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Chromosome Breakage by 1-Methyl-2-benzylhydrazine in Mouse Cancer Cells[1]

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SUMMARY

The induction of chromatid translocations by 1-methyl-2-benzylhydrazine was studied in a number of malignant and normal mammalian cell types and in narcissus root cells. In three different mouse ascites tumors (Ehrlich, 2BF, and L1210) treated *in vivo*, translocations were induced. In the Ehrlich tumor, they were induced with unusually high frequency, involving, in some cells, a majority of the chromosomes. None were found in the Ehrlich tumor or HeLa cells *in vitro*, nor were any found in untreated controls. No translocations were observed in treated or untreated normal tissues *in vivo* (mouse bone marrow and spleen, narcissus roots) or *in vitro* (human lymphocytes, mouse spleen).

INTRODUCTION

Methylhydrazine derivatives have been found to inhibit considerably the growth of a number of mouse and rat tumors (4). This effect has been repeatedly confirmed (*cf.* Ref. 7) in cancers of the human lymphoid system, especially in Hodgkin's disease, whereas the results on solid tumors have not been promising.

The cytological mechanism of these effects was first investigated by Rutishauser and Bollag (10) who found that MBH[2] phosphate broke the chromosomes to an unusual extent in the hypotetraploid Ehrlich mouse ascites tumor *in vivo*. It was therefore thought worthwhile to compare the cytological effects of this substance on several malignant and normal cell types, *in vitro* and *in vivo*. For a maximal effect, the highest concentration tolerated by the host or the tissue culture was used.

MATERIALS AND METHODS

For each experiment a fresh solution of MBH[3] (Hoffmann-La Roche, Inc. Basel, Switzerland) was prepared in double-distilled water. This was added to the culture medium (final concentration, 0.015 to 0.06%) or was injected i.p. into the mouse (Table 1).

The following cell types were treated with MBH *in vitro*: human lymphocytes, mouse spleen cells, HeLa cells, and Ehrlich cells. The lymphocytes were grown in the usual short-term culture. The same medium was used for the mouse spleen cells. The HeLa and Ehrlich cells were grown in a medium consisting of 10% fetal calf serum, 5% chicken embryo extract, and 85% Tissue Culture 199.

For the *in vivo* experiments (Table 1), 3 mouse ascites strains were used: the hypotetraploid Ehrlich strain and 2 diploid lines of leukemic origin, L1210 and the 5-fluorodeoxyuridine-resistant strain of L5178Y, 2BF. (All 3 strains were from the McArdle Institute for Cancer Research, Madison, Wis.) In addition, the effects of MBH on bone marrow and spleen cells, often in the same animal as the tumor, were studied. The Ehrlich ascites tumor was grown mainly in white Swiss hybrid mice; the diploid tumors were grown in either BDF_1 or C57 mice.

Some 5 hr before an animal was killed, 0.5 ml 0.025% colchicine solution was injected i.p. The cells were treated for 20 min in 1% sodium citrate solution before they were fixed in acetic acid:methanol (1:3). To spread the chromosomes better, the fixative was replaced either with acetic acid:methanol (1:1) or acetic acid:methanol:water (2:1:1) from which the cells were dried on the slide. In the *in vitro* experiments, the cells were treated correspondingly. The slides were stained with 1% orcein (George T. Gurr, London, England) in 45% acetic acid and were made permanent (6).

One experiment used the roots of *Narcissus tazetta* L. When the roots were about 4 cm long, the bulb was placed over a jar that contained 100 ml 0.004% MBH solution in double-distilled water. The roots were fixed in acetic acid:ethanol (1:3) and made into Feulgen squash slides (6). Chromosome anomalies were checked in anaphases.

RESULTS

The experiments with the narcissus roots gave the following results. After a 48-hr treatment, 1 of 152 anaphases showed a laggard. With a 48-hr treatment followed by a 24-hr recovery in tap water, the ratio was 4:77. In the control for the 2nd treatment, the ratio was 1:172. There was no evidence of chromosome breakage in any of these experiments.

In all other experiments, metaphases were examined for chromosome breakage. MBH seems to cause exclusively G_2-type chromosome damage. In other words, all the breaks

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[2] The abbreviation used is: MBH, 1-methyl-2-benzylhydrazine.

[3] For a sample of 1-methyl-2-benzylhydrazine phosphate, the author is grateful to Dr. W. Bollag, Hoffmann-LaRoche, Inc., Basel, Switzerland.

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Table 1

The effect of MBH on mammalian cells in vivo and in vitro

The dose per animal was 0.016 mg, except in Experiments 2 and 14 (0.004 mg) and in Experiment 16 (0.008 mg).

