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Marvin D. Anderson

Herbert S. Rosenkranz

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Marvin D. Anderson, MD* and Herbert S. Rosenkranz, MD**

A simple screening method, in which a bacterial mutant deficient in its ability to repair DNA, was used to detect genetic damage from a widely-used fungicide. The finding strengthens the suspicion that it may be a potent human carcinogen. The unique role of the physician in eliciting a history of exposure from a patient with cancer is emphasized in the case report.

*Medical Clinic II, Department of Medicine

**Department of Microbiology, College of Physicians and Surgeons, Columbia University

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Address reprint requests to Dr. Anderson at Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202

NATIONAL vital statistics show more persons die from cancer than from any other disease except heart disease. Every two minutes an American dies from cancer. The age-adjusted national death rate from cancer has been rising steadily.[1]

If, in fact, at least 85% of human cancer is attributable to cancer-causing agents in our environment, then today's vital challenge is the eradication of cancer through the identification and elimination of these environmental carcinogens. Thus cancer may be prevented even before we unravel the mystery of how a particular cell becomes malignant. Cancer prevention is better than cancer cure.[2] This approach requires that we scrutinize closely the chemical and physical agents to which we are constantly exposed.

For years it has been recognized that physical and chemical agents capable of causing cancer in mammals frequently cause genetic damage in a number of simpler biological systems. Conversely, these bench-top experiments have been used to indicate potential mammalian carcinogens and to investigate their mode of action.[3-11] As a result of such work, many investigators now feel that a chemical reaction with the genetic molecule DNA (deoxyribose nucleic acid) is the basis of drug-induced carcinogenesis.

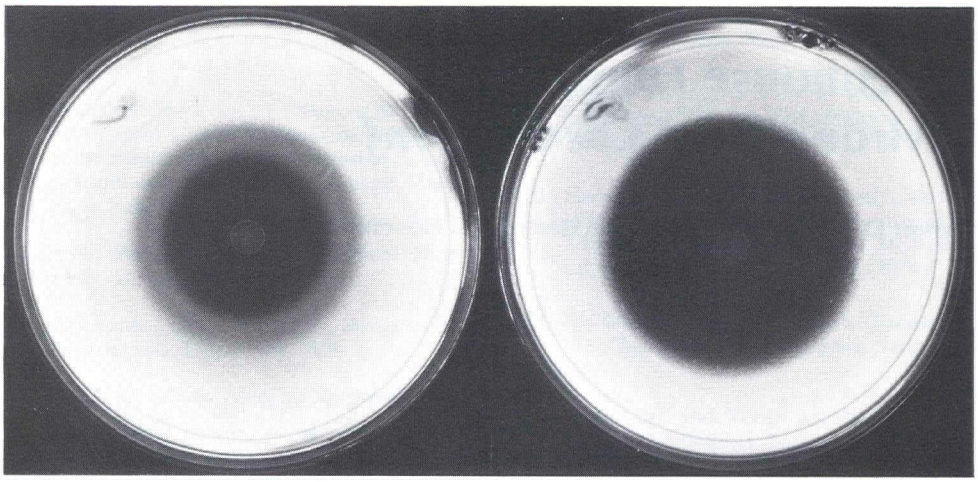


Figure 1
Effect of methylmethanesulfonate on E. Coli pol A+ (*left*), and its repair-deficient pol A− mutant (*right*). The latter exhibits a larger central clear zone of growth inhibition (photograph used with permission from *Cancer Research*).

We report how a simple screening method[12] in which a bacterial mutant, deficient in its ability to repair DNA, was used to detect genetic damage from a widely-used fungicide, thus strengthening the suspicion that it is a potent human carcinogen. The test is based on the finding that cells exposed to agents which modify their DNA tend to protect themselves by excising the altered DNA portion and resynthesizing the correct sequence of nucleotides in the double helix. The enzyme *DNA polymerase* has been implicated in this repair process, which has been elucidated in Kornberg's laboratory using ultra-violet (U-V) irradiated bacterial DNA.[13] U-V damage causes dimerization of adjacent thymine molecules. The steps in this proposed model are:

1. A thymine dimer introduces distortion into one strand of a DNA helix.
2. Endonuclease specific for damaged DNA recognizes the distortion and makes a single-strand break close to the dimer.
3. By adding nucleotides covalently DNA polymerase restores the duplex until it reaches the opposite side of the gap.
4. Exonuclease excises the damaged oligonucleotide.
5. Polynucleotide-joining enzyme (ligase) closes the nick, completing repair.

