Tumour Inhibitory Effects of a New Class of Cytotoxic Agents: Methylhydrazine Derivatives

1-Methyl-2-benzyl-hydrazine has been found to inhibit the growth of transplantable tumours in mice and rats. As this compound did not show a satisfactory therapeutic index and as it led furthermore to liver damage, a large series of derivatives has been synthesized by ZELLER et al.[1] in order to find substances having better chemotherapeutic properties combined with lower toxicity. Only compounds with the group $-NH-NH-CH_3$ show marked cytotoxic effects. In this paper the tumour inhibiting properties of 1-methyl-2-*p*-(isopropylcarbamoyl)benzyl-hydrazine hydrochloride (I[2]) and 1-methyl-2-*p*-allophanoylbenzyl-hydrazine hydrobromide (II[3]) are described. A detailed report on these investigations will be given elsewhere.

Methods. The experiments were done on the following transplantable tumours: Ehrlich carcinoma (solid form), Ehrlich carcinoma (ascitic form), Crocker sarcoma S 180, Walker carcinosarcoma 256 and uterus epithelioma (Guérin) T 8.

In the case of tumours growing in solid form, small tumour fragments were implanted subcutaneously, whereas of the Ehrlich ascites carcinoma ascites cell suspensions were injected intraperitoneally. Groups of ten animals were used for each dose. Daily administration of the compounds in aqueous solution was started on the day after the implantation, either by intraperitoneal or oral route. 5 to 9 daily doses were given, varying according to the kind of tumours. The day after the last dose had been administered the tumours were excised, weighed, and compared with the controls. In the case of the ascites carcinoma, the survival time was determined.

Results. Tables I and II illustrate the tumour inhibitory effects of I and II. In these experiments I was administered by parental route and II was fed by stomach tube.

As can be seen from Tables I and II the tumour growth of the neoplasms listed was markedly inhibited. With the Walker carcinosarcoma, experiments have been carried out also on established tumours. 40% of these tumours regressed completely, whereas in the other 60% a resistance developed against the administered drug after the tumour had almost completely regressed. A line of Ehrlich ascites carcinoma resistant to I could be obtained after 7–9 passages in mice treated with this compound.

The tumour inhibition tests with intraperitoneal and oral administration gave similar results.

Organs with a high proliferation rate like the haematopoietic tissues and the gonads are also affected by these substances[4]. The testing of a large series of methyl-

[1] P. ZELLER et al., Exper. *19*, 129 (1963).
[2] I = Ro 4-6467/1.
[3] II = Ro 4-6824.
[4] K. SCHÄRER and E. THEISS, to be published.

Table I. Effect of I on a spectrum of 5 tumours
Intraperitoneal administration

Ehrlich carcinoma, solid form, I i.p., 5 times within 7 days

Dose	Average tumour weight in mg	% tumour inhibition
Controls	529	0
100 mg/kg	158	70.2
200 mg/kg	50	90.6
300 mg/kg	40	92.5

Ehrlich ascites carcinoma, I i.p., 7 times within 9 days

Dose	Average survival time	Prolongation of survival in %
Controls	10.2	0
100 mg/kg	18.6	80
200 mg/kg	23.4	130
300 mg/kg	42.2	310

Crocker sarcoma S 180, I i.p., 5 times within 7 days

Dose	Average tumour weight in mg	% tumour inhibition
Controls	1554	0
300 mg/kg	589	62.1
400 mg/kg	281	81.9

Walker carcinosarcoma 256, I i.p., 6 times within 8 days

Dose	Average tumour weight in mg	% tumour inhibition
Controls	4905	0
5 mg/kg	954	80.6
10 mg/kg	0	100.0

Uterus epithelioma (Guérin) T 8, I i.p., 9 times within 11 days

Dose	Average tumour weight in mg	% tumour inhibition
Controls	15973	0
25 mg/kg	14165	11.3
50 mg/kg	6817	57.3

Table II. Effect of II on a spectrum of 5 tumours
Oral administration

Ehrlich carcinoma, solid form, II per os, *5 times within 7 days*

Dose	Average tumour weight in mg	% tumour inhibition
Controls	735	0
100 mg/kg	126	82.9
200 mg/kg	141	80.8
300 mg/kg	71	90.4

Ehrlich ascites carcinoma, II per os, *7 times within 9 days*

Dose	Average survival time	Prolongation of survival in %
Controls	14.5	0
100 mg/kg	24.6	69.7
200 mg/kg	41.6	186.9
300 mg/kg	52.6	262.8

Crocker sarcoma S 180, II per os, *5 times within 7 days*

Dose	Average tumour weight in mg	% tumour inhibition
Controls	2314	0
400 mg/kg	921	60.2
500 mg/kg	701	69.7

Walker carcinosarcoma 256, II per os, *6 times within 8 days*

Dose	Average tumour weight in mg	% tumour inhibition
Controls	6920	0
5 mg/kg	458	93.4
10 mg/kg	0	100.0

Uterus epithelioma (Guérin) T 8, II per os, *9 times within 11 days*

Dose	Average tumour weight in mg	% tumour inhibition
Controls	15647	0
50 mg/kg	10712	31.5
75 mg/kg	3642	76.7
100 mg/kg	1097	93.0

hydrazines revealed that the degree of damage to the various tumours mentioned above and to the haematopoietic systems showed striking variations. Selective effects either on tumours or on leukopoiesis alone have been noticed. Clinical studies are under way to establish the possible usefulness of these compounds in therapy of human malignant diseases. To obtain information on the mechanism of action of these compounds cytological and physico-chemical investigations have been undertaken[5,6].

