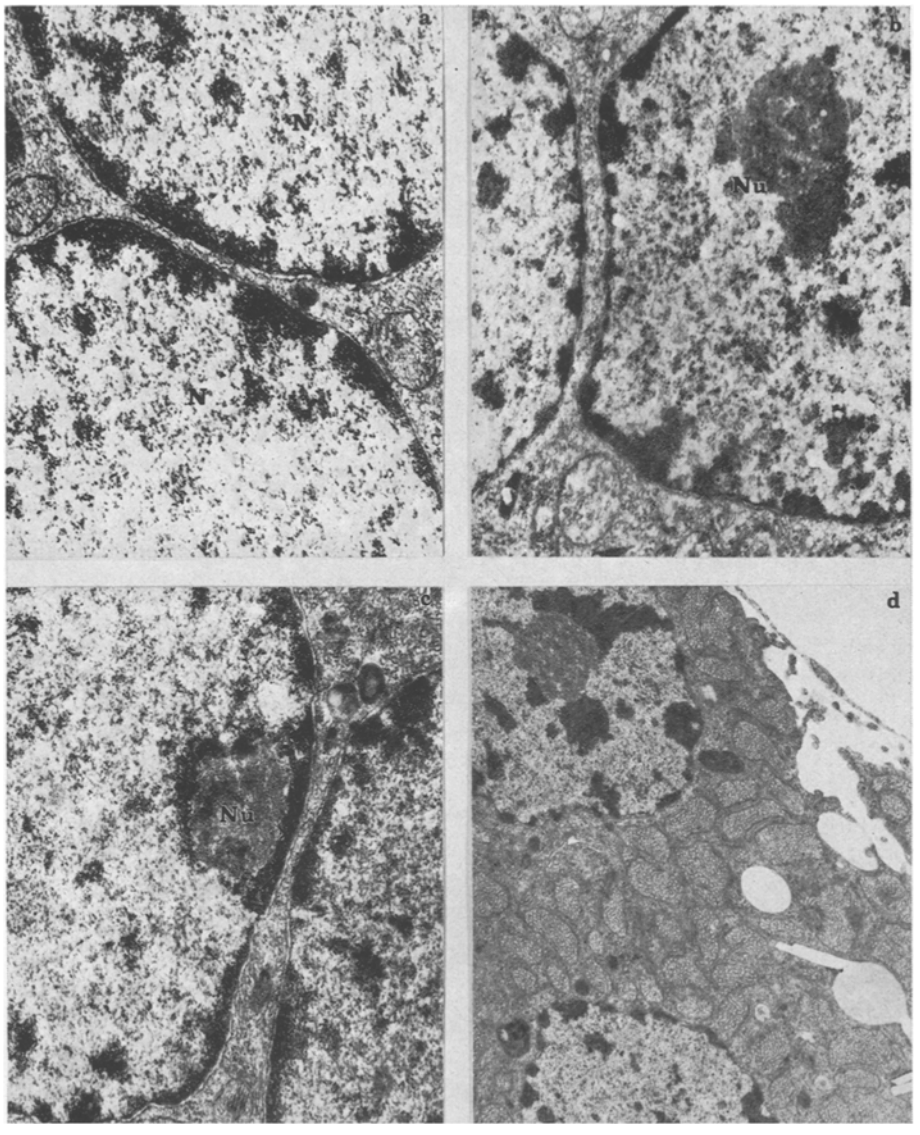

Fig. 4. a) Binucleate cell with confronted nuclei (N). ×15,000. b) and c) binucleate cells, the nucleolus (Nu) with the satellite heterochromatin can be seen in some of the nuclei. ×15,000. d) Binucleate cell in the sudanophobic region. ×6000.

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Mitostatic Action of 4,6-Dimethyl-2-Amino-3,4,5-Trimethoxyphenyl-Pyrimidine on Mammalian Cells[1]

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Summary. 4,6 dimethyl-2-amino-3,4,5-trimethoxyphenylpyrimidine arrests the mitotic cycle of mammalian cells in metaphase, both in vitro and in vivo. The mitostatic effect is promptly reverted by interruption of drug treatment.