A bacterial mutant deficient in DNA polymerase (pol A−) has been isolated in Cairns' laboratory from *Escherichia coli* W3110 (pol A+).[14] This mutant *(E coli* p 3478) is extremely sensitive to U-V rays, radio-mimetic agents, and agents known to react with cellular DNA.[13-15] Our method capitalizes on the inability of the mutant to grow in an area where agents which damage DNA are present. A filter paper disk impregnated with the compound to be tested is placed in a Petri dish on top of a nutrient agar layer containing either the pol A− or pol A+ strains. The plates are incubated overnight as the chemical diffuses outward

from the disk. If it is toxic there will be a central clear zone of growth inhibition in the creamy bacterial lawn found on the plate the next morning. The diameter of this clear zone is measured (Figure 1).

If the test agent acts by damaging DNA, the plate containing the repair-deficient pol A− strain will have a larger clear zone than that which occurs with pol A+. Methylmethanesulfonate, a known carcinogen and alkylating agent, is used as a positive control.

In this test carcinogens are found to give a much greater inhibition zone for the mutant strain. In fact, there is a high correlation between inhibition of pol A− by selected chemicals and the carcinogenicity of these chemicals as established by animal tests.[12] Although this method is not a direct test for carcinogenicity, it is a simple and rapid means for identifying substances which preferentially alter DNA.

In this fashion we tested the eight pesticides recovered from the greenhouse of a 72-year-old man. The patient worked almost all of his adult life commercially growing orchids and carnations. He had developed faltering speech over a five-year period. An epileptic seizure prompted his hospitalization. Cerebral angiography disclosed a tumor strain in the left temporal lobe. At craniotomy, a grade III malignant astrocytoma was found and partially excised. Post-operatively the patient survived two years in a nursing home. He died in March, 1971.

Prior episodes of documented pesticide intoxication in this patient, and the finding of brain tumors in other workers spraying pesticides in an enclosed environment,[16] aroused our suspicion that these aerosols may be carcinogenic. Some chemically resemble known mutagens.

TABLE I
INHIBITION OF E. COLI GROWTH (mm)

	W3110 Pol A+	Pol A− MUTANT
Isotox	20	20
Malathion	0	0
Morsodren	48	62
Fulex	10	10
Formula Ch-19	12	12
Captan	13	17
Parzate	17	32
Tetrachlor	0	0
MMS Positive Control	35	70

Effect of pesticides from the patient's greenhouse and methylmethanesulfonate (MMS) on the two bacterial strains. Numbers are diameters of central clear zones which delineate extent of irreparable damage caused by the compound tested. Identical zones indicate that the toxic effect did not involve DNA damage. Zero inhibition indicates chemical inactivity of the agent under these test conditions.

As indicated in Table I, three of the pesticides preferentially inhibited the mutant strain. In order to investigate the type of damage, mammalian DNA was exposed to these three mutagenic pesticides *in vitro* and analyzed. Samples of calf thymus DNA were dissolved in saline, mixed with the pesticides, and incubated in a water bath at 56°C. At hourly intervals aliquots were withdrawn, diluted in saline, and analyzed for sedimentation velocity in a Spinco Model E analytical ultracentrifuge. The normal DNA sedimentation pattern was altered by two of the three pesticides. One of them, the fungicide captan, was found to irreversibly denature DNA.

Captan's mode of action was further investigated with ascending paper chromatography. Each of the four purine and pyrimidine nucleotides present in DNA (deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine), dissolved in saline, was incubated in a water bath, as described, both in the presence and in the absence of captan. The reaction mixtures, each with its own control, were then spotted on Whatmann paper and chromatographed using an ethanol:ammonia:water solvent. The

