concentration of unlabeled compound required to reduce the 11-cis retinaldehyde binding to 50% of the control value.
None of the retinoids were found to compete significantly at any of the tested concentrations, except 11-cis retinol. This was not unexpected when it is considered that, in the retina, the CRALBP is found to carry 11-cis retinaldehyde and 11-cis retinol as the endogenous ligands, and in the pigment epithelium is also able to bind 11-cis retinol, in addition to the endogenous 11-cis retinaldehyde, following exposure to the light[3].
Although the different compartmentalization of the 11-cis retinoids in the retina and in the pigment epithelium[17] could be sufficient to explain the different complement of its endogenous ligand, the competition profile would also indicate for the CRALBP some difference in the affinity towards 11-cis retinaldehyde and 11-cis retinol.

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Induction of chromosomal aberrations by the anthracycline antitumor antibiotics N,N-dimethyldaunomycin and aclacinomycin A

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Summary. The clastogenic effect of the anthracycline antitumor antibiotics, N,N-dimethyldaunomycin and aclacinomycin A, was studied in a murine hemopoietic cell line (Friend leukemia cells). A dose-dependent increase in chromatid lesions, i.e., achromatic lesions, chromatid breaks, chromatid deletions and triradial or quadriradial chromosomal exchange figures, was found. It appears that the clastogenicity of N,N-dimethyldaunomycin and aclacinomycin A is lower than that of the classic anthracycline, daunomycin, which is also a potent mutagen and carcinogen. The data demonstrate that the capacity of chemicals to induce point mutations and chromosomal aberrations may not necessarily be correlated: aclacinomycin A is devoid of mutagenic activity in bacterial (Salmonella typh.) and mammalian cell (HGPRT) mutagenesis assays, and is non-carcinogenic in rats. Nevertheless, it was now found to possess clastogenic activity.
Key words. Aclacinomycin A; anthracyclines; chromosome aberrations; daunomycin; N,N-dimethyldaunomycin.

The anthracycline antitumor antibiotics, adriamycin and daunomycin, are potent mutagenic, clastogenic and carcinogenic compounds[1,2]. On the other hand, N-substituted anthracyclines are only weakly mutagenic or nonmutagenic[3,4]. However, some of these N-substituted anthracyclines, such as cyanomorpholino-adriamycin[3], are potent crosslinking agents and induce unscheduled DNA-synthesis and chromosomal aberrations[3,5]. It appears, therefore, that a discrepancy may exist between the mutagenic properties of N-substituted anthracyclines (as determined with bacterial or mammalian cells) and their clastogenic potential. To further clarify this, we studied the clastogenicity of the N-alkylated anthracycline N,N-dimethyldaunomycin, and the N-alkylated oligosaccharide anthracycline, aclacinomycin A. The latter is of particular biological and clinical interest because of its differentiation-inducing capacity in vitro[6-8] and possibly in vivo[9]. It should be noted that both N,N-dimethyldaunomycin and aclacinomycin A are nonmutagenic in bacterial and mammalian cells[3,4] and that, furthermore, aclacinomycin A is also noncarcinogenic in rats[10].
Materials and methods. Chemicals. Aclacinomycin A and N,N-dimethyldaunomycin were kindly provided by Dr T. Oki, Sanraku-Ocean Co. Ltd., Tokyo, Japan, and by Dr E. Acton, Stanford Research Institute, Menlo Park, California, USA, respectively. Daunomycin was obtained by courtesy of Dr F. Arcamone, Farmitalia-Carlo Erba, Milano, Italy. Alpha medium without nucleosides and fetal calf serum were purchased from Gibco Co., Darmstadt, FRG.
Before use the anthracyclines were dissolved in 0.9% NaCl solution. A stock solution (1 mg/ml) was prepared and further dilutions were carried out using alpha medium without fetal calf serum.
Cell line. The Friend erythroleukemia clone F4-6 employed by us has been described[11,12].
Induction of chromosomal aberrations. Cell cultures were set up in duplicate by plating 2.5–3 × 10^5 viable cells in plastic petri dishes (diameter, 5 cm), containing 5 ml of cell culture medium supplemented with 10% fetal calf serum. Three days later, the cultures were treated for 1 h with different concentrations of the test compounds. Thereafter, the cultures were rinsed twice with the medium to remove these compounds, and fresh culture medium was added. After 24 h, the cells were incubated for 2 h in the presence of colcemid (1 μg/ml) and harvested by decanting suspended cells or by scraping off the adherent cells with a rubber policeman. The cells were centrifuged, resuspended in the residual volume of 0.25–0.5 ml medium and treated for 12 min at room temperature with 4 ml of a hypotonic solution of KCl (0.075 M). After hypotonic treatment the cells were spun down again, resuspended in the remaining fluid and fixed by the addi-

tion of 4 ml of freshly prepared fixative (3 parts ethanol to 1 part glacial acetic acid, incubation for 15 min at room temperature). The cells were then spun down again and the fixation procedure was repeated once. Fixed cells (2–3 drops) were placed on a glass slide which had been stored in cold methanol (60–70%). The slide was then passed quickly through a flame. After drying, the slides were flooded for 10 min with Giemsa stain (pH 6.8).

