Summary. Suspension cultures of *Vicia hajastana* and *Haplopappus gracilis* were maintained in B5 medium containing 0.1, 1.0 and 10.0, and 0.1, 0.5, 1.0 and 5.0 µg/ml 2,4–D, respectively. Anaphase analyses showed that the frequency of anomalies, especially bridges, was negatively associated with the 2,4–D concentration.

B. D. SINGH[19] and B. L. HARVEY

Department of Genetics and Plant Breeding, Faculty of Agriculture, Banaras Hindu University, Varanasi 221005 (India), and Crop Science Department, University of Saskatchewan Saskatoon (Canada), 17 February 1975.

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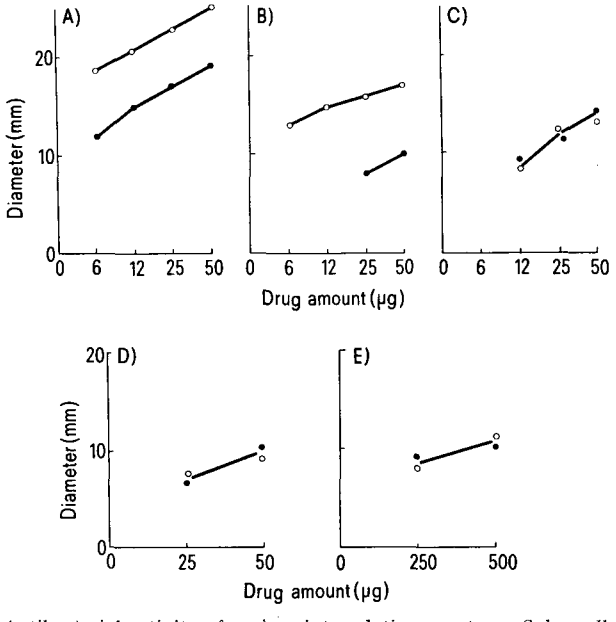
Effect of Excision Repair System on Antibacterial and Mutagenic Activity of Daunomycin and Other Intercalating Agents in *Salmonella typhimurium*

Anthracycline antibiotics and acridine dyes are known to interact with DNA as intercalating agents, thereby displaying antibacterial, antitumor and frameshift mutagenic activity[1–5]. It has been claimed that these activities are enhanced if the heterocyclic ring is substituted by different chemical reactive groups, which allow the formation of covalent bonds between DNA and the intercalating polycyclic ring[6–8]. It has also been supposed that the excision repair system possesses different capability to repair DNA molecules damaged by simple or reactive intercalators[8].

Daunomycin and adriamycin, two anthracycline antibiotics, interact with DNA by simple intercalation, but the aminosugar residues seem to be important for the stabilization of the complex and for the biological effects of the substances[1–4].

In the present paper we describe the antibacterial and mutagenic effect of daunomycin, adriamycin, and various acridine dyes on isogenic strains of *Salmonella typhimurium* with a normal or defective excision repair system.

Daunomycin and adriamycin were from Farmitalia, acridine orange and ethidium bromide from Sigma, and 2,8-diamino-10-methyl-acridine from K & K.



Antibacterial activity of various intercalating agents on *Salmonella typhimurium* strain TA1978 *uvr*$^+$ (●) and strain TA1538 *uvrB* (o). Diameters of zone of inhibition were measured as described in the text. A) daunomycin; B) adriamycin; C) acridine orange; D) 2,8-diamino-10-methyl-acridine; E) ethidium bromide.

Salmonella typhimurium strain TA1538 and TA1978 received from B.N. Ames were used. Both strains have the *his D3052* frameshift mutation[9], and a mutation conferring the deep rough character, which makes the bacteria much more permeable, and hence sensitive to mutagens[8]. The two strains differ in their ability to repair damaged DNA, as TA1538 has a deletion through the *uvrB* gene, while TA1978 has a normal excision repair system[8].

For the antibacterial test, 50 µl of appropriate dilutions of substances were pipetted in 6 mm holes cut into pour plates with a lawn of bacteria. Minimal agar with addition of histidine 1 m*M* and biotine 5 µ*M* was used. The plates were then placed in a 37 °C incubator for 24 h and the zone of killing measured.

For testing of mutagens, the method described by AMES et al.[8] was followed. Briefly, 2 ml of molten soft agar, containing 0.05 m*M* histidine and 0.05 m*M* biotine, were mixed with appropriate amounts of chemicals and with 0.1 ml of an overnight culture of the tester bacteria, and poured onto the surface of a minimal agar plate with Vogel-Bonner E medium. Plates were incubated at 37 °C for 2 days, after which the number of revertant colonies was counted.

