Genetic Effects of Povidone-Iodine

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Abstract □ Povidone-iodine is capable of specifically altering the DNA of living cells. This alteration may result in the induction of mutations of the base-substitution type. Because of the known relationship between mutagenic potential and the ability to induce cancer in animals, the present findings raise serious questions concerning the safety of this topical disinfectant.

Keyphrases □ Povidone-iodine—genetic effects □ Mutagenicity—povidone-iodine □ Genetic effects—povidone-iodine

Povidone-iodine[1], an organic iodine complex reported to contain 1% free iodine (1), is widely used as a topical disinfectant. Because halogens, including iodine, are capable of reacting with nucleic acids and their constituents (2–5) and in view of the known relationship between the ability to react with DNA and mutagenesis and/or carcinogenesis (6–8), the potential genetic effects of povidone-iodine were investigated. The ability of povidone-iodine to modify intracellular DNA and to induce mutations in *Salmonella typhimurium* is now reported.

EXPERIMENTAL

Mutagenicity Testing—A series of histidine-requiring mutants of *Salmonella typhimurium* was constructed, which can be used to detect and classify mutagens. Two of these strains, TA1530 and TA1538, were used in this study; they can be induced to revert to histidine independence by base substitution and frameshift mutagens, respectively (9–11). To determine the mutagenicity of povidone-iodine, two procedures were used.

In the standard mutagenicity assay (9), bacteria are incorporated into an agar overlay. When the agar surface has solidified, a paper disk impregnated with the test agent is deposited on it. The plates are incubated for 54 hr at 37°, and the number of mutants (revertants to histidine independence) is determined. The diameters of the zones of growth inhibition are also measured.

A second, more quantitative, procedure was also used. Bacteria in liquid medium (Columbia base-broth-BBL) were chilled to 4°, and povidone-iodine was added. At intervals, portions of the cells were removed, the povidone-iodine was decomposed by addition of ascorbic acid (final concentration 0.1 *M*), and 10-fold serial dilutions of the treated bacteria were spread onto the surface of agar plates for the determination of the number of revertants to histidine independence (mutants) as well as of the number of viable cells. The medium for scoring mutants was a minimal medium supplemented with biotin (9) and containing a trace of histidine sufficient to support approximately two or three divisions (9). For the determination of the total number of viable cells, a nutrient broth base medium was used. Plates were incubated in the dark for 54 hr, and the number of colonies was counted. The first dilution tube used to make 10-fold serial dilutions for the determination of the number of viable cells also contained ascorbic acid (0.1 *M*); ascorbic acid is devoid of mutagenic activity and has no effect on the viability of the bacteria (12).

[1] Betadine.

Table I—Preferential Inhibition of DNA Polymerase-Deficient *E. coli*

Agent	Amount, μl	Diameter of Zone of Inhibition, mm: pol A^+	pol A_1^-
Povidone-iodine solution	20	73.0	>86
	10	50.6	81.4
	5	26.5	31.0
	3.3	19.2	25.6
	2	16.6	18.9
	1	14.2	15.7
Methyl methanesulfonate	10	37.4	55.1
β-Propiolactone	10	36.5	58.7
Propyleneimine	10	11.5	21.5
Chloramphenicol	30 μg	31.8	31.9

DNA Modifying Activity—Following exposure to agents capable of altering DNA, cells attempt to overcome this damage to their genetic material by a variety of DNA repair systems. One reaction involves the excision of the modified DNA segment, followed by "patching" of the removed section. The enzyme DNA polymerase I is active in several of these DNA repair processes (13). Therefore, mutant cells deficient in this enzyme (pol A_1^- bacteria) are more sensitive than the pol A^+ parents from which they were derived to the lethal action of agents capable of modifying the cellular DNA (13). Indeed, this prediction was fulfilled when DNA polymerase-deficient *Escherichia coli* (pol A_1^-) became available (14, 15).

