Adriamycin-Induced Chromosome Damage: Elevated Frequencies of Isochromatid Aberrations in G_2 and S Phases[1]

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Summary. Analysis of chromosomes from cells treated with adriamycin during G_2 and S phases showed a high frequency of isochromatid-type of breaks, in addition to the expected chromatid breaks. These are interpreted as independent breaks on sister chromatids because of preferential effects of the drug in specific chromosome regions. The break points are likely to be different, but morphologically such breaks would be indistinguishable from isochromatid or chromosome breaks.

Generally when an agent capable of inducing chromosome breakage (clastogen) causes a chromosome break, the resulting configuration in the ensuing metaphase reflects the phases of the cell cycle during which the lesion was induced. When a break occurs in a chromosome containing a single chromatid (G_1 phase), a chromosome break will result after the cell completes its replication and enters mitosis. The break points of the two sister chromatids should be precisely the same. When a chromosome already consists of two sister chromatids (G_2 phase), or when a chromosome is replicating (S phase), breaks at the identical locations in the two sister chromatids (isochromatid breaks) should be a rare event if the agent induces random chromosome damage[2].

In the course of our studies on the cytological effects of the antitumor antibiotic adriamycin on several mammalian cell lines in vitro, we noted a relatively high frequency of isochromatid breaks in cell populations harvested after a short exposure (2–6 h) to the drug. The cells should have been in the G_2 or at the earliest in mid-S phases when the clastogen was introduced, since most mammalian cells in vitro have a G_2 phase of approximately 2 h and an S phase of 6–8 h. An isochromatid break should produce two fragments, one centric and the other acentric, and the two pieces should add up to be equivalent to the missing chromosome. However, in most materials where the diploid number is high and recognition of individual chromosomes is difficult without banding, the origin of a fragment is difficult to trace, especially in cells with a large number of aberrations. We therefore chose the diploid male Chinese hamster cell line Don as material for this set of experiments.

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[2] T. C. HSU, J. cell. Biol. *23*, 53 (1964).

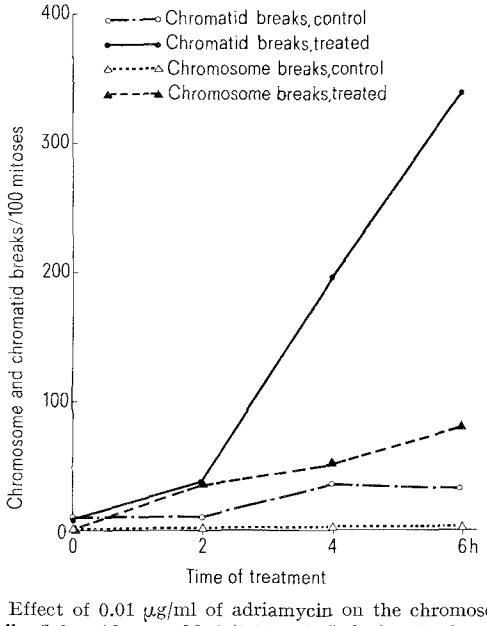

Fig. 1. Effect of 0.01 µg/ml of adriamycin on the chromosomes of Don cells. Colcemid was added (0.04 µg/ml) during the last hour of incubation. Each sample consists of 100 mitoses.

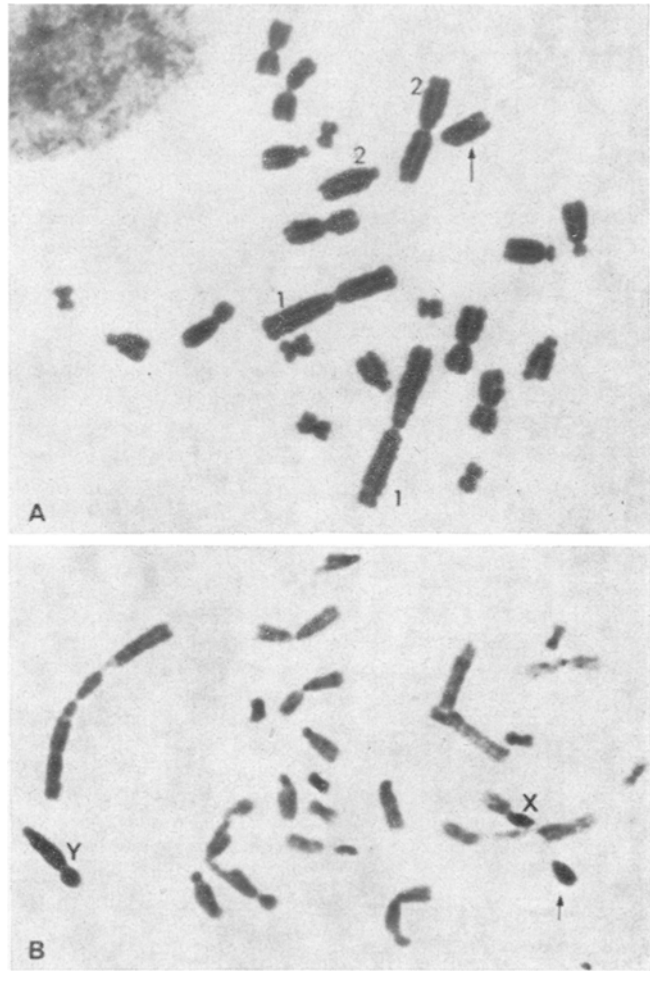

Fig. 2. Metaphases of male Chinese hamster 4 h after the introduction of adriamycin (0.1 µg/ml). A) Giemsa-stained metaphase showing 'isochromatid' break in one of the No. 2 chromosomes. Arrow indicates the acentric fragment. B) C-banded metaphase, arrow indicates the acentric heterochromatic fragment of the *X*-chromosome. Note also numerous chromatid aberrations.

