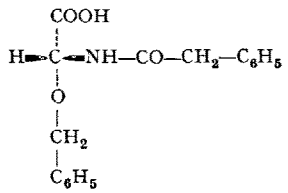
phenylacetyl-α-amino isobutyric acid, DL-α-benzyloxy-α-phenylacetamidopropionic acid[5], DL-α-methoxy-α-phenylacetamidopropionic acid methyl ester[5], chloroacetyl-α-amino isobutyric acid.

With the amino acid derivatives tested, it was possible to observe cleavage only in those belonging to the L-series, the acyl group being phenylacetic or chloroacetic acid and the carbon atom not being completely substituted.

For the above-mentioned compounds, which undergo enzymatic hydrolysis and have the asymmetric carbon atom containing the amide group, the configuration at asymmetric centre is known. We, however, also utilized α-benzyloxy-phenaceturic acid[5], whose configuration is not known. In our experiments with the DL-compound, one of the two forms was completely hydrolysed after 65 h, while the other was unchanged. This latter gave m.p. 106–108°, $[\alpha]_D = -2.05°$ (c = 3.8%)[6]. The configuration of the asymmetric carbon atom present in the isolated optically active compound is probably as follows:



Amino acid derivatives	Time in h	% of phenylacetic acid produced
Phenylacetyl-L-alanine	48	77
Phenylacetyl-L-valine	100	10
Phenylacetyl-L-leucine	48	50
Phenylacetyl-L-α-amino-*n*-butyric acid	48	66
Phenylacetyl-L-phenyl-alanine	48	48

Riassunto. L'azione delle acilasi presenti nelle cellule di *E. coli* A.T.C.C. 9637 è stata studiata utilizzando diversi substrati. Si è così trovato che alcuni cloroacetil e fenilacetil amino acidi subiscono un'idrolisi asimmetrica.

G. LUCENTE, A. ROMEO, and D. ROSSI

Istituto di Chimica Farmaceutica Applicata, Università di Roma (Italy), December 4, 1964.

[5] We shall report on the preparation of the α-alkoxy-α-phenylacetamido acids in a further note.

[6] Satisfactory elemental analyses were obtained for each new compound and optical rotation values were determined in ethanol solution.

Synergism Between Ionizing Radiation and a Cytotoxic Methylhydrazine Derivative: Effect on DNA-Degradation

The degradation of deoxyribonucleic acid (DNA) by methylhydrazine derivatives has recently been described[1]. It has been pointed out that the mechanism of action of these antitumour agents seems to show a similarity with the indirect effect of ionizing radiation[2], e.g. formation of strongly oxidizing and reducing free radicals. It therefore appeared desirable to investigate the effect of the combined action of the above mentioned compounds and of ionizing radiation on deoxyribonucleic acid.

The experiments were carried out with a 0.05% solution of sodium deoxyribonucleate prepared from calf thymus glands in 1/30 *M* phosphate buffer of pH 7, with the addition of 10% sodium chloride[3] as well as 0.002 moles/l sodium pyrophosphate to eliminate iron ions[4]. The cytotoxic compound[5] N-isopropyl-*p*-(2-methylhydrazinomethyl)benzamide hydrochloride (Natulan®) was added in solid form. The degradation of the DNA was checked by viscosity measurements with an Ostwald type viscometer (sheer stress about 200 to 500 sec^{-1}) and by the determination of sedimentation constants (extrapolation to concentration limes zero) with a Spinco analytical ultracentrifuge type E. Further experimental details have been described in previous papers[1]. For the irradiation experiments a cobalt-60 γ-ray source of 112 curies was used[6]. 10 ml of the solutions in cylindrical flasks were placed at a distance of 11.5 cm from the radiation source, the dose rate being 11,000 rad/h. The irradiation was carried out at room temperature. About 30 min after termination of the irradiation, the sampels were put in a thermostat and kept at 37°C.

Figure 1 presents the viscosity decrease of the DNA solution initiated by the following treatments: I = irradiation with 11,000 rad, II = addition of 0.0005 moles/l Natulan, III = irradiation with 11,000 rad followed immediately by addition of 0.0005 moles/l Natulan. According to the experimental results, the decrease in specific viscosity caused by the combined action of irradiation and of Natulan is substantially greater than that expected from a simple addition of both treatments. The maximum effect was obtained when Natulan was added immediately after termination of the irradiation. The effect decreased gradually as the interval between the termination of irradia-

[1] K. BERNEIS, M. KOFLER, W. BOLLAG, A. KAISER, and A. LANGEMANN, Exper. *19*, 132 (1963). – K. BERNEIS, M. KOFLER, W. BOLLAG, P. ZELLER, A. KAISER, and A. LANGEMANN, Helv. chim. Acta *46*, 2157 (1963).