Accordingly, a simple bioassay system was developed for detecting agents with the potential for altering cellular DNA. Numerous known mutagens and carcinogens gave positive results in this system; *i.e.*, they preferentially inhibited the growth of the pol A_1^- bacteria (15 and unpublished results). The technical aspects of the procedure were described in detail elsewhere (15) and basically involve the spreading of *E. coli* pol A^+ and *E. coli* pol A_1^- onto the surface of agar plates. When the surface of the agar has dried, paper disks impregnated with the test agent are deposited on top of the bacterial layer. The cultures are incubated at 37° for 12 hr, and the diameters of the zones of inhibition are measured. Agents known to modify cellular DNA produce a larger zone of growth inhibition on the pol A_1^--containing plate than on the corresponding pol A^+ one. Substances known to interfere with structures other than the DNA inhibit the two strains to the same extent (15).

RESULTS AND DISCUSSION

Povidone-iodine preferentially blocked the growth of the DNA polymerase-deficient *E. coli* strain (Table I), a property it shared with known mutagens and carcinogens (methyl methanesulfonate, β-propiolactone, and propyleneimine), which are known to derive activity from their ability to react with the cellular DNA. Chloramphenicol, which affects protein synthesis, inhibited the two strains to the same extent (Table I).

The mutagenicity of povidone-iodine for *S. typhimurium* could not be demonstrated in the standard mutagenicity assay (Table II), even though there was a zone of growth inhibition around the disks impregnated with this disinfectant. A number of known mutagens capable of inducing base substitution (methyl methanesulfonate, β-propiolactone, and ethyl methanesulfonate) and frame-

Table II—Mutagenicity Testing Using a Standard *S. typhimurium* Assay System[a]

Agent	Amount, μl	TA1530 Zone of Inhibition, mm	TA1530 Mutants per Plate	TA1538 Zone of Inhibition, mm	TA1538 Mutants per Plate
Povidone-iodine solution	0.2	0	17	0	13
	2	11.3	16	11.5	14
	20	31.0	22	32.3	16
Ethyl methanesulfonate	10	0	8000	0	7
Methyl methanesulfonate	10	22.2	530	24.2	14
β-Propiolactone	10	31.0	2652	25.9	13
2-Nitrofluorene	250 μg	0	13	0	87
Water (control)	10	0	17	0	12

[a]The "standard" procedure developed by Ames (9) was followed. Bacteria were incorporated into the agar overlay while the test substance was present in a paper disk which was placed on the surface of the inoculated plates. The plates were incubated in the dark at 37° for 54 hr whereupon the diameters of the zones of inhibition were measured and the number of mutants (revertants to histidine independence) was determined.

shift (2-nitrofluorene) mutations were active in this standard assay (Table II).

Recent studies have detected a number of agents that act in the same manner as povidone-iodine; *e.g.*, they are positive in assays designed to detect *in vivo* modifications of the DNA but they do not respond in the standard *Salmonella* mutagenesis assay. Substances behaving in this manner include nitrofurans (16 and unpublished results), sodium hypochlorite (12, 17), and some haloalkanes (18). It was demonstrated that this behavior is due to the fact that agents in this group are strongly bactericidal [as evidenced by large zones of growth inhibition (Table II)], and the frequency of mutations is not detectable due to devitalization of the cells (12, 18).

In this connection, it must be stated that results in the standard mutagenicity assay are expressed as mutants per number of bacteria added to the plate and not as mutants per surviving cell. This difficulty was overcome readily if the cells were pulsed for a short duration with the potential mutagen, the latter was then removed, and the cells were processed for the enumeration of mutants and survivors by plating on selected media. Moreover, cellular survival could be increased by carrying out the procedure in an ice bath (4°). The mutagenicity of nitrofurans (unpublished results), haloalkanes (18), and hypochlorite (12) was demonstrated by this procedure.