Zusammenfassung. Die tumorhemmende Wirkung einer neuen Klasse von Cytostatica (Methylhydrazinderivate) wird beschrieben. Das Wachstum des Ehrlich-Carcinoms in solider und ascitischer Form, des Crocker Sarkoms S 180, des Walker-Carcinosarkoms 256 und des Uterus-Epithelioms T 8 wird deutlich gehemmt. 1-Methyl-2-*p*-(isopropylcarbamoyl)benzyl-hydrazin-hydrochlorid (I) und 1-Methyl-2-*p*-allophanoylbenzyl-hydrazin-hydrobromid (II) zeichnen sich durch besonders starke cytostatische Aktivität aus.

W. Bollag and E. Grunberg

Medizinische Forschungsabteilung, F. Hoffmann-La Roche & Co. AG., Basel (Switzerland), and Medical Research Department, Hoffmann-La Roche Inc., Nutley (New Jersey, U.S.A.), December 17, 1962.

[5] A. Rutishauser and W. Bollag, Exper. *19*, 131 (1963).
[6] K. Berneis et al., Exper. *19*, 132 (1963).

Cytological Investigations with a New Class of Cytotoxic Agents: Methylhydrazine Derivatives

Methylhydrazine derivatives have been found as a new class of tumour inhibitory compounds[1]. In this paper experiments to elucidate the mechanism of action of these antitumour substances are described. We hoped to learn from cytological investigations whether and how the mitotic cycle and the chromosomes are affected.

The test substance used in our experiments was 1-methyl-2-benzyl-hydrazinephosphate (MBH). The Ehrlich ascites carcinoma was chosen as a test model for the following reasons: (1) The Ehrlich ascites tumour is markedly inhibited by MBH and (2) this transplantable neoplasm in its ascitic form is very convenient for a cytological analysis.

Albino mice, weighing 22–24 g, were inoculated with fresh ascites. 0.2 ml of a cell suspension, containing 10–15 million cells, were injected intraperitoneally. 4 days after the implantation a single injection of an aqueous solution of MBH was administered intraperitoneally in varying doses.

The characteristic data of the hypertriploid ascites tumour (strain B) and the cytological methods of the Feulgen technique and the orcein staining are described elsewhere[2]. For some analyses mitoses were arrested in metaphase by 24 γ of colchicine usually given 4 h before taking an ascites sample.

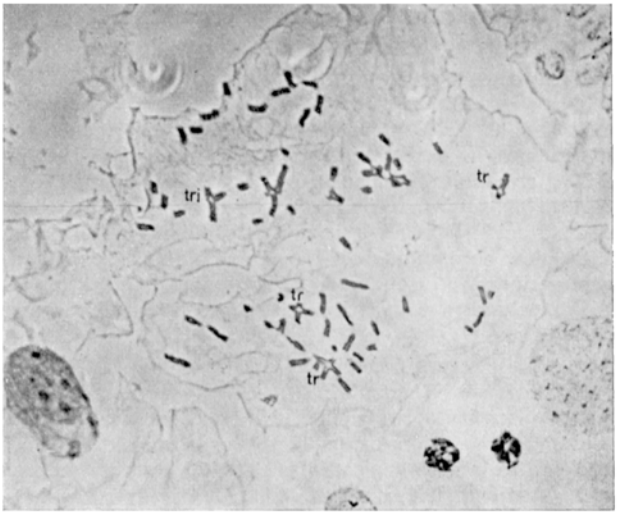
Results. (1) Mitotic index: In the non-treated ascites tumour the percentage of mitoses in 1000 counted cells varies between 9.0 and 5.3%. In the treated animals the percentage of cells being in mitosis decreases markedly down to 0.5% varying according to the dose of the cytotoxic agent and to the lapse of time after the administration of the drug (Table I).

(2) Phase ratio: The distribution of the various phases of the mitotic cycle can be seen in Table I. In untreated tumours the number of prophases exceeds that of the metaphases. The analysis of the phases of treated ascites tumours reveals a noticeable shift from prophase to metaphase. Now the metaphases predominate. The percentage of ana- and telophases does not change significantly.

(3) Chromosome number: The ascites tumour used in our experiments has a rather stable chromosomal variation pattern. The stemline number (s) is 66 and there exist two metacentric marker chromosomes. Under the treatment with MBH no significant change in the chromo-

Table I. Mitotic index and phase ratio

Dose	h after single injection	Mitotic index in %	Phase ratio in % Pro-phase	Meta-phase	Ana- and telophase
Controls	8	9	53	38	9
	24	8.4	53	41	6
	48	5.3	62.3	33.9	3.8
	72	6.4	55.4	30.8	13.8
200 mg/kg MBH i.p.	8	0.6	28	58	14
	24	5.4	50	41	9
	48	3.4	28	62	10
	72	5.5	29.1	52.7	18.2
300 mg/kg MBH i.p.	24	0.8	22.5	64.9	12.6
	48	0.5	14.7	71.3	14.0



Metaphase plate, 48 h after 400 mg/kg MBH. Chromosomal aberrations, in which some of the recombinations are pointed out as tr = translocation (interchange) and tri = triradial.

[1] W. Bollag and E. Grunberg, Exper. *19*, 130 (1963).
[2] A. Rutishauser, Neujahrsblatt der Zürcher Naturforsch. Ges. *1963*, 1.