[2] F. WEGMÜLLER, Dissertation Universität Bern (1942), p. 74. – J. WEISS, Nature *153*, 748 (1944). – R. A. COX, W. G. OVEREND, A. R. PEACOCKE, and S. WILSON, Proc. Royal Soc. *149*, 511 (1958).

[3] R. SIGNER and H. SCHWANDER, Helv. chim. Acta *32*, 853 (1948).

[4] W. C. SCHUMB, C. N. SATTERFIELD, and R. L. WENTWORTH, *Hydrogen Peroxide* (Reinhold Publishing Corp., New York 1954), p. 540.

[5] P. ZELLER, H. GUTMANN, B. HEGEDÜS, A. KAISER, A. LANGEMANN, and M. MÜLLER, Exper. *19*, 129 (1963). – W. BOLLAG and E. GRUNBERG, Exper. *19*, 130 (1963). – A. RUTISHAUSER and W. BOLLAG, Exper. *19*, 131 (1963).

[6] H. LÜTHY and H. MOHLER, Atompraxis, in press (1965).

tion and the addition of Natulan increased. The effect of Natulan decreased also when it was added *before* the irradiation. This was probably due to competitive radioprotective reactions of Natulan during irradiation resulting from its reducing power, e.g. 'repair' of DNA radicals.

In Figure 2 the correlation between specific viscosity of the DNA solution and the sedimentation constant of the DNA is given. These results were obtained with DNA degraded by different doses of Natulan with and without preceding irradiation. The results suggest that the same correlation exists between specific viscosity and sedimentation constant for both DNA degraded by Natulan alone and by a combination of Natulan and irradiation.

In Figure 3 the specific viscosities of Figure 1 were converted to 'average molar concentrations' of DNA. For this calculation the relationship given in Figure 2 between specific viscosity and sedimentation constants was used. The latter were transformed into average molecular weights using the relationship of Doty et al.[7]. The increase in 'average molar concentration' is a measure of the number of double breaks that have occurred in the DNA molecule[8]. Figure 3 thus represents the increase in 'average molar concentration' of DNA with time after the following treatments: I = irradiation with 11,000 rad, II = addition of 0.0005 moles/l Natulan, III = irradiation with 11,000 rad *and* addition of 0.0005 moles/l Natulan immediately after termination of the irradiation. According to these results the combined action of ionizing radiation and of Natulan causes more double breaks in the DNA molecule than would be expected from a simple summation of the effects of radiation and of the cytotoxic agent.

A remarkable feature is the synergistic effect of ionizing radiation and of the cytotoxic methylhydrazine compound on DNA fragmentation and the fact that the maximum effect was observed only when Natulan was added immediately after the irradiation. No such effects were obtained with a nitrogen mustard [methyl-bis(β-chlorethyl)amine hydrochloride]. Added hydrogen peroxide had a much smaller enhancing effect on the degradation than the equimolar amount of Natulan. Interestingly enough, the effect of hydrogen peroxide was greater when it was already present during irradiation than when added afterwards.

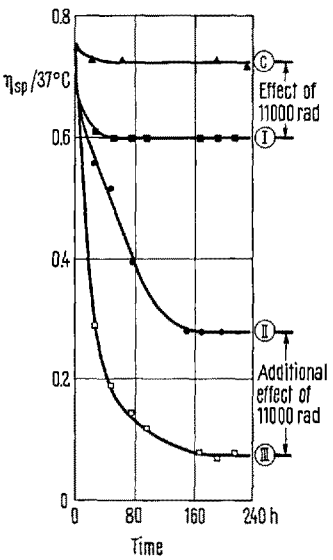


Fig. 1. Change in the specific viscosity of a 0.05% solution of DNA after the following treatments: (C) Control (without treatment). (I) Irradiation with 11,000 rad. (II) Addition of 0.0005 moles/l N-isopropyl-*p*-(2-methylhydrazinomethyl)benzamide hydrochloride. (III) Irradiation with 11,000 rad *and* addition of 0.0005 moles/l N-isopropyl-*p*-(2-methylhydrazinomethyl)benzamide hydrochloride *immediately after* termination of irradiation.