In vivo experiment no.	Mouse cell strain	Duration (hr)	Treated cells with translocations/total	Control cells with translocations/total
2	Ehrlich	48	192/319	
	Bone marrow		0/18	
15	Ehrlich	48	36/315	
	Spleen		0/60	
16	Ehrlich	48	103/221	0/89
14	Spleen	72	0/50	
25	2BF	24	20/600	0/600
		48	37/400	
13	L1210	48	3/105	0/351
	Spleen		0/87	0/19
27	L1210	48	8/400	0/400
	Spleen		0/200	
28	L1210	72[a]	11/200	
In vivo	Total cancer (mouse)		410/2560	0/1440
	Total normal (mouse)		0/415	0/19
In vitro	Total cancer (mouse, human)		1/604	0/132
	Total normal (mouse, human)		0/199	0/125

[a] MBH was given at Hr 0, 24, and 48.

were of the chromatid type, as seems most often to be the case with chromosome breaks induced by chemical agents.

The damage consists mainly of rearrangements, often involving more than 2 breaks. Compared with the translocations, the frequency of gaps and chromatid breaks was negligible. The number of translocations per cell is often so high (Fig. 1) that it would be senseless to try to determine it. A chromatid translocation between 2 chromosomes involves 2 breaks, not 4, as assumed by Bollag (3). In the present scoring, the metaphases have been divided into those with and those without translocations. None of the *in vitro* experiments (Table 1) revealed any chromosome-breaking effect of MBH; the 1 observed translocation has, of course, no significance.

The *in vivo* experiments yielded striking results (Table 1). Whereas translocations were found neither in the controls nor in the normal cells in the treated animals, MBH had induced translocations in significant numbers in all 3 ascites strains. With a 48-hr treatment, the frequency of occurrence of cells with translocations was 2.2% in L1210 (Fig. 4), 9.2% in 2BF (Fig. 3), and ranged from 11 to 60% in the hypotetraploid Ehrlich strain. The impression was that, in the latter, the less ascites fluid there was in the animal, the more pronounced was the effect. The data from the diploid strains suggest that the effect increased with the duration of treatment (*cf.* Ref 10). Cells from the same sample varied enormously in their response to treatment. Thus, in the Ehrlich samples in which many cells had a spectacular number of translocations (Fig. 1), some 40 to 89% of cells did not show a single translocation. In the case of the 2BF tumor, of 200 cells scored from 1 mouse, 191 had no exchanges, 8 had just 1, whereas in 1 cell there were more than 20.

A phenomenon that was caused sporadically by MBH in all 3 tumors was the occurrence of one or more uncoiled chromosomes in an otherwise normal metaphase plate. This ranged from a degree of uncoiling (Fig. 2) to an almost interphase-like appearance.

DISCUSSION

The previous and present observations that should be taken into consideration in any attempt to explain the chemical pathways leading to the cytological effects of methylhydrazine derivatives are the following. (*a*) The duration of metaphase relative to that of prophase is increased in Ehrlich ascites cells after MBH treatment (10). Rutishauser and Bollag (11) also showed that the duration of either S or G_2 is increased and concluded that the decreased mitotic rate is a result of this increase. (*b*) MBH has no effect on the chromosomes of several normal or malignant cell types *in vitro* or on those of normal mammalian cells and narcissus roots *in vivo*. On the other hand, MBH breaks the chromosomes in the 3 investigated mouse ascites tumors *in vivo*. All of the chromosome breaks are of the G_2 type. Cells from the same tumor differed greatly in their response to the treatment.

At present it is not feasible to decide among the following possibilities, all of which presuppose that some mouse tissue changes MBH into another substance, which either is itself chromosome breaking or can be converted to such a compound: (*a*) The substance is chromosome breaking, but only the cancer cells are permeable to it; (*b*) the substance breaks chromosomes but is destroyed by an enzyme of which the cancer cells have less; (*c*) the substance does not in itself break chromosomes but is converted into a short-lived radiomimetic compound by the cancer cells, whereas the normal cells lack the enzyme for this conversion. Experiments are under way to test these possibilities.

Methylhydrazine derivatives are reactive in a number of

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ways. They undergo autoxidation, producing H_2O_2 (2). They are alkylating agents (5, 8, 9) along more than 1 pathway (12). One of the products is formaldehyde (12), which may combine with peroxides into further active compounds (1). It is, however, doubtful whether any of these reactions suffice to account for the cytological effects. Although these substances in some ways resemble alkylating agents in their biological effects, and indeed have been shown to be alkylating, it is possible that their main mode of action involves some other mechanism, since they do not show any cross-resistance to other antitumor drugs. It is obvious that this very reactive group of substances merits further study.

