inhibitory signal provided by FSH is very specific; it has been impossible to duplicate it by using 2 other pituitary hormones (ACTH + LH).

These results and their interpretation are also supported by the data of NEGRO-VILAR and MEITES[16] and of CORBIN (personal communication, 1967) which have shown that hypophysectomy is followed in the rat by the appearance of measurable amounts of FSH-RF into the peripheral blood; treatment of hypophysectomized animals with pituitary FSH makes FSH-RF plasma levels disappear. Another recent result points to the same conclusion[17]: treatment of hypophysectomized-castrated rats with FSH has been shown to reduce the size of the nuclei of several hypothalamic cells[18,19].

Résumé. Les auteurs ont montré que l'administration s.c. d'hormone folliculo-stimulante (FSH) entraîne, chez le rat mâle castré, une diminution significative de quantités de FSH présentes dans l'hypophyse et de FSH-releasing factor (FSH-RF) stockées dans l'hypothalamus. Ces résultats suggèrent qu'un mécanisme de «short feedback» peut contrôler la sécrétion de FSH; ce mécanisme de «contre-régulation courte» agirait au niveau de l'hypothalamus.

F. FRASCHINI, M. MOTTA and L. MARTINI

Department of Pharmacology, University of Milano (Italy), 23 October 1967.

[16] A. NEGRO-VILAR and J. MEITES, Progm 49th Meet. Endocr. Soc. (1967), p. 88.
[17] J. D. IFFT, Neuroendocrinology *1*, 350 (1966).
[18] This work was supported by grant No. AM-10119 of the National Institutes of Health, Bethesda, Md., whose help is gratefully acknowledged.
[19] The authors would like to thank the Endocrinology Study Section of the National Institutes of Health, Bethesda, Md., for the generous gift of FSH and LH preparations.

Chromosome Aberrations Induced by Daunomycin in Human Leucocyte Cultures, with the Apparent Synergistic Effect of Arginine

Daunomycin is an antibiotic isolated from *Streptomyces peucetius* (GRIEN et al.[1]) which has lately seen some success in experimental use against childhood leukemias and certain solid tumors (TAN et al.[2]). It is a glycoside with an aglycone chromophore linked to an amino sugar, which binds to DNA both in vivo and in vitro (GOLDBERG[3]). A few studies have been reported on the adverse action of Daunomycin on mitotic activity (DIMARCO et al.[4,5]). Cessation of DNA and RNA synthesis (DIMARCO[4,6], GOLDBERG[3]) and speculations on the role of Daunomycin in blocking other biochemical processes have been reported but there have been no reported studies on the action of this antibiotic on chromosomes except for negative results noted in HeLa cells (OSTERTAG and KERSTEN[7]). We may postulate that a drug which can cause cessation of DNA synthesis and inhibit protein synthesis (DIMARCO et al.[6]) may have the capacity to cause chromosomal aberrations. This paper reports confirmation of this postulate.

Cultured human leucocytes from 3 normal females were treated with Daunomycin during the last 12–48 h of a 72 h

[1] A. GRIEN, C. SPELLA, A. DIMARCO and G. CANEVAZZI, G. Microbiol. *11*, 109 (1963).
[2] C. TAN, H. TASAKA, K. P. YU, M. L. MURPHY and D. A. KARNOFSKY, Cancer *20*, 333 (1967).
[3] I. H. GOLDBERG, Am. J. Med. *39*, 722 (1965).
[4] A. DIMARCO, M. GAETANI, L. DORIGOTI, M. SOLDATI and O. BELLINI, Tumori *49*, 203 (1963).
[5] A. DIMARCO, M. SOLDATI, A. FIONETTI and T. DASDIA, Tumori *49*, 253 (1963).
[6] A. DIMARCO, R. SILVESTRINI, S. DIMARCO and T. DASDIA, J. Cell Biol. *27*, 545 (1965).
[7] W. OSTERTAG and W. KERSTEN, Expl Cell Res. *39*, 296 (1965).

Table I. Analysis of chromosome aberrations induced by daunomycin

Treatment	No. of cells analysed	% of cells with aberrations	Frequency and types of aberrations		
			Fragments	Interchanges	Aberration frequency/cell
Control	100	0	0	0	0
Daunomycin μg/ml					
12 h 0.02 and less	100	0	0	0	0
24 h 0.01 and less	100	0	0	0	0
0.02	70[a]	57.1	60	92	2.17
48 h 0.01 and less	100	0	0	0	0
0.02 and more[b]	no mitosis				

[a] Only 70 cells were available for study. [b] Complete cessation of mitotic activity was observed with 0.03 μg/ml of Daunomycin for 12, 24, or 48 h.

culture period. Final drug concentration ranged from 0.005–5 μg/ml (precisely, 0.005, 0.01, 0.02, 0.03, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.8, 1.0, 2, and 5 μg/ml). Mitosis was arrested by using vinblastine during the last 3 h of culture.

A proportionate reduction in mitotic index was observed in all the concentrations used except 0.005 μg/ml.

Fig. 1. Part of a cell treated with Daunomycin showing chromatid break and interchanges involving 2 chromosomes.

No aberrations were recorded in any treatment except with 0.02 μg/ml of Daunomycin for 24 h (Table I). The concentration of 0.01 μg/ml was ineffective in bringing about chromosomal aberrations even when the drug was in contact with the calls for 48 h. The concentration of 0.03 μg/ml proved totally inhibitory even when the cells were exposed to the drug for only 12 h. Thus aberration production is apparently dependent on concentration of drug rather than duration of exposure.

