Multiplication of *Mycobacterium leprae* in hairless mice

Strain of mouse	Route of inoculation	No. of bacilli inoculated	No. of bacilli harvested
Hairless	Footpad	1.0×10^4	$7.0 \pm 1.0 \times 10^6$/footpad
Hairless	Subcutaneous	1.0×10^7	Skin: negative Footpad: negative Spleen: negative
Hairless	Intravenous	1.0×10^7	Skin: negative Spleen: negative Footpad: negative
NIH	Footpad	1.0×10^4	$5.3 \pm 0.93 \times 10^6$/footpad

infection[5]. This has been attributed to the induced deficiency of immune response in the host animals. The nine-banded armadillo is the only species in which at least some of the animals have been shown to be naturally susceptible to the systemic form of leprosy[6]. Hairless (nude) mice are characterized by hypoplastic thymus glands and a consequent depletion of T-lymphocytes[7,8]. Congenitally athymic (nude) mice have been found to accept for their life-time skin grafts from distantly related animals species including man[9], demonstrating an impairment of thymus-dependent immunity in these mice.

Methods. To study whether there would be increased multiplication of *M. leprae* in hairless mice, the animals were inoculated in the footpads as well as i.v. and s.c. with suspensions of viable bacilli. The nude mice were offspring heterozygotes (brown, black and nonpigmented strains), obtained from the Jackson Laboratory, Bar Harbor, Maine, USA. The suspension of *M. leprae* was prepared from skin biopsies of lepromatous patients. To test the viability of the organisms, the bacilli were inoculated into the footpads of an NIH strain of Swiss mice. 2 experiments were completed using 30 hairless mice and 20 NIH mice. 6 months after inoculation, the animals were sacrificed and their footpads as well as skin and spleen tissues were examined, and the organisms present were enumerated. (In autopsies of lepromatous patients, we have detected greater numbers of leprosy bacilli in the spleen than in other internal organs.) Several of the hairless mice died in the course of the experiment; however, a number of animals survived for more than 6 months. The life span of the nude mouse is approximately 7 months[10]. The mice that died were also examined for any evidence of bacterial multiplication.

Results and discussion. Typical results presented in the Table show that there is no significant increase in proliferation of *M. leprae* in hairless mice as compared to 'normal' mice. Despite their proven T-cell deficiency[8], the nude mice do not promote generalized infection with *M. leprae*. Immune deficiency disorders are usually accompanied by high incidence of malignancies. However, RYGAARD and POVLSEN[10] found that hairless mice do not develop spontaneous tumors. These authors propose a third (as yet unknown) expression of immunological surveillance in nude mice, separate from cell-mediated and humoral immunity. Our observations suggest that deficient T-lymphocyte function alone might not provide a satisfactory explanation for excessive susceptibility to leprosy.

Zusammenfassung. Die Vermehrung und Ausbreitung von *Mycobacterium leprae* in einem haarlosen Mäusestamm wurde untersucht. Trotz Fehlen der T-Lymphocyten (zelluläre Immunität) wurden gleiche Resultate wie beim Mäusestamm mit T-Lymphocyten-Aktivität erzielt.

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[5] R. J. W. REES, M. F. R. WATERS, A. G. M. WEDDEL and E. PALMER, Nature, Lond. *215*, 597 (1967).
[6] W. F. KIRCHHEIMER and E. E. STORRS, Int. J. Lepr. *39*, 693 (1971).
[7] A. C. CORDIER, J. Ultrastructure Res. *47*, 26 (1974).
[8] E. M. PANTELOURIS, Nature, Lond. *217*, 370 (1968).
[9] D. D. MANNING, N. D. REED and C. F. SHAFFER, J. exp. Med. *138*, 488 (1973).
[10] J. RYGAARD and C. D. POVLSEN, Acta path. microbiol. scand., Section B, *82*, 99 (1974).
[11] The study was supported in part by the U.S.-Japan Cooperative Medical Science Program of the National Institute of Allergy and Infectious Diseases, Department of Health, Education and Welfare No. AI-07890.

Does 2,4-D Induce Mitotic Irregularities in Plant Tissue Cultures?

An exogenous growth regulator is required for initiation and maintenance of plant tissue cultures; 2,4-D is, perhaps, the most commonly used chemical for this purpose. It induces both mitotic and meiotic irregularities in vivo in a number of plant species[1-3]. Earlier reports suggested that exogenous 2,4-D induces mitotic irregularities in plant tissue cultures[4,5]. Recently BAYLISS[6] concluded that 2,4-D induces anaphase anomalies in carrot

[1] J. UNRAU and A. N. LARTER, Can. J. Bot. *30*, 22 (1952).
[2] B. H. CROCKER, Bot. Gaz. *114*, 274 (1953).
[3] T. SHOJI, Y. ODA and Y. MATSURA, La Chromosomo *45–46*, 1531 (1960).
[4] G. MELCHERS and L. BERGMANN, Ber. dt. bot. Ges. *71*, 459 (1959).
[5] Z. B. SHAMINA, Proc. Symp. on the Mutational Process. Mechanism of Mutation and Inducing Factors (Ed. Z. LANDA; Academia, Prague 1966), p. 377.
[6] W. M. BAYLISS, Nature, Lond. *246*, 529 (1973).

