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*CHROMOSOME DAMAGE INDUCED BY HYDROXYLAMINE IN MAMMALIAN CELLS**

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Cytologically detectable damage at specific chromosome regions, resulting from different agents of known chemical action, should yield information relating to the molecular architecture of chromosomes. Recently, it was demonstrated that replacement of thymidine moieties of deoxyribonucleic acid (DNA) by 5-bromodeoxyuridine (BUDR) induces breakages of several specific localities along chromosome No. 1 of Chinese hamster cells grown *in vitro*.[1] These specific vulnerable regions are interpreted to be sites of DNA molecules containing relatively high ratios of adenine-thymine (A-T) base pairs.

Freese and his collaborators have shown that in T4 phage hydroxylamine (HA) is a mutagen with a high degree of specificity of action, and the genetic damage produced by HA is thought to be through its initial action on cytosine.[2, 3] Studies of the direct action of hydroxylamine with free bases have shown that among DNA bases, although there is some reaction with hydroxymethylcytosine, there is a preferential action of HA with cytosine. There is very little or no reaction of HA with 5-methylcytosine or thymine, and purine bases are unaltered.[2–5] Treatment of thymus DNA with HA leads to a loss of cytosine but not of thymine.[4]

The present study describes chromosome damage produced by HA in Chinese hamster cells grown *in vitro*. Breakages in specific chromosome regions resulting from HA treatment are compared with those induced by BUDR incorporation as well as by a physical agent, X-ray.

Material and Methods.—The Chinese hamster cell line 11dFAF28[6] was used to test the effects of HA, BUDR, and X ray on mammalian chromosomes.

The McCoy 5*a* medium[7] supplemented with 15 per cent fetal calf serum was used throughout the experiments. The techniques for routine cultivation and for cytological preparations were the same as those described by Hsu and Kellogg.[8]

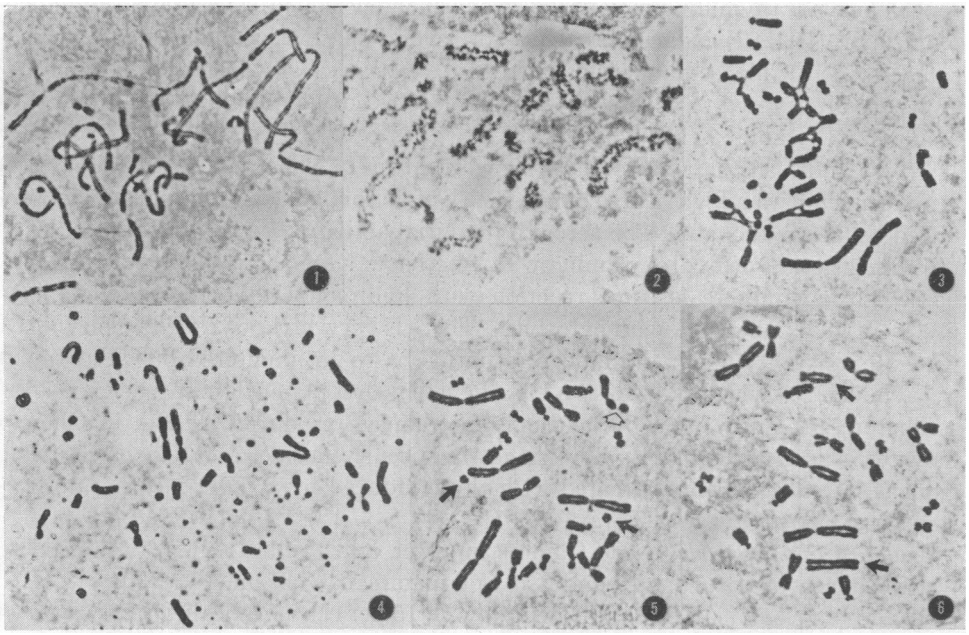
Stock solutions of 5-bromodeoxyuridine or hydroxylamine ($NH_2OH \cdot HCl$), 1 mg/ml, were prepared in Hank's balanced salt solution (BSS) immediately

before use. Cells were treated with 5 and 25 μg/ml HA for 24 and 48 hr and with 25 μg/ml BUDR for 24 hr.