When using the modified assay, the mutagenicity of povidone-iodine for *S. typhimurium* TA1530 was easily demonstrated. This effect was a function of both the povidone-iodine concentration and the duration of exposure (Fig. 1, curves A and B). Under experimental conditions similar to those described in Fig. 1A, povidone-iodine had no mutagenic effect on strain TA1538. At the dilutions plated, no mutations were detected at all (*i.e.*, even less than in untreated control). This finding presumably reflected the devitalizing effect of povidone-iodine. When the concentration of the latter was diminished (*i.e.*, situation similar to Fig. 1B) so as to increase the number of survivors, histidine-independent mutants could be scored. Their frequency, however, was not significantly different from that of the untreated control (Fig. 1C). These findings indicate that in *S. typhimurium*, povidone-iodine induces mutations of the base substitution but not of the frameshift type.

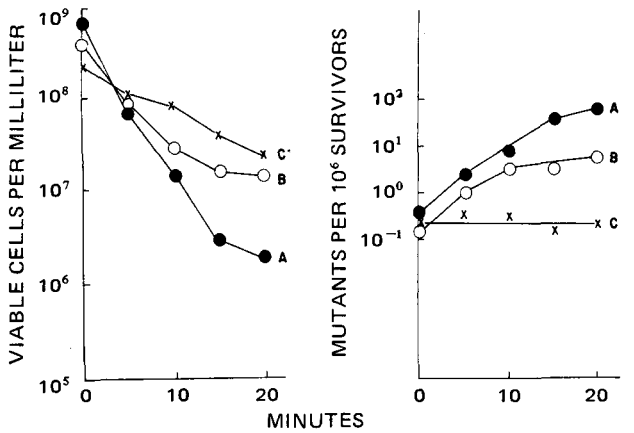

Figure 1—*Mutagenicity of povidone-iodine for* S. typhimurium. *The effects of povidone-iodine on the viability of* Salmonella *are given in the left panel, while the numbers of mutants induced per 10^6 survivors are given in the panel on the right. Key: A, effect of a 1/250 dilution of povidone-iodine solution on strain TA1530; and B and C, effects of a 1/1000 dilution of povidone-iodine solution on strains TA1530 and TA1538, respectively.*

CONCLUSIONS

The present results indicate that povidone-iodine, a widely used disinfectant, is capable of modifying the DNA of living cells and this modification, in turn, may result in mutagenic events. In view of the known relationship between mutagenic potential and the ability to induce cancer in mammals, the present findings raise some serious questions about the safety of povidone-iodine, especially in view of the residual iodine deposited at the site of application which may generate active iodine for prolonged periods. An evaluation of the potential genetic and carcinogenic hazards posed by the widespread use of povidone-iodine appears to be in order. These hazards will have to be balanced against the availability of a disinfectant of equal effectiveness and less potential toxicity.

The present findings reemphasize the importance of including tests that detect DNA modifications as well as mutations in the battery of standard microbial assays used to detect potential environmental mutagens and carcinogens.

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GLC Determination of Heroin and Its Metabolites in Human Urine

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Abstract □ Heroin and its metabolites, 6-monoacetylmorphine, morphine, and normorphine, were determined in human urine with a GLC procedure. Heroin was extracted with chloroform at pH 4.5 and chromatographed at a temperature programmed from 200–250° by 8°/min. 6-Monoacetylmorphine and morphine were extracted with ethylene dichloride containing 30% isopropanol at pH 8.5, and normorphine was extracted at pH 10.4 with the same solvent. The extract was derivatized with trimethylsilylimidazole and chromatographed at 230° for the determination of 6-monoacetylmorphine and morphine and at 220° for normorphine and morphine.

Keyphrases □ Heroin and metabolites—GLC analysis in human urine □ Morphine, normorphine, and 6-monoacetylmorphine (heroin metabolites)—GLC analysis in human urine □ GLC—analysis, heroin and metabolites in human urine

Previous studies on laboratory animals *in vitro* and *in vivo* have indicated that heroin (3,6-diacetylmorphine) is rapidly deacetylated to 6-monoacetylmorphine and then to morphine (1–7). The major portion of a given dose of heroin can be accounted for in the urine as free morphine and morphine conjugate (8–13). Heroin, 6-monoacetylmorphine, and morphine have previously been estimated in biological materials with a methyl orange dye procedure, a Folin–Ciocalteu phenolic reagent (3), radioactive tracers (5), paper chromatography (7, 14), and GLC (12). This article presents an improved GLC procedure for the estimation of heroin and its metabolites, 6-monoacetylmorphine, morphine, and normorphine.

