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INFLUENCE OF MUTAGENS ON THE INITIATION OF SKIN CARCINOGENESIS

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Abstract—In order to test the hypothesis that there is a general correlation between mutagenesis and the initiation stage of carcinogenesis, some of the recently developed mutagens, whose mechanisms of interaction with DNA are partly understood, were tested for initiation of skin carcinogenesis in mice.

Substances used were:

(*a*) Aminoacridines: acridine yellow and acridine orange.

(*b*) DNA base analogues: 5-bromodeoxyuridine, 5-iododeoxyuridine, 2-aminopurine and 2,6-diaminopurine.

(*c*) The weak β emitter, ^3H, in the form of tritiated thymidine.

These compounds failed to induce skin tumours when the treated animals were subsequently painted with croton oil. Neither did they significantly increase the spontaneous lung adenoma incidence in these mice.

DESPITE the growing body of evidence which has been accumulating against a close correlation between mutagenicity and carcinogenicity,[1, 2] the idea persists that carcinogenesis and mutagenesis share a common site of action, usually thought to involve DNA. To explain the poor correlation between these two phenomena which has been observed to date, it is possible to invoke the 'two-stage mechanism' of carcinogenesis[3] and to suggest that mutagens act essentially as initiators of carcinogenesis rather than as complete carcinogens. However, previous studies[4–7] have shown only a partial overlapping in the spectra of initiating and mutagenic agents among the small number of mutagens tested.

The recent development of several classes of mutagenic agents, whose mechanisms of interaction with DNA have been at least partially elucidated, prompted this reinvestigation. These studies revealed that neither mutagenic aminoacridines, mutagenic purine or pyrimidine analogues, nor tritiated thymidine, initiated skin tumours in mice.

MATERIALS AND METHODS

Animals

The mice used were male and female mice of a Swiss/NIH non-inbred line obtained from the National Institutes of Health, Bethesda, Maryland, through the courtesy of Dr. L. W. Law. For the tritiated thymidine experiment, SWR/Jax inbred females were used. At the beginning of the experiment mice were $2\frac{1}{2}$–$3\frac{1}{2}$ months old. They were

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randomized among the experimental groups and kept in metal cages in an air conditioned room at 21–24°. Their diet consisted of Purina Laboratory Chow pellets and water *ad libitum*.

Chemicals

The mutagens used in this experiment might be classified in three categories: (a) aminoacridines (the use of which was suggested by Dr. S. Brenner, Cambridge), (b) DNA base analogues, and (c) tritiated thymidine.

Acridine yellow (2,8-diamino 3,7-dimethylacridine, AY) and acridine orange (2,8-bisdimethylaminoacridine, AO) were obtained from the National Aniline Division of Allied Chemical and Dye Corporation, New York. AO was used as obtained commercially, but only the water soluble fraction of AY (75 per cent) was used. 5-Bromodeoxyuridine, 5-iododeoxyuridine, thymidine, 2-aminopurine nitrate, 2,6-diaminopurine sulphate and adenine were obtained from the California Corporation for Biochemical Research, Los Angeles. Tritiated thymidine was obtained from Schwartz Biochemicals, Inc., Mt. Vernon, New York, as an aqueous solution containing 1 mc/ml. It was diluted tenfold before use. Urethane (ethyl carbamate) was obtained from British Drug Houses Ltd. and croton oil from Magnus, Maybee and Reynards, New York.

Procedure

Compounds tested as initiators were dissolved in water (with the exception of adenine, which was used as an aqueous suspension). They were injected intraperitoneally as a single dose in volumes of 0·5 ml or less. Animals injected with aminoacridines were protected from light to prevent against possible photodynamic damage.[8]

For promoting action, croton oil was applied as a 5 per cent solution in medicinal liquid paraffin. Before each application, the hair over an area of approximately 1·5 cm^2 in the region of the shoulder blades was clipped with fine scissors. The solution was delivered by a glass rod, the first time 4 days after injection of the test agent, and the procedure repeated twice weekly for 20 weeks, i.e. 40 paintings in all. In the case of tritiated thymidine injected mice, croton oil painting began 24 days after the thymidine injection, by which time the daily excretion of ^3H was less than 1 per cent of the initial rate.

The resulting skin papillomas were charted at their first appearance and fortnightly thereafter. At the completion of the experiment, the animals were autopsied and examined under a dissecting microscope for the presence of lung adenomas. Histological sections of all questionable tumours were examined for confirmatory diagnosis.

RESULTS

Preliminary toxicity trials of the chemical mutagens were performed to ensure that maximal levels were tested. The doses used (Table 1) were all close to or slightly exceeded the minimum lethal dose. Adenine and thymidine, normal DNA constituents, were tested as controls.

With the exception of the known initiator urethane (see discussion) all the substances tested failed to initiate skin papillomas (Table 1). The yield of lung adenomas which appeared in the different experimental groups was not significantly different from that of the control group (group 1), except again in the case of urethane.

A group of 10 mice, from the same lot as group 11, were given similar injections of 1·0 μc/g thymidine ^3H, but not painted with croton oil. Among 8 survivors, no papillomas and 2 lung adenomas were found.

In all except group 2 (urethane treated) no mouse bore more than a single adenoma.