For irradiation experiments, cell suspensions in BSS received 250 rads X irradiation from a 220-kvp constant potential Westinghouse X-ray machine operated at 15 ma, with a half-value layer of 1.4 mm Cu. The dose rate was 250 to 260 rads/min. Irradiated cells were immediately returned to growth medium and sampled four hours later.

Chromosome damage resulting from various treatments was recorded by visual estimation of regions in chromosomes No. 1, No. 2, and the X. Both chromatid and chromosome breaks were registered as single breaks. In order to estimate any possible differential susceptibility to various treatments among chromosomes, a crude end point was chosen. With HA or X-ray, as many cells as necessary were examined to obtain 200 breaks in chromosome No. 1, and with BUDR, 300 breaks were analyzed in the same chromosome. The frequency of breaks in chromosomes No. 2 and the X was recorded in the same samples.

Results.—As noted previously by Hsu and Somers,[1] there are two major observable effects on mammalian chromosomes following BUDR treatment, namely, an enhancement of constrictions and chromatid breakages. Following the treatment of Chinese hamster cells with hydroxylamine, there are also marked changes in chromosome morphology. Constrictions are common. In some cells, chromosomes may show a multiplicity of constrictions throughout the entire length, so that they assume a banded pattern (Fig. 1). Occasionally, the chromosomes become highly despiralized (Fig. 2).



FIGS. 1–6.—Chinese hamster cells treated with hydroxylamine (5 μg/ml) for 48 hours. FIG. 1.—Numerous constrictions in chromosomes. FIG. 2.—All metaphase chromosomes are despiralized. FIG. 3.—Numerous chromatid breaks and translocations. FIG. 4.—Multiple chromosome breaks. FIG. 5.—Note the centromeric chromosome break in a small metacentric (solid arrows) and a chromatid break in the short arm of a submetacentric (open arrow). FIG. 6.—Chromosome break at the centromere of chromosome No. 1 (arrows).

Although chromosome damage is not usually extensive with low levels of HA (5 μg/ml, 48 hr), some heavily damaged cells are noted. Figure 3 represents a cell with multiple chromatid breakages, many of which are involved in translocations. Chromosome breakages in this cell can also be noted. A single chromatid break in the short arm of a submetacentric chromosome is shown in Figure 5 (open arrow).

One effect of HA treatment, which is in striking contrast to that of BUDR, is the production of a large number of chromosome breakages. In Figure 4 is shown a heavily damaged cell with numerous chromosome breaks. Chromosome breakages were frequently observed to occur at centromeric regions of all elements, as exemplified by Figures 5 and 6. Figure 5 depicts the separation of the two arms of a small metacentric chromosome (solid arrows) as the result of a chromosome break at the centromere. Similar damage to chromosome No. 1 (arrows) is represented in Figure 6.

Since individual chromosomes in Chinese hamster cells can be recognized, it is possible to test the relative amounts of damage produced in specific chromosome regions by different treatments. Frequencies of breaks occurring in specific regions of chromosomes No. 1, No. 2, and the X are compared following the three treatments.

Although pictorial examples of damaged chromosome regions are not completely presented, Figures 7 and 8 show representative regions of chromosomes No. 1

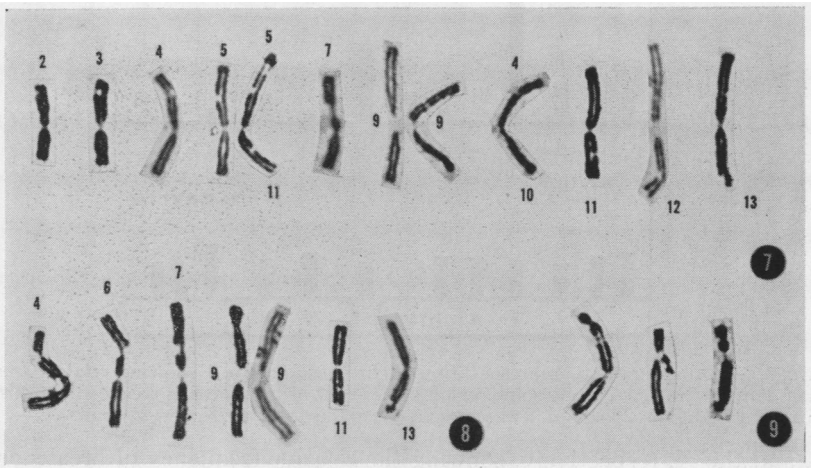


FIG. 7.—Pictorial examples of some chromosome regions of chromosome No. 1 which can be damaged by HA treatment (5 μg/ml, 48 hr). Numerals at the ends of chromosome arms correspond to the regions showing the chromatid breaks. Region 9 (centromere) is represented by both a chromosome and a chromatid break.

