- 12 h incubation. – · – · – 15 min incubation.

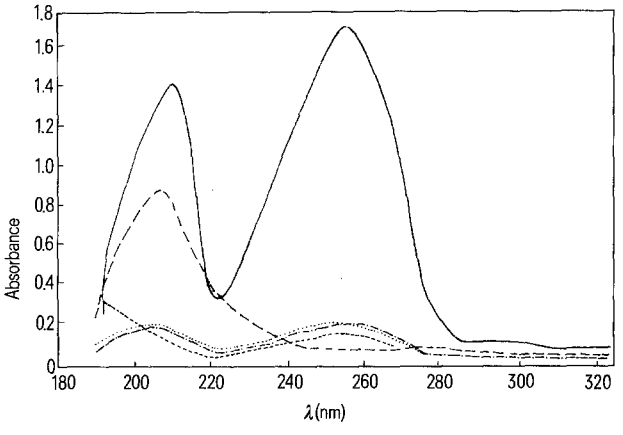

Fig. 2. Absorption spectra of 50 ppm sulphite and 5 ppm adenosine. – – – – sulphite, ····· 1 h incubation, ——— adenosine, -------- 12 h incubation, – · – · – 15 min incubation.

zoate, inhibits DNA synthesis[23]. Micronuclei formation leads to a loss of genetic material and is a true mutagenic effect[24]. There is a strong correlation between chromosome aberrations and mutagenicity[25]. Although benzoate and sulphite are not mutagenic in *Drosophila* and *Salmonella*, the fact that they cause chromosome aberrations leading to a loss of genetic material, inhibit DNA synthesis, and might interact with DNA and bases, calls for a closer look at their genetic toxicological effects.

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Nosiheptide, a sulfur-containing peptide antibiotic isolated from *Streptomyces actuosus* 40037

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Summary. Nosiheptide (9671 R.P.) isolated from *Streptomyces actuosus* 40037 (NRRL 2954) is a sulfur-containing polypeptidic antibiotic, quite different from all the other members of this family. Very active in vitro against gram-positive bacteria, it is inactive in vivo in experimentally infected mice. Not toxic, even at high dose, it may be used as a feed additive for chickens and pigs and it shows a favourable effect on the growth and conversion index.

In the course of a study on the production of antimicrobial agents by microorganisms, an antibiotic, nosiheptide (also known as 9671 R.P.), was discovered in the culture broths of *Streptomyces actuosus* 40037 (NRRL 2954)[1]. The main features of this strain have been reported by Shirling and Gottlieb[2].

Nosiheptide is obtained by culture of *Streptomyces actuosus* 40037 on aerated and stirred media as follows: the strain, stored as a spore-soil mixture, is grown in test tubes on Bennett's agar medium[3] for 3 weeks at 26 °C. It is brought to a suitable development by 2 successive transfers, first into 250 ml of liquid medium (composition in g/l: corn-steep liquor 20, saccharose 30, ammonium sulfate 2, calcium carbonate 7.5) in a 2-l flask, incubated for 48 h at 27 °C on a rotary shaker, then into 500 l of the following medium (in g/l): peptone 10, yeast extract 5, glucose monohydrate 10, agar 2, in a 800-l stainless steel fermenter in which the culture is agitated and aerated for 24 h at 27 °C. The final culture is obtained by seeding 50 l of the previous culture into a 800-l stainless steel fermenter containing 500 l of the following medium (in g/l): soybean flour 40, distillers' solubles 5, soybean oil 20, sodium chloride 5, heptahydrated magnesium sulfate 0.5, monohydrated manganese sulfate 0.3, heptahydrated ferrous sulfate 0.2, pentahydrated copper sulfate 0.02, hexahydrated cobalt chloride 0.02. The culture is agitated, aerated and kept at

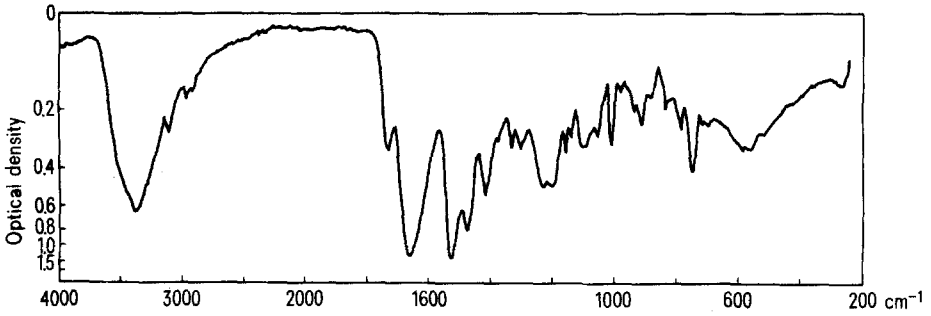

Fig. 1. IR-absorption spectrum of nosiheptide (KBr pellet).