Chromosomal aberrations (Figures 1 and 2) included chromatid or isolocus fragments, intrachromosomal rearrangements and interchromosomal exchanges involving

Fig. 2. Part of a Daunomycin treated cell with an intrachromosomal rearrangement and complex interchanges.

Table II. Analysis of chromosome aberrations induced by Daunomycin and arginine

Treatment	No. of cells analysed	% of cells with aberrations	Frequency and types of aberrations				
			Fragments	Interchanges	Dicentrics	Caps	Aberration frequency/cell
Control (with 0.16% arginine)	100	0	0	0	0	0	0
Daunomycin μg/ml + 0.16% arginine – 24 h							
0.01	250	26.0	63	40	7	6	0.46
0.02[a]	143[b]	70.7	274	177	16	17	3.39

[a] Concentration of Daunomycin more than 0.02 μg/ml resulted in complete cessation of mitosis. [b] Only 143 cells were available for study

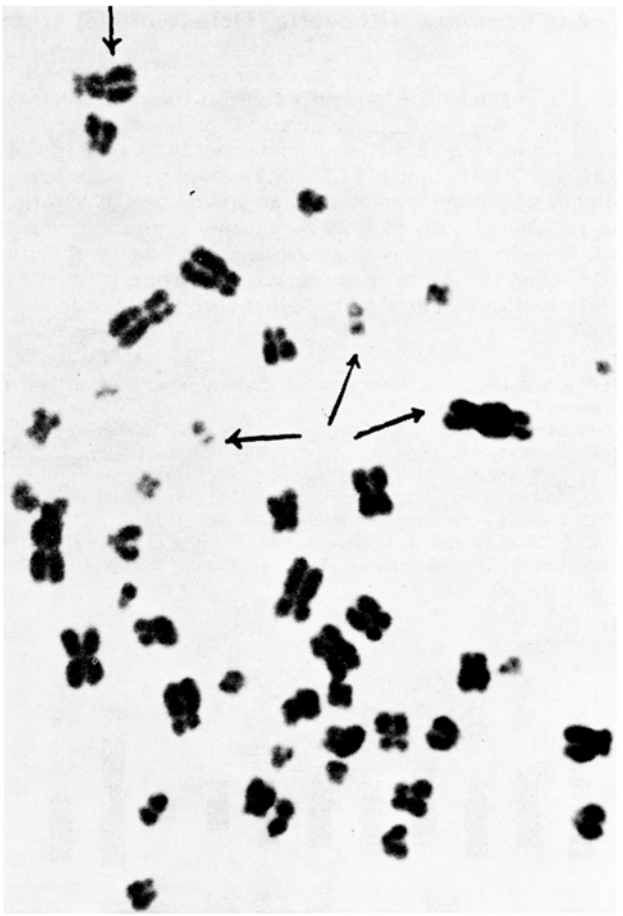

Fig. 3. A cell treated with Daunomycin + arginine with 2 dicentric chromosomes and double fragments.

Fig. 4. A Daunomycin + arginine treated cell showing partial dicentrics (exchanges), double fragments and an intrachromosomal rearrangement.

2 or more chromosomes. The exchanges were at chromatid or isochromatid level and appeared to result from aberrations originating at post-duplication stages. This idea was postulated from absence of symmetric or complete dicentrics accompanied by double acentric fragments.

The data in Table II reveal the increased frequency of aberrations when Daunomycin was supplemented with the amino acid arginine (L[+]-monohydrochloride). Whereas 0.01 μg/ml of Daunomycin was ineffective in producing aberrations, its supplementation with 0.16% arginine resulted in chromosomal aberrations in 26% of cells. With 0.02 μg/ml of Daunomycin + 0.16% arginine, an increase both in mitotic index and aberration frequency was observed. These studies suggest that cessation of mitotic activity and chromosomal aberrations may result from 2 different types of actions. Obviously, mitotic activity is required for latent chromosome aberrations to become manifest.

Besides the usual types of aberrations as observed with Daunomycin, combined Daunomycin and arginine produced dicentrics, double fragments (Figures 3 and 4) and lesions or gaps in the continuity of the chromatids. Does this indicate that the Daunomycin-arginine mixture can act on single-stranded chromosomes at the pre-DNA synthetic period, whereas Daunomycin alone cannot?

The appearance of gaps is attributed to inadequate thymidylate activity (TAYLOR[8]). This might mean that a combination of Daunomycin and arginine is capable of affecting the thymidylate activity whereas Daunomycin alone is not. Further work is in progress[9].

Zusammenfassung. Das Antibiotikum und Zytostatikum Daunomycin induziert beträchtliche Chromosomenaberrationen in menschlichen Leukozyten in der Konzentration von 0,02 μg/cm^3. Dieser Effekt wird durch Zusatz von Arginin zur Zellkultur wesentlich verstärkt, und die Aberrationen werden auch qualitativ verändert. Es ist nicht bekannt, wie Daunomycin und Arginine aufeinander einwirken.

B. K. VIG, STELLA B. KONTRAS and L. D. SAMUELS

Department of Pediatrics, Ohio State University, The Children's Hospital, Columbus (Ohio 43205, USA),
1 December 1967.

[8] J. H. TAYLOR, J. cell. comp. Physiol. Suppl. I *62*, 73 (1963).

[9] The Daunomycin used was prepared by Farmitalia Research Laboratories, Milano, Italy and provided by CR, NCI, NIH, PHS, U.S. Dept. HEW, Bethesda, Maryland. The tissue culture media used was No. 1A from Grand Island Biological Co. Address requests for reprints to Dr. B. K. VIG, The Children's Hospital, Columbus, Ohio 43205.