FIG. 8.—Pictorial representation of some regions of chromosome No. 2 damaged by HA treatment (5 μg/ml, 48 hr). Numerals at the ends of chromosome arms correspond to the regions showing the chromatid breaks. Region 9 (centromere) is represented by both a chromosome and a chromatid break.

FIG. 9.—Examples of telomeric translocations between chromosomes from cells treated with BUDR (25 μg/ml, 24 hr). Note that the telomere of one chromosome may join either with another telomere or with a broken chromatid or another chromosome.

and No. 2, respectively, damaged by HA treatment. All regions showing breaks are numbered in consecutive order, beginning with the telomere of the long arm and ending with the telomere of the short arm. The centromeric region (region 9)

is illustrated by a chromosome as well as a chromatid break for both elements. Figure 9 portrays three sets of telomeric translocations which are commonly found following BUDR treatment. Note that the telomere of one chromosome may join either with another telomere or with a broken chromatid of another chromosome.

Figures 10, 11, and 12 are diagrammatic representations of chromosomes No. 1,

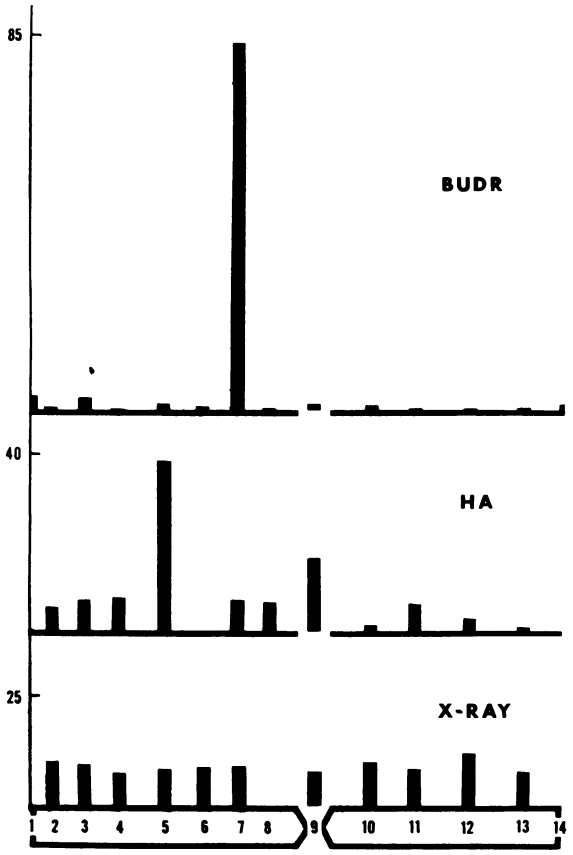


FIG. 10.—A diagrammatic representation of chromosome No. 1 showing relative frequencies of damage for each chromosome region in per cent of total breaks recorded. Cells were treated with BUDR (25 μg/ml, 24 hr) or HA (5 μg/ml, 48 hr) or X ray (250 rads).

No. 2, and the X, respectively, showing the relative frequency of breaks occurring at each chromosome region following treatment of cells with BUDR, HA, or X ray. Each frequency is represented as per cent of total breaks recorded from a chromosome.

When the damage produced by X ray is examined in regard to the distribution of breaks for chromosomes No. 1 and No. 2, one readily notes its randomness, i.e., no region is particularly sensitive to effects of ionizing irradiation. The telomeres, however, appear to be most invulnerable to X ray since no damage was observed in these localities. Also, no damage was observed for region 8 in chromosome No. 1 (Fig. 10).