In the course of studies on the toxic effects of pyrimidine derivatives on mammalian cells, the mitostatic activity of 4,6-dimethyl-2-amino-3,4,5-trimethoxyphenylpyrimidine (B 31) has been reavealed. Results from these experiments are reported here.

Material and methods. B 31 and other pyrimidine derivatives were synthesized by Istituto Chemioterapico Italiano (I.C.I.) Lodi; vinblastine (Lilly) and colchicine (Simes) were also used. In vitro tests were carried out in cells of the human aneuploid line HEp2 (American Type

Table I. Mitotic effect of B 31 on HEp2 cells

Compound in the medium (μg/ml)	Cytotoxic effect at 10 h	Neutral red uptake at 10 h [a]	Mitoses at 30 h (%)				Teloph.
			Total	Proph.	Metaph. [b]	Anaph.	
—	—	+++	4.5	0.6	3	0.5	0.4
B 31							
300	+	+	48.5	2.6	45.9	0	0
100	—	+++	68.9	3.9	65	0	0
33.3	—	+++	63.8	2.8	61.0	0	0
11.1	—	+++	68	1.5	66	0.3	0.2
3.7	—	+++	16.2	1	12.6	1.3	1.3
Colchicine							
0.1	—	+++	59.6	3	56.6	0	0
0.033	—	+++	73.5	2.5	71	0	0
0.011	—	+++	72	1	71	0	0
0.0037	—	+++	35.9	1.4	32	1.4	1.1

[a] Cell cultures were supplemented with 100 μg/ml of neutral red (Merck) at 9 h after drug-treatment. 1h later monolayers were washed 3 times in Earle's solution and examined at microscope. [b] At microscope observation, B 31 induced metaphases were indistinguishable from C-mitoses produced by colchicine.

Culture Collection, Rockville), which were grown on coverslips in Eagle's MEM (Earle's base, pH 7.3, supplemented with 7% calf serum) at 37 °C for 16 h before drug addition. Mitotic index was determined by microscope examination of Giemsa stained monolayers. Toxic effects on interkinetic cells were established by microscope observation of Giemsa stained cells and by intracellular uptake of neutral red (100 μg/ml of medium, 1 h pulses). In vivo experiments were carried out on Swiss male mice, weighing about 20 g, which were injected i.p. with the substance under study. Because of its low solubility in water, B 31 was injected suspended in olive oil. At given time intervals, mice were sacrificed and the mitotic index of femoral bone marrow cells, previously stained with Giemsa, was evaluated. More details of technique are given in the Tables.

Results. At concentrations 10 times lower than the maximum tolerated by interkinetic cells, B 31 produces a remarkable arrest of the mitotic period of HEp2 cells, leading to accumulation of 'C metaphases' morphologically indistinguishable from those produced by colchicine[2]

Table II. Reversibility of the mitostatic effect of B 31 on HEp2 cells

Compound in the medium (μg/ml)	Mitoses at 30 h (%)	Mitoses 24 h after drug removal from the medium (%) [a]
—	2.5	2
B 31		
100	62.6	8
33	70.6	2.8
11	65.8	2
3.7	11.9	2.6
Vinblastine		
0.003	78.5	18
0.001	79.6	16
0.00033	72	7.6
0.00011	59	4.5
0.000033	16.9	2.4

[a] At 30 h after treatment cell monolayers were washed 3 times in Earle's solution and incubated at 37 °C in drug free Eagle's MEM.