(*Daucus carota* L.) cell suspension cultures. However, in their studies only one concentration of 2,4–D, was used and therefore, a proper comparison was not possible. Effects of different concentrations of 2,4–D on mitotic abnormalities in suspension cultures of *Vicia hajastana* Grossh. ($n = 5$) and *Haplopappus gracilis* (Nutt.) Gray ($n = 2$) are reported here. Selection for diploid cells has been reported in both *Vicia*[7] and *Haplopappus*[8] suspension cultures maintained in B5 medium[9] containing 1 µg/ml 2,4–D.

Cells from *V. hajastana* and *H. gracilis* suspension cultures maintained in B5 medium with 1 µg/ml and 0.5 µg/ml 2,4–D, respectively, were transferred to media containing different concentrations of 2,4–D (Tables I and II). Anaphase analyses from both the species showed a number of abnormalities. The concentration of 2,4–D in the culture medium had a marked effect on the frequency of abnormal anaphase configurations, i.e. the higher the concentration the lower the frequency. This relationship existed because the frequency of anaphase bridges, including interlocking in the case of *Vicia*, showed a strong negative association with the 2,4–D concentration. In the case of *Vicia*, the frequency of dicentric chromosomes at metaphase also showed a similar trend. However, no dicentric chromosomes were observed at metapahse in the suspension cultures of *H. gracilis*. The anaphase bridges in *H. gracilis* were most likely produced by breakage-fusion-bridge cycle[10]. Apparently 2,4–D reduced the chances of reunion of damaged chromosome ends, probably by stabilizing them in some way. Formation of complexes between 2,4–D and histones has been observed during callus initiation in peas[11–13]. The two phenomena may be related in some way, which needs further investigation.

Table I. Frequencies (%) of various anaphase configurations (average of 2 cultures) in suspension cultures of *Vicia hajastana* (4 days)

Description	2,4-D concentration (µg/ml)		
	0.1	1.0	10.0
Normal	29.0	46.6	71.2
Fragment	3.0	0.9	1.4
Bridge	51.1	43.4	21.6
Bridge + fragment	2.6	1.8	0.7
Bridge + laggard	1.3	0.9	0.7
Laggard	0.9	0.9	—
Interlocking	12.1	5.4	4.3
Number of cells observed	231	221	239

The cultures were kept in a room with constant temperature (27–28 °C) and continuous light (2,000 lux) and subcultured every 3 days. Cytological techniques were those of SINGH et al.[7]. 2 subcultures were maintained on each 2,4-D concentration. The results from the 2 cultures agreed closely, hence only averages are presented.

Table II. Frequencies (%) of various anaphase configurations in *H. gracilis* suspension cultures (4 days)

Description	2,4-D concentration (µg/ml)			
	0.1	0.5	1.0	5.0
Normal	84.0	86.8	88.4	92.6
Bridge	15.1	12.4	10.3	6.5
Bridge + fragment	—	—	—	0.5
Bridge + laggard	0.5	—	0.4	—
Fragment	0.5	0.9	0.9	—
Unequal distribution of chromatids	—	—	—	0.5
Number of cells observed	212	234	224	217

The cultures were subcultured every 2 days. Only 1 culture was maintained on each of the 2,4-D concentrations. Anaphase analyses after 90 days in culture presented a similar trend.

The present findings are at variance with the earlier reports[4–6]. MELCHERS and BERGMANN[4] observed various mitotic abnormalities after replacing naphthaleneacetic acid (NAA) with 2,4–D. They did not maintain a control in the NAA medium. The age of the culture may be a potent factor, as in some cases abnormalities appear only after a period of in vitro culture[14,15]. SHAMINA[5] studying *H. gracilis* calli, used 1 µg/ml NAA and 0.5 µg/ml 2,4–D. The differential response to the 2 growth regulators could have been due to the differences in their concentrations[16]. BAYLISS[6] compared the frequencies of anaphase anomalies in carrot suspension cultures in MS medium[17] containing 0.1 µg/ml 2,4–D with those in differentiating cultures (in absence of 2,4–D) and in root tips. Such a comparison, however, has a limited validity since root tips and differentiating cultures differ from cell suspension cultures with respect to a number of factors other than 2,4–D, e.g., organized versus unorganized growth, undifferentiated state compared to various degrees of differentiation, occurrence of endoreduplication etc. The differentiation-division cycle imposed by subculturing of plant tissue cultures may be an important factor in this regard. Furthermore, the suggestion by BAYLISS[6] that 2,4–D induces mitotic abnormalities was based on the fact that it produces mitotic aberrations in root tip cells when used in high concentrations (50 µg/ml and above)[1–3,18]. It should be noted that these concentrations are too high compared with those generally used in plant tissue cultures (0.1 to 2 µg/ml), and root tips and tissue cultures also differ physiologically, at least with respect to the hormonal balance. These factors may be important in determining the cytogenetic effects of 2,4–D on plant cells. The ideal comparison would be between tissue cultures maintained on 2,4–D with those cultured without any growth regulator. Since initiation and maintenance of plant tissue cultures is not possible without a growth regulator, a study of a number of 2,4–D concentrations would be the next best alternative. The present results show that 2,4–D does not induce mitotic irregularities in plant tissue cultures. In fact, the higher the concentration of 2,4–D in the culture medium the lower the frequency of mitotic irregularities.