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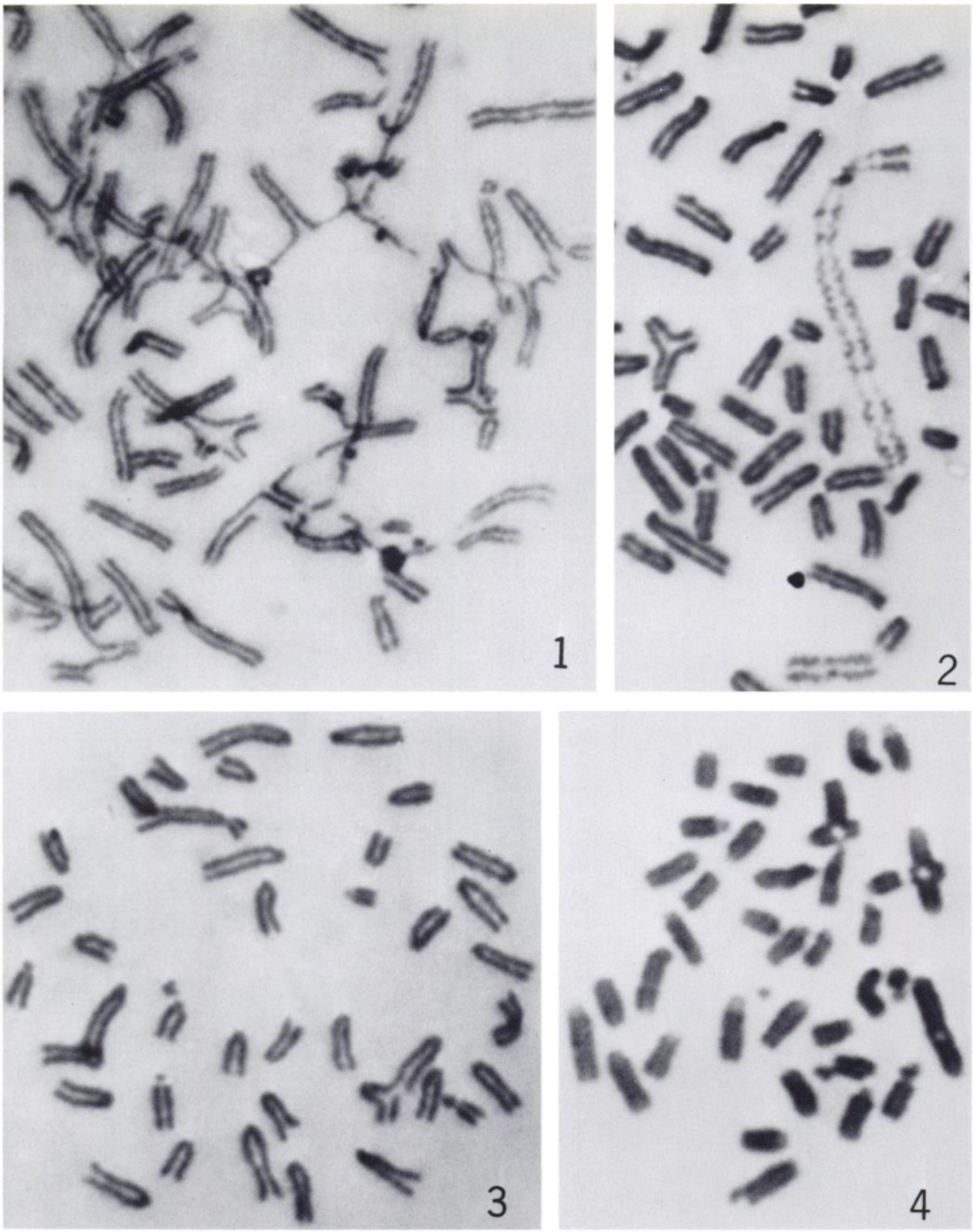

Figs. 1 to 4. All 4 cells were treated with MBH and stained with orcein. × 2600.

Fig. 1. Part of a metaphase in an Ehrlich ascites cell with numerous translocations (Experiment 16).

Fig. 2. Part of a metaphase in an Ehrlich ascites cell with 2 despiralized chromosomes and a translocation (Experiment 16).

Fig. 3. Metaphase from a 2BF ascites cell with 1 translocation (Experiment 25).

Fig. 4. Metaphase from a L1210 ascites cell with 3 translocations (Experiment 28).

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