Table III. Mitostatic activity of pyrimidine derivatives structurally related to B 31 on HEp2 cells

Compound in the medium (μg/ml)		Cytotoxic effect at 10 h	Mitoses at 30 h (%)
		—	2.5
B 31	100	—	64.6
	30	—	69
	10	—	62
	3	—	9.9
B 33	30	+	2
	10	—	2.8
	3	—	3
	1	—	2
B 28	30	±	2.1
	10	—	2.4
	3	—	2.5
	1	—	2.8
B 32	30	+	3.6
	10	—	8.5
	3	—	2.7
	1	—	2.9

[1] This work has been supported by a Grant of the Consiglio Nazionale delle Ricerche (Roma).

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Table IV. Mitostatic activity of B 31 on mouse bone marrow cells in vivo

No. of mice	Compound injected i.p. (one single dose, mg/ml)	Lethality at the 3rd day	Mitoses % ° (mean values of 2 mice) after														
			2 hours					6 hours					18 hours				
			Total	P	M	A	T	Total	P	M	A	T	Total	P	M	A	T
6	—	0	11.5	3.0	4.5	2.5	1.5			n.d.					n.d.		
6	B 31 3000	5			n.d.					n.d.					n.d.		
6	B 31 1000	0	53	16.5	35.5	1	0	71	22	44.5	2.2	2.3	28.5	13	13.5	1	1
6	B 31 333	0	30.5	9.2	16.3	3	2	40	12.5	23.5	3	1	14	4	6	2	2
6	Vinblastine 10	6	92	13	76	2	1	105	17	88	0	0	60	10	50	0	0

n.d., not determined.

(Table I). Upon drug removal from the medium, the mitostatic effect of B 31 is easier to reverse than that of vinblastine[3] (Table II). Preliminary tests on structure-activity relationship have shown that compounds such as 4,6-dimethyl-2-amino-4-methoxyphenylpyrimidine (B 33), 2-amino-3,4,5-trimethoxyphenylpyrimidine (B 28) and 5-methyl-2-amino-3,4,5-trimethoxyphenylpyrimidine (B 32) have very little or no mitostatic effect on HEp2 cells, even at the maximum non-toxic concentrations for interkinetic cells (Table III). These data indicate that the mitostatic effect of B 31 calls for a trimethoxyphenyl ring and, in addition, for methyl groups, possibly in either or both 4,6 positions in the pyrimidine moiety.

Injected i.p. in mice, B 31 has a clear mitostatic effect on bone marrow cells. This in vivo effect, however, is far less pronounced than that observed in vitro, and is produced only by high doses of B 31 (Table IV).

Research is in progress to establish whether and which modifications in the B 31 structure may enhance the mitostatic effect. At present, B 31 can be considered as a promizing tool for cell synchronization.

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Epidermal Growth Factor Enhancement of Skin Tumor Induction in Mice

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Summary. Subcutaneous injection of epidermal growth factor 1. significantly shortened the latency period for the appearance of methylcholanthrene induced skin tumors and 2. increased the average number of papillomas elicited per mouse in both the Swiss Webster and C3HeB/FeJ strains.

A number of agents which stimulate hyperplasia in target tissues have been found to enhance the tumorigenicity of chemical carcinogens in these tissues. For example, in the rat mammary gland system the induction of tumors by combined administration of the chemical carcinogen methylcholanthrene (MCA), and prolactin, a protein hormone, has been well characterized[3]. Neither MCA nor prolactin administered individually could produce mammary tumors at the concentrations used.

A variety of physical and chemical agents[4–8], which have in common the ability to stimulate epidermal hyperplasia, have been reported to promote the induction of skin tumors by chemical carcinogens. Epidermal growth factor (EGF), isolated from the mouse submaxillary gland, is a polypetide growth factor which stimulates epidermal growth when injected into neonatal mice[9] and epithelial cellular proliferation when added to chick embryo skin fragments[10] and mouse mammary gland or mammary carcinoma[11,12] in organ culture. EGF also shares with phorbol myristate acetate, a potent promoter of skin tumor formation[13] derived from Croton oil, the ability to initiate cell division in non-dividing cultures of murine 3T3 cells[14,15].

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