Evaluation of chromosomal aberrations. Using 1000-fold microscopic magnification, the mitotic index and the chromosome number of individual metaphases was determined. In addition, the metaphases were examined for structural chromosomal aberrations, such as 'gaps' (achromatic lesions), chromatid breaks and deletions, chromosome breaks, chromosomal exchange figures (i.e., triradial or quadriradial translocations) and other chromosomal aberrations[13].

The results were expressed in absolute numbers of chromosomal aberrations as well as in frequencies, i.e., aberrations per number of chromosomes. In addition, the percentage of damaged mitoses (irrespective of the type or the number of aberrations per individual metaphase) was calculated.

Results. The induction of chromosomal aberrations by N,N-dimethyldaunomycin and aclacinomycin A was studied in Friend leukemia cells (tables 1 and 2). In general, the experiments were carried out thrice and yielded reproducible results. Both compounds, N,N-dimethyldaunomycin and aclacinomycin A, induced aberrations of the chromatid type, i.e., achromatic lesions, chromatid breaks, chromatid deletions as well as triradial and quadriradial chromosomal exchange figures (translocations)[13].

Following treatment of cells with N,N-dimethyldaunomycin, an increase of the aberration rate was seen. The total number of damaged mitoses showed a dose-dependent increase (table 1) while achromatic lesions and chromatid breaks reached a plateau level at the concentration range of 1–2 µg/ml. The numbers of translocations induced by N,N-dimethyldaunomycin were too low to permit conclusions about the dose-dependency of their induction (table 1).

The increase of damaged mitotic figures and chromosomal aberrations was dose-dependent after aclacinomycin A treatment (table 2). Chromosome pulverization and/or chromosome clumping was also observed following daunomycin treatment at a concentration of 0.1 µg/ml or treatment with N,N-dimethyldaunomycin or aclacinomycin A at higher concentrations.

Discussion. Our data demonstrate a dose-dependent increase in chromosomal aberrations of the chromatid type after treatment of Friend leukemia cells with N,N-dimethyldaunomycin or aclacinomycin A. From previous and the present data, it appears that the clastogenicity of N-substituted anthracyclines is correlated with their cytotoxic activity: a) cytotoxicity and clastogenicity of cyanomorpholinoadriamycin was about 1000-fold higher than that of adriamycin; b) N,N-dimethyldaunomycin and aclacinomycin A were less toxic and less clastogenic in Friend leukemia cells than daunomycin[5,14]. The present data, indicating that daunomycin has a higher clastogenic potential than N,N-dimethyldaunomycin or aclacinomycin A, are in accord with this suggestion.

The mechanism by which anthracyclines exert their clastogenic effects remains unknown. The anthracyclines, adriamycin and daunomycin, bind strongly to DNA by intercalation[15] and may exert their cytotoxic, mutagenic and clastogenic effects by interfering with DNA structure, replication and template activity[16,17]. However, alternative mechanisms, such as conversion of anthracyclines to semiquinone free radicals which might cause damage to DNA either directly or by formation of oxygen radicals, have been proposed[18]. In addition, topoisomerase II activity may be involved[19–20].

As pointed out in the introduction, N,N-dimethyldaunomycin and aclacinomycin A are devoid of mutagenic activity[3,4]; our data thus demonstrate that, at least in the case of aclacinomycin A and possibly also in the case of other N-alkylated anthracyclines, the activities of chemicals to induce point mutations and chromosomal aberrations are not necessarily correlated. Since aclacinomycin A was shown by us to be non-carcinogenic in rats, clastogenicity may be a poor indicator of carcinogenic potential (at least in the case of anthracyclines). It should be emphasized that aclacinomycin A is of considerable clinical importance[22,23].

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Table 1. Chromosomal aberrations induced in Friend leukemia cells by daunomycin and N,N-dimethyldaunomycin

Compound	Concentration (µg/ml)	Mitotic index (‰)	No. of mitoses evaluated	Achromatic lesions	Chromatic lesions[a]	Translocations	Total No. of damaged mitoses (%)
Control	–	60	50	0	0.001 (2)[b]	0	4
Daunomycin	0.1	42	50	0.007 (11)	0.01 (17)	0.019 (32)	58
N,N-Dimethyldaunomycin	0.5	42	50	0.0015 (3)	0.0005 (1)	0	6
	1.0	42	50	0.01 (20)	0.007 (13)	0.006 (11)	40
	2.0	24	41	0.01 (18)	0.007 (12)	0.002 (4)	44

[a] Breaks and deletions (chromosome breaks were not observed). [b] Aberrations per number of chromosomes; in parentheses, absolute number of aberrations.

Table 2. Chromosomal aberrations induced in Friend leukemia cells by daunomycin and aclacinomycin A

Compound	Concentration (µg/ml)	Mitotic index (‰)	No. of mitoses evaluated	Achromatic lesions	Chromatid lesions[a]	Translocations	Total No. of damaged mitoses (%)
Control	–	124	50	0	0.0014 (3)[b]	0	4
Daunomycin	0.1	72	36	0.01 (19)	0.02 (39)	0.018 (34)	88
Aclacinomycin A	0.5	60	50	0.0014 (3)	0.0005 (1)	0.002 (5)	8
	1.0	70	47	0.005 (11)	0.006 (14)	0.011 (25)	42
	1.5	30	40	0.012 (26	0.016 (33)	0.031 (66)	80

[a] Breaks and deletions (with the exception of one chromosome break induced by daunomycin, chromosome breaks were not observed). [b] Aberrations per number of chromosomes; in parentheses, absolute number of aberrations.

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