The Figure shows the antibacterial effect of daunomycin, adriamycin, acridine orange, ethidium bromide and 2,8-diamino-10-methyl-acridine on *Salmonella typhimurium* with and without excision repair system. It is evident that strain TA1538, which lacks the product of *uvrB* gene, is much more sensitive to daunomycin and adriamycin that strain TA1978, which has a normal repair system. In contrast, the 3 acridine dyes do not show any differential effect on the 2 strains.

When tested for mutagenic activity in amounts ranging from 1 to 1000 µg acridine orange, 2,8-diamino-10-methyl-

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acridine, and ethidium bromide showed little, if any, effect in the test system used. This is in accordance to previous reports[6-8], that acridines without an alkylating side chain are poor mutagens. Daunomycin and adriamycin, on the contrary, showed definite mutagenic activity which is more pronounced on strain TA1538 lacking the excision repair function (Table).

The above results clearly show that, for the antibacterial and mutagenic activity of anthracycline antibiotics daunomycin and adriamycin, it is important whether the tester bacterial strain has a normal or a defective excision repair system. In contrast, the acridine dyes do not show any differential effect on *uvrB* and *uvr*$^+$ strains.

Mutagenic activity of anthracycline antibiotics

Compound added	Amount (μg)	Revertant colonies from tester strain	
		TA1978 (*uvr*$^+$)	TA1538 (*uvrB*)
None (control)		2	3
Daunomycin	1	2	20
	3	5	46
	10	10	65
	30	11	400
Adriamycin	1	12	4
	3	10	10
	10	4	70
	30	7	33

Figures show the number of revertant colonies (histidine non-requiring) per petri plate and are the mean of 2 separate experiments.

Both groups substances are known to interact with DNA by simple intercalation without covalent bonds[1-5]. It has been claimed that DNA damaged by certain acridines with alkylating side chains is subject to repair by the bacterial excision repair system, while damage caused by simple intercalators is not[7,8].

The present results show that even some noncovalent intercalators, such as anthracycline antibiotics, may interact with DNA in a way which is relevant for the excision repair system. This fact may be related to the aminosugar residue of the two antibiotics, since antibacterial and mutagenic activity of simple acridine dyes is equally pronounced on bacteria with and without the excision repair system.

Studies are in progress for determining which kind of side chain, apart from the cases already known[6-8] and this report) confers to an intercalating agent a reactivity with DNA which is significant for the excision repair system of bacteria.

Summary. Daunomycin and adriamycin, are more mutagenic and antibacterial for a strain of *Salmonella typhimurium* defective for the *uvrB* gene than for its *uvr*$^+$ counterpart. Other intercalating agents, as some acridine dyes, affect equally the two bacterial strains.

B. PANI, C. MONTI-BRAGADIN and L. SAMER[10]

Istituto di Microbiologia dell'Università, Via Valerio 34, I-34127 Trieste (Italy), 18 February 1975.

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Tsetse Fly *Glossina morsitans morsitans* Produces Ultrasound Related to Behavior*

Several aspects of the behavior of the tsetse fly are unexplained. It is unknown how tsetse flies can survive low host densities and how, from an almost evenly and widely dispersed population in the bush[1] they can find each other for mating and formation of the wellknown 'following swarm of Swynnerton'. This behavior would suggest the existence of some form of communication among tsetse flies. So far as is known, tsetse flies do not possess sex pheromones[2,3]. Evidence of the emission of sound by tsetse flies was gleaned long ago and con-

* ICIPE publication No. 24.

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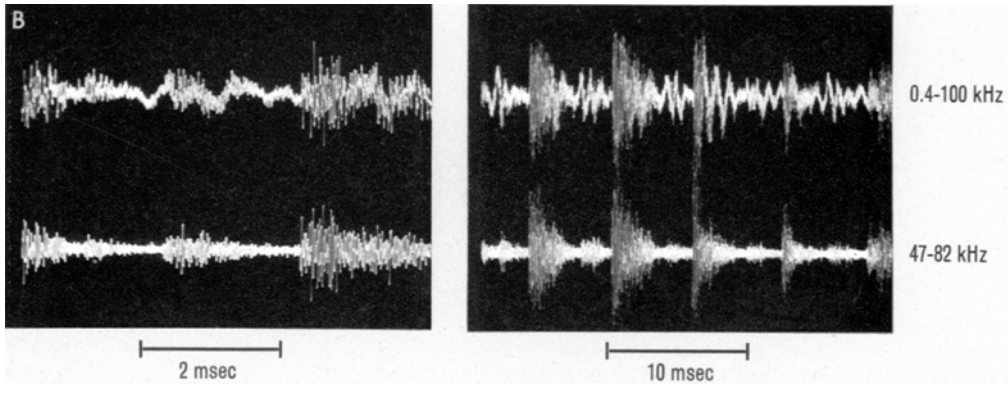

Fig. 1. Typical mating sounds. The amplification on the 47-82 kHz channel was twice that on the channel showing the full frequency range.