A different pattern emerges when one examines regional sensitivity of chromosomes following treatment with either of the two chemical agents. In general, the

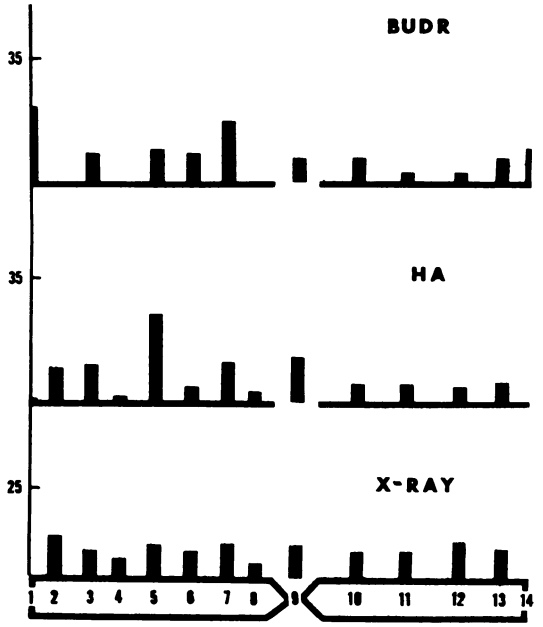


FIG. 11.—A diagrammatic representation of chromosome No. 2 showing relative frequencies of damage for each chromosome region in per cent of total breaks recorded. Cells were treated with BUDR (25 μg/ml, 24 hr) or HA (5 μg/ml, 48 hr) or X ray (250 rads).

most striking effect of HA treatment is the production of breakages at the centromeric regions of all chromosomes, while BUDR is relatively inactive (region 9, chromosomes No. 1 and No. 2, Figs. 10 and 11; region 7, X chromosome, Fig. 12). On the other hand, BUDR damages telomeric regions of all chromosomes (regions 1 and 14, chromosomes No. 1 and No. 2, Figs. 10 and 11; region 11, X chromosome, Fig. 12). Although these regions are preferentially damaged by one agent, the effects are not exclusive for each.

There are also other chromosome regions which are differentially sensitive to one of the two chemical agents tested. It can readily be seen that region 7 of the long arm of chromosome No. 1 (Fig. 10) is extremely susceptible to BUDR but not HA damage. This region corresponds to region 4 reported by Hsu and Somers.[1] Although the damage to chromosome No. 1 resulting from HA treatment is more generalized than that from BUDR, it is evident that regions 5 and 9 (centromere) are most heavily damaged (Fig. 10). These regions show little damage with BUDR.

Striking differences are also noted when BUDR and HA breakage frequencies are compared among regions of the X chromosome (Fig. 12). Region 3 shows 89 per

TABLE 1

RELATIVE FREQUENCIES OF TOTAL BREAKS AMONG THREE CHROMOSOMES FOLLOWING TREATMENTS WITH DIFFERENT AGENTS

Treatment	Chromosome No. 1	Chromosome No. 2	X chromosome
BUDR	100	24.6	56
HA	100	69.5	44
X ray	100	58	—

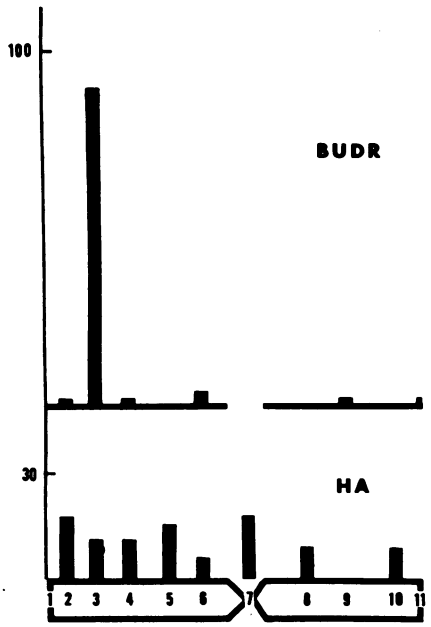


FIG. 12.—A diagrammatic representation of the X chromosome showing relative frequencies of damage for each chromosome region in per cent of total breaks recorded. Cells were treated with either BUDR (5 μg/ml, 24 hr) or with HA (5 μg/ml, 48 hr).

cent of all BUDR-induced breaks, while regions 5, 7 (centromere), 8, and 10 do not break following BUDR treatment but are damaged by HA.

