Genetic Activity of 1,2-Dibromo-3-Chloropropane, A Widely-Used Fumigant

by HERBERT S. ROSENKRANZ
Department of Microbiology
College of Physicians and Surgeons
Columbia University
New York, N.Y.

INTRODUCTION

It has been estimated that up to 90% of all human cancers are caused by chemicals present in the environment (EPSTEIN, 1974). In view of this large proportion of presumably preventable malignancies and because at the present time it appears that a preventive program will be more effective than the therapeutic modalities currently available for the treatment of cancer, it would seem that elimination of offensive agents from the environment might result in a dramatic decrease in the incidence of cancer. In order to achieve this it will be necessary to recognize environmental agents responsible for cancer causation. Classically this has taken the form of animal testing. However to be effective such assays must use a large number of animals of different species (EPSTEIN, 1974). The cost of such an endeavor may be very large, possibly even prohibitive (MAUGH, 1974).

As it has been found that many carcinogens are endowed with mutagenic activity, either because carcinogenesis originates as a genetic event or because carcinogens as well as mutagens act on a common structure - the cellular DNA - it has been suggested (MRAK, 1969; DHEW, 1974a, 1974b; ROSENKRANZ, 1973) that bioassays based upon the ability to detect agents which modify the cellular DNA or which induce mutagenic changes be developed to screen for potential carcinogens. Microbial assays because of their low cost, simplicity, rapidity and sensitivity appear well-suited for such screening purposes and indeed a number of such systems have been developed which appear to have promise for detecting potential carcinogens (ROSENKRANZ, 1973).

In a recent study, we investigated (BREM et al, 1974) the mutagenicity and DNA-modifying activity of a number of widely-used haloalkanes. One of these, ethylene dibromide (1,2-dibromoethane), a widely-used fumigant and intermediate in many industrial processes, was shown by others (OLSON et al, 1973) to be carcinogenic for rodents. These same investigators also found another widely-

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used fumigant and nematocide, 1,2-dibromo-3-chloropropane (DBCP, fumazone, nemagon, nemafume), to induce tumors in rodents. Since DBCP was not tested in microbial systems in our original study, it was thought of importance to conduct tests on this widely-used fumigant in order to determine further the reliability of short-term microbial assays in predicting carcinogenicity.

Experimental

DNA-modifying capacity was tested using a set of Escherichia coli strains: one deficient in DNA polymerase I (E. coli pol A_1^-) and the other with a normal complement of this enzyme (E. coli pol A^+) (BREM et al, 1974; SLATER et al, 1971). Agar plates were inoculated with the tester strains and filter discs impregnated with the test chemical were placed on top of the bacterial lawn. The plates were incubated at 37°C for 9 hours and the diameters of the zones of growth inhibition were determined. In this assay agent which alter the cellular DNA will inhibit the E. coli pol A_1^- strain preferentially. This will be expressed as a larger zone of growth inhibition. On the other hand, agents which affect structures other than the DNA will inhibit the two tester strains to the same extent (SLATER et al, 1971).

The tester strains and procedure developed by Ames and his associates (AMES, 1971; AMES et al, 1973) were used to determine mutagenicity. In this assay system histidine-requiring strains of Salmonella typhimurium are induced to mutate back to histidine independence. Some of the strains (e.g. TA1530) revert as a result of base-substitution mutations (AMES, 1971; AMES et al, 1973) while other (e.g. TA1538) do so in response to frameshift mutagens (AMES et al, 1973; ISANO and YOURNO, 1974). The bacteria together with various dilutions of the test agent were incorporated into an agar overlay containing minimal medium, glucose, biotin and a trace of histidine (AMES, 1971). The plates were incubated in the dark at 37°C for 54 hours whereupon the number of revertants to histidine-independence was determined.

Result and Discussion

DBCP preferentially blocked the growth of E. coli pol A_1^- (Table 1) which is indicative of an effect on the cellular DNA. This reaction was expressable as a mutagenic effect on Salmonella typhimurium (Table 1) and presumably provides a basis for its reported carcinogenicity (OLSON et al, 1973). It should be noted that DBCP is mutagenic for strain TA1530 and not TA1538 which indicates that it induces mutations of the base-substitution but not of the frameshift type. This suggests that DBCP acts as an alkylating agent.

TABLE 1

Genetic Effects of 1, 2-Dibromo-3-Chloropropane

Agent	Amount (mg)	Revertants per Plate TA1530	Revertants per Plate TA1538	Diameter of Zone of Inhibition (mm) Pol A^+	Diameter of Zone of Inhibition (mm) Pol A_1^-
1, 2-Dibromo-3-chloropropane	0	17	10		
	0.4	48	14		
	1.1	122	12		
	2.1	153	10		
	4.2	242	9		
	6.3	469	6		
	10.5	751	9		
	15.7	870	13		
	20.9	1218	9	8.9	13.7
β-Propiolactone	11.5	384	12	34.4	52.0
Chloramphenicol	0.03	14	8	32.8	32.7

β-Propiolactone and chloramphenicol were included as positive and negative controls, respectively.

However, further chemical studies will be required to establish this fact.

The present findings establish that DBCP, a known carcinogen, is positive in microbial assays designed to detect mutagens and agents capable of altering the cellular DNA. This not only reinforces the reliability of microbial screening procedures but it suggests that such bioassays be introduced for the routine detection of potential harmful environmental agents. Agents which give positive tests in these systems could then be subjected to carcinogenicity testing in animals. Some of the agents which see wide-spread household use could be withdrawn pending the completion of animal studies.

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