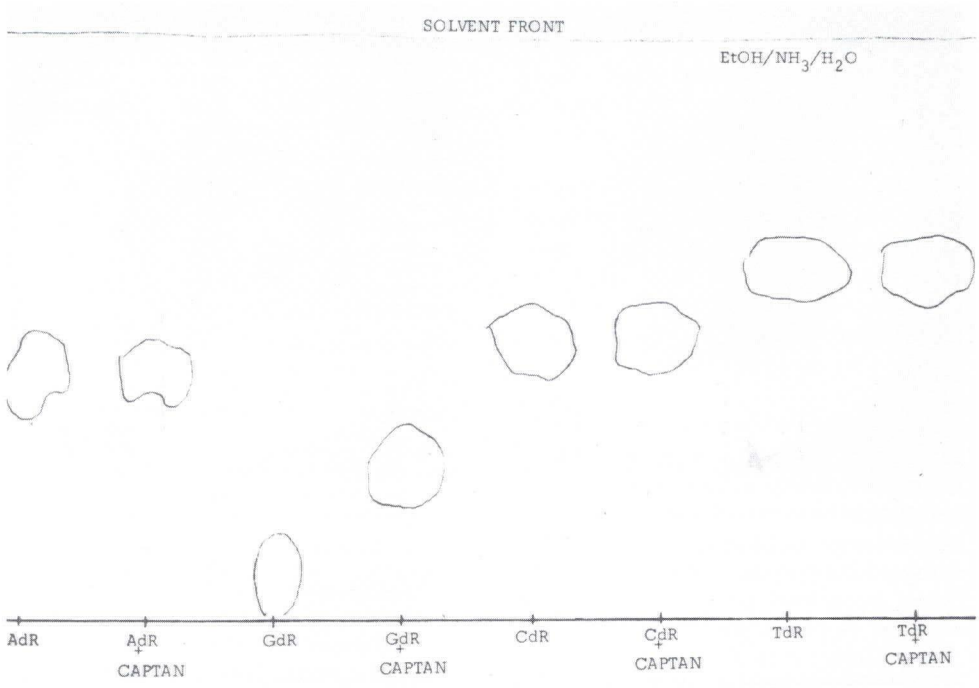


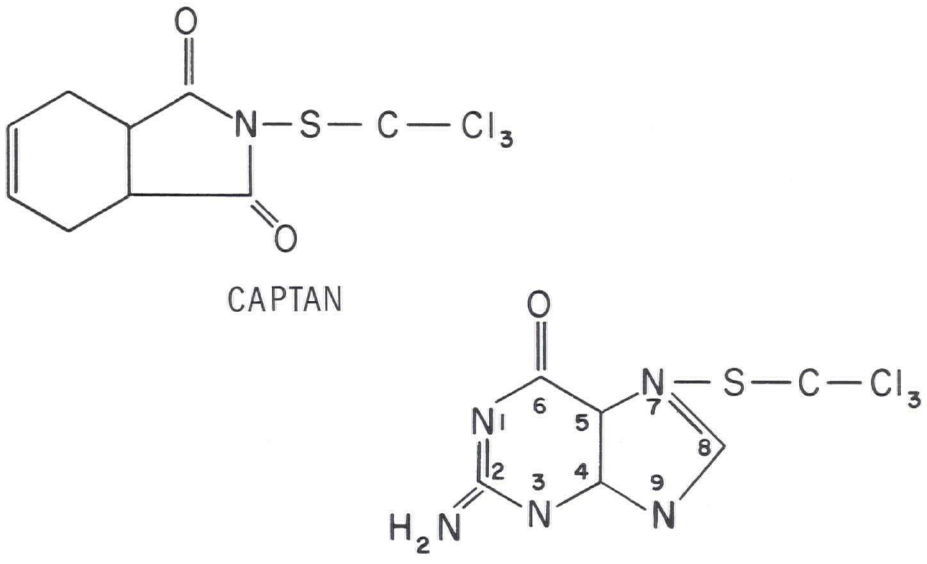
Figure 2
Reaction between captan and deoxyadenosine (AdR), deoxyguanosine (GdR), deoxycytidine (CdR) and thymidine (TdR). Captan affects only the rate of migration of GdR thereby indicating that a reaction between GdR and captan has taken place.

chromatographs were dried and examined under ultraviolet light. Migrant spots were identified and compared.

The migrations of deoxyadenosine, deoxycytidine, and thymidine were unaffected by captan, but captan was found to react preferentially with deoxyguanosine. A different migrant spot was found representing the newly formed reaction product between the purine base guanine and the fungicide (Figure 2). The heat lability and spectral properties of this new compound allowed its identification as an alkylation-like reaction of the number seven position of guanine by the trichloro-methyl-sulfenyl radical from the captan molecule. Thus the reaction product is presumed to be 7-(trichloromethylsulfenyl) guanine (Figure 3).

To determine whether or not this *in vitro* reaction also occurred *in vivo*, radioactive captan-S^{35} was obtained and administered to a live mouse. Several hours later the animal was sacrificed and its DNA isolated. By means of paper and Sephadex column radiochromatography the *in vivo* and *in vitro* alkylation products were shown to be identical.

Others have found captan to be toxic. Verrett and associates[5] have demonstrated that captan is teratogenic. It causes severe malformations in developing chick embryos.[17] Recently, Bridges et al have found captan to be mutagenic



7 (TRICHLOROMETHYLSULFENYL) GUANINE

Figure 3
Presumed reactions product from alkylation of guanine at position No. 7 by the trichloromethylsulfenyl radical of captan.

using another type of repair deficient strain of *E coli*. They allude to the potential hazard of carcinogenesis by inhalation of captan.[18] Cancer has not been described as bacteria, but deficient DNA repair in humans does greatly enhance their oncogenic susceptibility. An excellent review of this genetic mutant state has appeared recently.[19]

In summary, the fungicide captan is a potent mutagen in bacteria, teratogen in bird embryos, and an alkylating agent in mammals. As such it is highly suspect to be a carcinogen, especially in light of the circumstances which brought it to our attention. However, it cannot be inferred from this data alone that a cause and effect relationship has been established between exposure to captan and tumorigenesis in the brain or elsewhere. What can be emphasized is the unique role of the physician in eliciting a history of exposure from a patient with cancer, for it may uncover yet unrecognized harmful environmental agents.

Acknowledgment

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