[7] B. D. SINGH, B. L. HARVEY, K. N. KAO and R. A. MILLER, Can. J. Genet. Cytol. *14*, 65 (1972).
[8] B. D. SINGH and B. L. HARVEY, Nature, Lond., *253*, 453 (1975).
[9] O. L. GAMBORG, R. A. MILLER and K. OJIMA, Expl. Cell Res. *50*, 151 (1968).
[10] B. D. SINGH and B. L. HARVEY, Can. J. Genet. Cytol., in press.
[11] Y. YAMADA, T. YASUDA, M. KOGE and J. SEKIYA, *Les Cultures de Tissus de Plantes* (Colloques Internationaux, C.N.R.S. No. 193, Paris 1970), p. 137.
[12] Y. YAMADA, T. YASUDA, M. KOGE and J. SEKIYA, Agr. biol. Chem. *35*, 99 (1971).
[13] T. YASUDA and Y. YAMADA, Biochem. biophys. Res. Commun. *40*, 649 (1970).
[14] S. YAZAWA, Bot. Mag., Tokyo *80*, 413 (1967).
[15] B. D. SINGH, Indian J. exp. Biol. *12*, 289 (1974).
[16] A. BENNICI, M. BUIATTI, F. D'AMATO and M. PAGLIAI, *Les Cultures de Tissus de Plantes* (Colloques Internationaux C.N.R.S. No. 193, Paris 1970), p. 245.
[17] T. MURASHIGE and F. SKOOG, Physiologia. plant. *15*, 473 (1962).
[18] T. MOHANDAS and W. F. GRANT, Can. J. Genet. Cytol. *14*, 773 (1972).

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.

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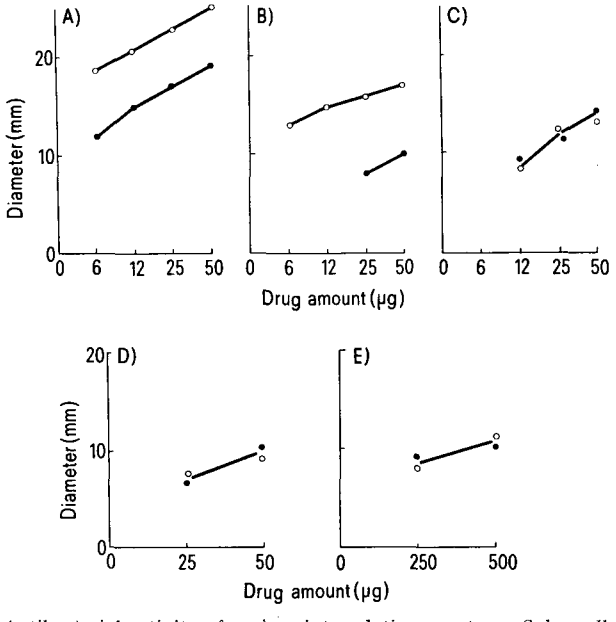
Effect of Excision Repair System on Antibacterial and Mutagenic Activity of Daunomycin and Other Intercalating Agents in *Salmonella typhimurium*

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-

[1] E. CALENDI, A. DI MARCO, R. REGGIANI, B. SCARPINATO and L. VALENTINI, Biochim. biophys. Acta *103*, 25 (1965).
[2] I. H. GOLDBERG and P. A. FRIEDMAN, A. Rev. Biochem. *40*, 775 (1971).
[3] F. QUADRIFOGLIO and V. CRESCENZI, Biophys. Chem. *2*, 64 (1974).
[4] E. F. GALE, E. CUNDLIFFE, P. E. REYNOLDS, M. H. RICHMOND and M. J. WARING, *The Molecular Basis of Antibiotic Action* (Wiley & Sons, London 1972), p. 187.
[5] F. QUADRIFOGLIO, V. CRESCENZI and V. GIANCOTTI, Biophys. Chem. *1*, 319 (1974).
[6] H. J. CREECH, R. K. PRESTON, R. M. PECK, A. P. O'CONNELL and B. N. AMES, J. med. Chem. *15*, 739 (1972).
[7] B. N. AMES, E. G. GURNEY, J. A. MILLER and H. BARTSCH, Proc. natn. Acad. Sci., USA *69*, 3128 (1972).
[8] B. N. AMES, F. D. LEE and W. E. DURSTON, Proc. natn. Acad. Sci., USA *70*, 782 (1973).
[9] P. E. HARTMAN, K. LEVINE, Z. HARTMAN and H. BERGER, Science *172*, 1058 (1971).