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.

A. RUTISHAUSER and W. BOLLAG

Cytologisches Laboratorium, Institut für allgemeine Botanik, Universität Zürich (Switzerland), and Medizinische Forschungsabteilung, F. Hoffmann-La Roche & Co. AG., Basel (Switzerland), December 17, 1962.

[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.

of hydrogen peroxide from a 0.01 molar solution of I in 1/15 molar phosphate buffer of pH 7 is presented as a function of time. The titanous sulphate method[10] was used for the determination of hydrogen peroxide. After 140 h about 78% of the theoretical amount of hydrogen peroxide is formed. It is supposed that the reaction follows a similar scheme as the autoxidation of hydrazobenzene[11].

The same pattern of behaviour as I both with respect to the degradation of DNA and to the formation of hydrogen peroxide is observed with 1-methyl-2-benzyl-hydrazine phosphate and with 1-methyl-2-*p*-allophanoyl-benzyl-hydrazine hydrobromide (II[12]). All these compounds have in common that they autoxidize rather slowly. It may be assumed that *slow* release of hydrogen peroxide is an essential requirement for cytotoxic activity.

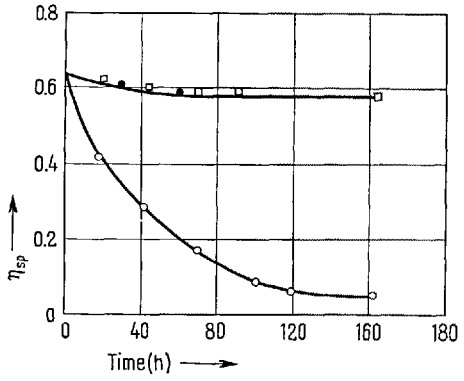

Fig. 1. Change of specific viscosity of a 0.0005 molar aqueous solution of I containing 0.07% w/v of DNA.
○ in air; ● in nitrogen; □ in air, solution contains catalase

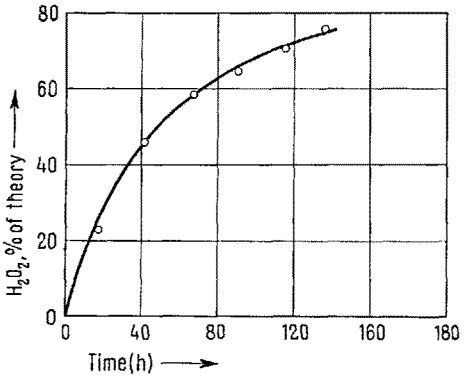

Fig. 2. Formation of hydrogen peroxide in a 0.01 molar aqueous solution of I in the presence of air.

From the experimental results it is concluded that the effect on the viscosity of aqueous DNA solutions of the above mentioned methylhydrazine derivatives is due to autoxidation of the latter compounds, leading to the formation of hydrogen peroxide. It is generally accepted that the action of hydrogen peroxide on DNA proceeds via OH radicals[8]. Therefore, an analogy of the effect of the methylhydrazine derivatives on DNA with the indirect effect of ionizing radiation is evident as the latter is assumed to be due mainly to the action of OH radicals[13]. The question remains open whether the inhibition of tumour growth depends on an effect on preformed DNA, on the synthesis of DNA, or on other biochemical effects of the hydrogen peroxide, like the inhibition of glycolysis[14].

Zusammenfassung. Methylhydrazinderivate wie 1-Methyl-2-*p*-(isopropylcarbamoyl) benzyl-hydrazin-hydrochlorid und 1-Methyl-2-*p*-allophanoylbenzyl-hydrazin-hydrobromid bewirken unter aeroben Bedingungen einen Viskositätsabfall der wässerigen Lösungen von Desoxyribonucleinsäure. Es wird gezeigt, dass dieser Effekt auf die Bildung von Wasserstoffperoxyd bei der Autoxydation der Methylhydrazinverbindungen zurückzuführen ist. Auf die Analogie zum indirekten Effekt von Röntgenstrahlen wird hingewiesen.

K. Berneis, M. Kofler, W. Bollag, A. Kaiser, and A. Langemann

Forschungsabteilung, F. Hoffmann-La Roche & Co. AG., Basel (Switzerland), December 17, 1962.

[10] A. C. Egerton et al., Anal. chim. Acta *10*, 422 (1954).
[11] W. Manchot and J. Herzog, Liebigs Ann. Chem. *316*, 331 (1901). – J. H. Walton and C. W. Filson, J. Amer. chem. Soc. *54*, 3228 (1932).
[12] II = Ro 4-6824.
[13] J. Weiss, Nature *153*, 748 (1944). – H. Engelhard, Second International Congress of Radiation Research (Harrogate 1962), Abstracts p. 67.
[14] O. H. Warburg, Z. Naturforsch. *13b*, 591 (1958).

Preliminary Studies on the Hemostatic Activity of the Isonicotinyl Hydrazone of Acetaldehyde

Introduction. The isonicotinyl hydrazone of acetaldehyde (IHA) was studied by us as a possible metabolite of isonicotinic acid hydrazide[1], arising from the decarboxilation of the isonicotinyl hydrazone of pyruvic acid[2]. Studies on the toxicity and bacteriostatic activity of the said hydrazones[3] led to the finding of a pronounced hemostatic activity of the acetaldehyde derivative, and these are the results we wish to describe.

Materials and Methods. IHA was prepared by dissolving isonicotinic acid hydrazide in commercial acetaldehyde, evaporating the excess aldehyde and precipitating the hydrazone by the addition of water. The precipitate was washed with water and with ethanol, being then dried under an infrared lamp. The purity of the product was controlled by paper chromatography[1,4] and its composition was confirmed by acid hydrolysis (HCl) and paper chromatography[1,4,5]. IHA is a yellowish powder, insoluble in

[1] R. C. R. Barreto, J. Chromat. *7*, 82 (1962).
[2] R. C. R. Barreto and D. B. Mano, Biochem. Pharmacol. *8*, 409 (1961).
[3] R. C. R. Barreto, Rev. Bras. Tub. *29*, 287 (1961).
[4] R. C. R. Barreto and S. O. Sabino, J. Chromat. *9*, 180 (1962).
[5] V. Sýkora and Z. Procházka, Chem. Listy *47*, 1674 (1953).