TABLE 1. INFLUENCE OF MUTAGENS ON THE INITIATION OF SKIN CARCINOGENESIS AND ON LUNG TUMOUR FORMATION

Group*	Test agent	Dose	No. of mice at start	No. of survivors	No. of papillomas	No. of adenomas	% mice with adenomas
1	None	—	41	39	0	3	8
2	Urethane	20 mg per mouse	25	16	6	64	87
3	Acridine yellow	50 μg/g	25	11	0	2	18
4	Acridine orange	45 μg/g	27	15	0	3	20
5	Thymidine	20 μmoles per mouse	26	15	0	3	20
6	5-Bromodeoxyuridine	20 μmoles per mouse	28	23	0	4	17
7	5-Iododeoxyuridine	20 μmoles per mouse	21	16	0	2	12
8	Adenine	30 μmoles per mouse	38	16	0	1	6
9	2-Aminopurine	40 μmoles per mouse	25	11	0	1	9
10	2,6-Diaminopurine	30 μmoles per mouse	35	13	0	2	17
11	Thymidine-^3H	1·0 μ/cg	10	7	0	1	14

* Animals in groups 1–10 were Swiss/NIH, and those in group 11 were SWR/Jax. Croton oil (5 per cent in liquid paraffin) was applied to the skin of the back, twice weekly for 20 weeks, after which the mice were killed and autopsied.

DISCUSSION

Shortly after the demonstration that mustard gas could induce mutations,[9] this agent was tested as an initiator of skin carcinogenesis,[4] and found to be inactive. Earlier work[10] showed that mustard gas is strongly *anti*carcinogenic for skin. Several years later it was discovered that urethane acted as a 'pure initiator' either when it was applied to mouse skin[11, 12] or when it was administered systemically.[13] Since urethane had previously been shown to be mutagenic for *Drosophila*[14] and *E. coli*,[15] several mutagenic agents were included among a series of compounds tested for initiating activity.[5–7] Only a small proportion of these mutagens showed initiating activity.

The development of the technique of fine structure genetic mapping[16] made possible tests, in bacteriophage systems, of chemical mutagens which are considered to act by causing errors in DNA replication, involving chromosomal lengths as small as a single pair of nucleotides. The chemical mutagens selected for the experiments reported in this paper were chosen mainly from among those successfully used as mutagens for the r_{II} locus of bacteriophage T4[17–19], since this system has provided data for proposals of specific types of base replacement to account for mutagenesis. A theory of acridine action involving intercalation of acridines between DNA base

pairs has been proposed.[20, 21] Both aminoacridines and base analogues have also been shown to be strongly mutagenic in other systems. For references to the mutagenic effect of acridine orange and acridine yellow on plant material see D'Amato.[22] A recent comparison of the induction of mutations in bacteria by an aminopurine and a halogenated pyrimidine has been made by Strelzoff.[23] Interactions of base analogues with nucleic acids have been reviewed by Handschumacher and Welch.[24] These authors also critically review the proposals for a specific interaction between urethane and DNA metabolism. As yet, such a specificity of action has not been demonstrated.[25]

The β radiation from thallium 204 (maximum energy 0·78 MeV), which penetrates tissue to a depth of approximately 2·5 mm, has been successfully used to initiate skin carcinogenesis when it was given locally in single doses of 800 rep and followed by repeated applications of croton oil to the same area.[26] Tritium-labelled thymidine was used [27] to induce leukaemia in mice at a dose of 1 μc/g, equivalent to a maximum initial activity of approximately 1 rep per day. This use of a very weak β emitter (maximum energy from $^3H = 0{\cdot}018$ MeV) as part of the molecule of thymidine, which is specifically incorporated into DNA, results in the selective irradiation of the cell nucleus. Tritiated thymidine has also recently been reported to cause lethal mutations in mice.[28]

In addition to the difficulty in attempting to compare mutagenic, carcinogenic or initiating agents on the basis of their actions in diverse biological systems, the toxicity of the agents used in the present study limited the dose which could be administered by intraperitoneal injection. However, 2,6-diaminopurine, which proved inactive as an initiator when it was tested in this series, in the form of a single injection of 4·5 mg, had also been shown to be inactive when it was given as a series of 10 skin paintings in which a total of 100 mg was applied.[6] Potent initiating action has been demonstrated, for example, for triethylene melamine[5] when a dose as low as 0·24 mg was painted once on mouse skin. In the present study, the minimum amount of aminoacridine injected was 0·95 mg. Base analogues were all injected at doses of 20 μmole per mouse or greater, which gave a minimum dose on a weight basis of 4·5 mg per mouse.

With the exception of urethane, none of the substances tested in the present experiments was able to initiate skin carcinogenesis in mice (chemical mutagens were tested in approximately minimum lethal doses and tritiated thymidine was administered in a dose sufficient to cause leukaemia in a longer term experiment). These results suggest that a *general* correlation between mutagenic and tumour initiating agents does not exist. However, the chemical induction of a specific pattern of mutations, which has shown, for example, that "the mutagenic effect of 5-bromouracil is not merely a general enhancement of spontaneously occurring mutations",[29] makes it probable that only a small proportion of mutagenic agents would share the common site or sites of action which one could postulate to be involved in the initiation of carcinogenesis.

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