The cells in exponential growth period were labeled with ^{3}H-thymidine (5 μCi/ml) for 10 min, washed twice with Hanks' balanced salt solution, and reincubated in growth medium containing adriamycin (0.01-0.1 μg/ml). Cell harvest for cytological preparations was made at 2, 4 and 6 h after the introduction of the drug, each with a 1-h Colcemid arrest. Autoradiographs showed that in the 2-h samples the metaphases were either unlabelled (G_2) or labelled with the typical late replicating pattern (heavy label over the long arm of the *X*, the *Y*, and the small metacentric elements)[3]. In the 4-h and 6-h samples, metaphases with late-replicating pattern were proportionally more than those found in the untreated control samples; but the majority of metaphases showed label over most chromosomes throughout their lengths.

Figure 1 shows that adriamycin induced both the chromatid- and the isochromatid-types of aberrations, and that their frequencies increased as the durations of treatment increased. The slight increase in the frequencies of chromatid gaps and breaks in the control samples harvested at 4 and 6 h was probably due to the radiation effects of ^{3}H-thymidine. However, aberrations of the isochromatid type, nonexistent in the controls, were also recorded with a relatively high frequency in the drug-treated samples. Most of these isochromatid aberrations were fragments, but dicentrics were occasionally observed. Figure 2A depicts a metaphase (0.1 μg/ml, 4 h) in which one of the No. 2 chromosomes had an isochromatid break near the centromere. The acentric fragment (arrow) is not connected with the centric fragment. In Figure 2B (also from 0.1 μg/ml, 4 h), a similar break occurred in the heterochromatic long arm of the *X* chromosome.

It should be pointed out that isochromatid gaps and isochromatid breaks are cytological terms. Breaks at the 'identical' cytological locations of the two sister chromatids may be thousands of nucleotides apart when DNA sequences are considered. On the other hand, the lesions for a chromosome break must be identical in the two sister chromatids because the break occurs prior to chromosome duplication. In our materials, the 'isochromatid' aberrations induced in the G_2 and S phases did not necessarily represent identical loci between the sister chromatids. Since daunomycin may have specific affinity for dAT base pairs[3] and since adriamycin is a derivative of daunomycin, adriamycin might also damage chromosome segments rich in dAT bases. If a break is induced in one chromatid in an AT-rich chromosome segment and another break is induced in the same segment of the sister chromatid, the locations of these two breaks probably are not identical, but the morphological characteristics of the isochromatid breaks would be indistinguishable from a true chromosome break. It is entirely possible that other chemical clastogens with base specificities may behave in a similar way but the employment of proper test materials and proper concentrations are necessary to detect such actions.

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Actinomycin-Resistant Antiviral Activity Associated with Preparations Containing Chicken Interferon[1]

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Summary. Crude and purified preparations containing chicken interferon show a 2fold antiviral activity. One is inhibited by actinomycin and leads to total inhibition if interferon is added to cells before actinomycin, the other is insensitive to actinomycin and leads to partial inhibition if interferon is added simultaneously with or after actinomycin.

Interferon is supposed to need an intact synthesis of ribonucleic acids (RNA) and protein in order to develop within hours a full state of inhibition of viral growth[4–6]. Therefore interferon is considered to be the inducer of a hypothetical antiviral substance[7]. The cellular gene activated by interferon can be discriminated from that specifying the interferon on basis of genetical evidence. Theoretically then, all crude preparations containing interferon (itself induced by a viral challenge) should also contain the antiviral principle. In the course of experiments on kinetics of interferon action, we found crude preparations to inhibit the synthesis of Semliki Forest Virus (SFV), despite the fact that host RNA biosynthesis had been blocked *before* the interferon application by actinomycin D. (The replication of SFV, an RNA-virus, is not harmed by actinomycin.)

Materials and methods. Cell cultures, media and infection with SFV (Kumba or Zurich strain) were as described[8]. Interferon preparations were from allantoic fluid and chicken embryos (II: 120 units/mg protein; V: 240 units/mg; 10/1: 20,000 units/mg) or from allantoic fluid (447/196: 33,420 units/mg; 489/24: 280,000 units/mg) according to methods established[9]. Antiviral activities were assayed either by measuring the incorporation of [^{3}H]-uridine[10] into acid-precipitable material of SFV-infected chick embryo fibroblast (CEF) monolayers treated with actinomycin D (RNA-test)[8] or with the plaque test[11]. The actinomycin-resistant (non-inductive)

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[9] The authors wish to thank Prof. H. Zuber, ETH, Zurich, for providing interferon 10/1, and Dr K. H. Fantes, Wellcome Res. Labs., Beckenham, England, for his preparations 447/196 and 489/24. One unit is defined as the amount of protein reducing plaque counts or uridine incorporation into viral RNA to 50% of the control value.

[10] Abbreviations and reagents: Actinomycin D (Calbiochem); medium Eagle MEM is minimum essential medium – Hanks (BBL, USA); Medium 199 – Hanks (Difco, Detroit); Fetal calf serum (Flow, Scotland); [^{3}H]-uridine, uniformly labeled, 3.5–6.3 Ci/m*M* (Amersham, England).

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