In contrast to the effects on chromosome No. 1 and the X, none of the regions of chromosome No. 2 show extreme sensitivity to either BUDR or HA (Fig. 11). However, the telomeres (regions 1 and 14) show a high degree of BUDR damage, and regions 5 and 9 (centromere) show the highest HA-induced breakage frequencies.

In addition to the differences in damage to specific chromosome regions, chromosomes differ in their total susceptibility to each agent. Using the number of breaks recorded in chromosome No. 1 as 100%, chromosomes No. 2 and the X appear more resistant (Table 1) than the No. 1. This cannot be solely explained by the differences in chromosome lengths, because the X chromosome is considerably shorter than the second chromosome, yet sustained more damage with BUDR. Similarly, while the damage in chromosome No. 1 remained 100, chromosome No. 2 seems more resistant to BUDR than to X ray and to HA.

Discussion.—Hsu and Somers[1] demonstrated that chromatid breakages in mammalian chromosomes are related to an instability induced by BUDR incorporation into the DNA molecule. It was further shown that this instability is related to the actual incorporation of BUDR, since breakages can be observed in cells which have undergone one DNA synthesis period in the presence of the analogue.[9] Therefore, it appears that breakage may be more closely related to altered physical properties of the chromosome which interfere with the regular spiralization cycle, as suggested by Hsu and Somers,[1] rather than a mechanism which requires replication of the altered DNA molecule, as suggested by Djordjevic and Szybalski.[10] Furthermore, it has been suggested that chomosome regions, showing preferential damage following BUDR incorporation, e.g., telomeres and some special loci, may indicate DNA of relatively high A-T ratio.[1]

Since hydroxylamine was observed to initiate chromosome damage also, it could be inferred that this damage results from alterations of the cytosine moieties of DNA molecules. Severe damage at specific regions, e.g., centromeres, suggests a high content of guanine-cytosine (G-C) of such localities. These regions are not preferentially damaged by BUDR. Although cytosine bases of cellular RNA should also be affected by HA treatment, it could be inferred from cytological data that chromosome damage results from alteration of DNA bases.

Relating these findings to chromosome architecture, it is suggested that (1) telo-

meric and certain other chromosome regions contain a high A-T ratio or even represent a short segment of A-T polymers, and (2) centromeric regions and certain intrachromosomal regions are of high G-C content. Nevertheless, a centromeric region is a fairly wide zone which may contain an assortment of DNA segments differing in base composition. The fact that BUDR occasionally breaks the centromeric regions indicates that this region is not totally composed of G-C polymers. Perhaps more favorable cytological materials or more sensitive methods should be chosen to further the investigation of the composition of centromeric regions.

It has been demonstrated by Hsu and associates[11] that mitotic cells of the Chinese hamster, sampled 3 or 4 hours following irradiation of 250 rads, represent those originally in the G_2 phase at the time of irradiation. Even though no labeled DNA precursor was used in this study, an abundance of chromatid breaks recorded from our sample confirms their findings. All regions of chromosomes seem to be equally sensitive to X-ray damage. Differences in total damage between chromosomes No. 1 and No. 2 can probably be directly related to the target area provided by each chromosome. In contrast, the low level of damage produced by BUDR in chromosome No. 2 can best be related to the chemical structure of this chromosome. Perhaps with the exception of the telomeres, there is a relatively even distribution of A-T base pairs in the DNA of this chromosome. This reduces the chances of BUDR incorporation to levels that would lead to chromosome damage.

Following X irradiation, breakages of the chromosome type can usually be ascribed to a lesion produced prior to chromosome replication.[11] A similar mechanism could explain the large number of chromosome breaks which were found following HA treatment. However, if damage results from direct chemical action of HA on the DNA molecule, an alternative is also possible, i.e., in some instances homologous regions of chromatids could be attacked simultaneously.

Although there is no chemical evidence for the direct action of hydroxylamine on chromosomes of mammalian cells, cytological data indicate that there is a specificity of action. DNA from treated cells is being examined in order to test this possibility.

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