EXPERIMENTAL

Materials—Heroin hydrochloride[1] (containing about 5% 6-monoacetylmorphine as an impurity, as determined with the GLC procedure described here), normorphine hydrochloride[1], and morphine sulfate[2] USP were obtained.

Gas Chromatograph—A gas chromatograph[3] equipped with dual flame-ionization detectors and a dual pen recorder[4] was used. A 0.9-m (3-ft) × 2-mm glass column (Column 1) was packed with 3% OV-17 coated on Gas Chrom Q (60–80 mesh) and conditioned at 270° by passing nitrogen (30 ml/min) for 1 hr, then at 340° without nitrogen for 4 hr, and finally at 290° with nitrogen (16 ml/min) for 72 hr. A 1.5-m (5-ft) × 2-mm stainless steel column (Column 2) was packed with 3% SE-30 coated on Varaport (100–120 mesh). The temperatures of the injector and detector were set at 255 and 295°, respectively.

Determination of Heroin—A 5-ml aliquot of urine was placed in a 40-ml centrifuge tube, adjusted to about pH 4.5 with acetic acid, buffered with 1 ml of 1 *M* sodium acetate buffer at pH 4.5, salted with 1.6 g sodium chloride, shaken with 15 ml of chloroform in a shaker[5] at 280–300 oscillations/min for 10 min, and centrifuged at 2000 rpm for 10 min. After removal of the aqueous phase by aspiration, a 13-ml aliquot of the organic phase was transferred to a 15-ml conical centrifuge tube and evaporated to dryness under a stream of nitrogen in a water bath at 60–70°.

The tube was rinsed with 1 ml of chloroform by mixing on a mixer[6] for about 10 sec, and the chloroform was evaporated to dryness. To the residue was added 50 μl of cholestane solution (0.05 mg/ml in ethyl acetate) as an internal standard, and 1 μl of the solution was injected onto Column 2. The temperature of the column was programmed from 200 to 250° at 8°/min. A standard curve was prepared by adding authentic heroin hydrochloride (0.25–10 μg) to 5 ml of drug-free cigarette smoker's urine and analyzing by the described procedure.

Determination of 6-Monoacetylmorphine and Morphine—A 5-ml aliquot of urine was placed in a 40-ml centrifuge tube, adjusted to pH 8.0–8.5 with sodium hydroxide solution, buffered with 1 ml of 1 *M* phosphate buffer at pH 8.5, salted with 1.6 g of sodium chloride, shaken with 15 ml of ethylene dichloride containing 30% isopropanol in a shaker[5] at 280–300 oscillations/min for 10 min, and centrifuged at 2000 rpm for 10 min. After removal of the aqueous phase by aspiration, a 13-ml aliquot of the organic phase was transferred to a 40-ml centrifuge tube.

The alkaloids in the organic phase were back-extracted into the aqueous phase with 5 ml of 0.1 *N* HCl by shaking in the shaker for 5 min and centrifuging for 5 min. The organic phase was removed by aspiration without removing any of the acid phase (leaving a small drop of the organic phase in the bottom of the tube). The acidic phase was adjusted to pH 8.0–8.5, buffered, salted with 1.0 g of sodium chloride, and extracted with the organic solvent as already described. After removal of the aqueous phase by aspiration, a 13-ml aliquot of the organic phase was transferred carefully (so as not to include any aqueous phase) to a 15-ml conical centrifuge tube and evaporated to dryness under a stream of nitrogen in a water bath at 60–70°.

[1] Through the courtesy of Dr. Everette May, National Institutes of Health.

[2] Commercial product.

[3] Varian Aerograph, Series 2700.

[4] Model A-25, Varian.

[5] International bottle shaker, International Equipment Co., Boston, Mass.

[6] Vortex.