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.

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[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 $\gamma$ of colchicine usually given 4 h before taking an ascites sample.

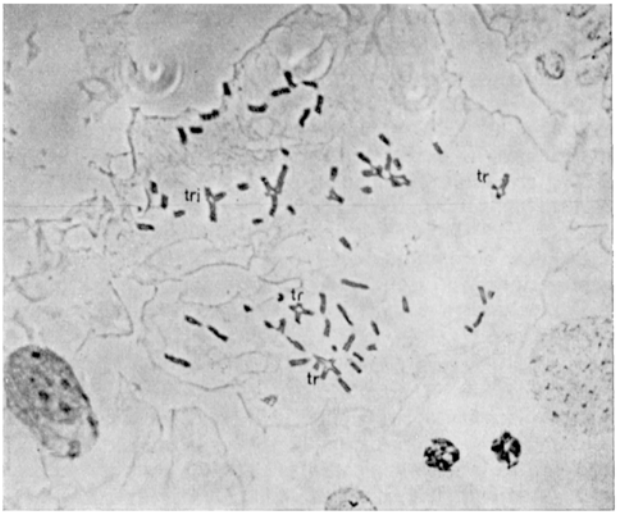
*Results.* (1) Mitotic index: In the non-treated ascites tumour the percentage of mitoses in 1000 counted cells varies between 9.0 and 5.3%. In the treated animals the percentage of cells being in mitosis decreases markedly down to 0.5% varying according to the dose of the cytotoxic agent and to the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

some number and in the number of marker chromosomes takes place.

(4) Chromosome breaks: In ascites carcinoma no chromosome or chromatid breaks can be observed under ordinary conditions of transplantation. After the treatment with MBH chromatid breaks and reunions occur. A few free chromatid breaks can be seen, but interchange and triradial recombinations prevail by far (Figure). From this fact it may be inferred, that only chromatid and no chromosome breakage occurs in consequence of the treatment with this cytotoxic drug. The number of breakages is dependent on the dose of the drug and the lapse of time after the injection of the cytotoxic agent. In Table II the mean percentage of chromatid breaks is indicated. 1% breaks mean that in one of 100 investigated metaphase-plates one chromatid break is found.

*Discussion.* From the low mitotic index, i.e. the suppression of mitosis, it may be inferred, that the interphase is markedly prolonged by this kind of cytotoxic agents. The shift in distribution from prophase to metaphase is not followed by a decrease of ana- and telophase. For this reason it cannot be interpreted as a C-mitotic effect. The chromosomal aberrations induced by the methylhydrazines show a specific pattern. Only chromatid and no chromosome breaks occur. Therefore the breaks seem to be induced during or after deoxyribonucleic acid (DNA) synthesis. The late appearance of breaks may be due to a prolongation of the interphase or to a delayed action of this type of cytotoxic agent. The investigations on the effect of methylhydrazines on isolated DNA[3], which show a very slow degradation of DNA, may explain the above mentioned cytological phenomenon.

Table II. Chromatid breaks

| Dose | h after single injection | Mean percentage of breaks |
|---|---|---|
| Controls | | 0 |
| 200 mg/kg | 8 | 0 |
| MBH i.p. | 24 | 3.3 |
| | 48 | 38.0 |
| | 72 | 29.0 |
| 400 mg/kg | 48 | 70.4 |
| MBH i.p. | 72 | 280.0 |
| | 168 | 255.6 |
| | 192 | 39.6 |

*Zusammenfassung.* Mittels cytologischer Untersuchungen wurde versucht, einen Einblick in den Wirkungsmechanismus der tumorhemmenden Methylhydrazinverbindungen zu gewinnen. 1-Methyl-2-benzyl-hydrazinphosphat bewirkt beim Ehrlich-Ascites-Carcinom der Maus einen Abfall des Mitoseindexes, eine Verschiebung des Mitosephasenindexes zugunsten der Metaphase sowie das Auftreten von Chromatidbrüchen. Diese Befunde werden diskutiert.

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[3] K. BERNEIS et al., Exper. *19*, 132 (1963).

## The Degradation of Deoxyribonucleic Acid by New Tumour Inhibiting Compounds: the Intermediate Formation of Hydrogen Peroxide

It has been demonstrated that 1-methyl-2-benzyl-hydrazine and derivatives have tumour inhibiting properties[1] and that they can cause chromosome breaks[2]. As the chromosomes contain a large amount of deoxyribonucleic acid (DNA) we were interested in the effect of these new compounds on DNA. With the following investigations we intended to obtain some information on the mechanism of action of these methylhydrazine derivatives.

We have examined the effect of 1-methyl-2-*p*-(isopropylcarbamoyl) benzyl-hydrazine hydrochloride (I[3]) on the viscosity of aqueous solutions of deoxyribonucleic acid[4]. The solution of 0.07% w/v sodium deoxyribonucleinate[5] and 10% sodium chloride to stabilize the DNA against denaturation[6] in 1/30 molar phosphate buffer of pH 7 was made 0.0005 molar with respect to I. The solution was stored at 37°C. The viscosity was measured periodically in an Ostwald type viscometer at 37°C (sheer stress between 300 and 600 $sec^{-1}$).

The results of the viscosity measurements are presented in Figure 1. In the presence of molecular oxygen I causes a steady decrease of the viscosity over a period of several days (circles), whereas the viscosity is practically not affected when oxygen is replaced by an inert gas (dots). If 0.001% peroxidase[7] or 0.001% catalase[7] is added to the DNA solution the viscosity remains almost constant even in the presence of molecular oxygen (squares). These results suggest that a reaction product of molecular oxygen with I, which can be destroyed by catalase or by peroxidase is responsible for the decrease of viscosity. We therefore assumed that hydrogen peroxide may be formed. It is known that hydrogen peroxide can degradate DNA in the presence of ferrous ions[8,9]. The experiments described below strongly support this hypothesis.

I is readily autoxidized at 37°C in aqueous solution with the formation of hydrogen peroxide. In Figure 2 the yield

[1] W. BOLLAG and E. GRUNBERG, Exper. *19*, 130 (1963).
[2] A. RUTISHAUSER and W. BOLLAG, Exper. *19*, 131 (1963).
[3] I = Ro 4-6467/1.
[4] Viscosity measurements of DNA solutions as a test for the reaction of 'radiomimetic compounds' with the DNA have first been carried out by F. C. GJESSING and A. CHANUTIN, Cancer Res. *6*, 593 (1946). – The results are presented as 'specific viscosities'; for definition see H. STAUDINGER and W. HEUER, Ber. deutsch. chem. Ges. *63*, 222 (1930).
[5] Supplied by Fluka AG, Buchs (Switzerland).
[6] R. SIGNER and H. SCHWANDER, Helv. chim. Acta *32*, 853 (1948).
[7] Supplied by Boehringer, Mannheim (Germany).
[8] J. A. V. BUTLER and B. E. CONWAY, J. chem. Soc. *1950*, 3418. – J. A. V. BUTLER and K. A. SMITH, Nature *165*, 847 (1950). – H. MOROSON and P. ALEXANDER, Radiation Res. *14*, 29 (1961).
[9] The DNA used in our experiments contained approx. 0.01% iron.