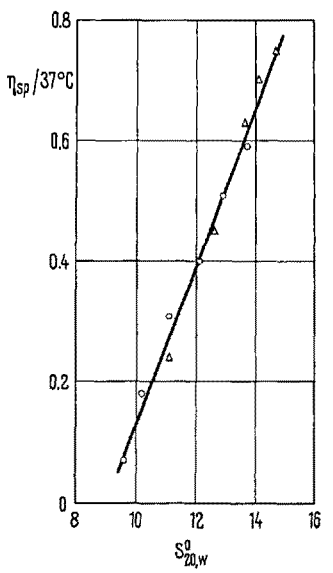


Fig. 2. Correlation between specific viscosity and sedimentation constant of DNA degraded with different doses of N-isopropyl-*p*-(2-methylhydrazinomethyl)benzamide hydrochloride with and without preceding irradiation. Circles: Degradation by 11,000 rad and N-isopropyl-*p*-(2-methylhydrazinomethyl)benzamide hydrochloride. Triangles: Degradation by N-isopropyl-*p*-(2-methylhydrazinomethyl)-benzamide hydrochloride alone.

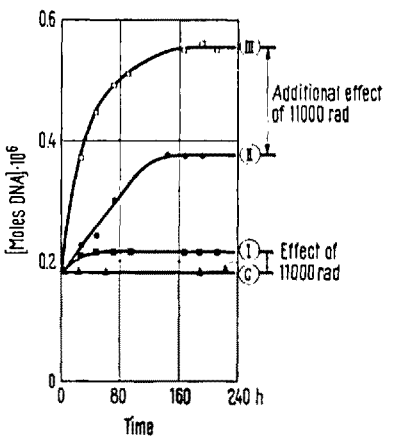


Fig. 3. Effect of ionizing radiation and of a cytotoxic methylhydrazine derivative and of their combination on the 'average molar concentration' of DNA. (C) Control. (I) 11,000 rad. (II) 0.0005 moles/l N-isopropyl-*p*-(2-methylhydrazinomethyl)benzamide hydrochloride. (III) 11,000 rad *and* 0.0005 moles/l N-isopropyl-*p*-(2-methylhydrazinomethyl)benzamide hydrochloride added *immediately after* termination of irradiation.

[7] P. Doty, B. Bunce McGill, and S. A. Rice, Proc. Nat. Acad. Sci. *44*, 432 (1958).

[8] A. R. Peacocke and B. N. Preston, J. Polymer Sci. *31*, 1 (1958).

According to the results, ionizing radiation seems to have a sensitizing effect on DNA subjected to the action of Natulan. This may be explained by the formation of unstable peroxides during irradiation[9], which yield free radicals as a result of their decomposition[10]. SCHOLES et al.[11] have demonstrated that the organic peroxides formed by X-irradiation of DNA in the presence of oxygen show a marked post-irradiation decay which mainly takes place within a few hours after termination of the irradiation. The free radicals formed during this decay may act as initiators[12] of the autoxidation of cytotoxic methylhydrazine compounds[1]. The higher rate of autoxidation will result in an increase of both the formation[1] and of the activation[13] of hydrogen peroxide and thus enhance the degradation of DNA.

Zusammenfassung. Die kombinierte Einwirkung von ionisierender Strahlung und N-Isopropyl-*p*-(2-methylhydrazinomethyl)benzamid hydrochlorid (Natulan®) führt zu einem wesentlich stärkeren Abbau von Desoxyribonucleinsäure (DNS) als auf Grund der linearen Superposition zu erwarten wäre. Der Synergismus ist am ausgeprägtesten, wenn der Zusatz von Natulan unmittelbar nach der Bestrahlung erfolgt. Dieser Effekt kann als Folge der Bildung instabiler Peroxide während der Bestrahlung erklärt werden. Bekanntlich zerfallen die bei der Bestrahlung entstandenen organischen Peroxide zum grössten Teil innerhalb der ersten zwei bis drei Stunden nach der Bestrahlung unter Bildung freier Radikale. Diese können als Startradikale bei der Autoxydation von Natulan wirken und daher den Abbau von DNS beschleunigen.

K. BERNEIS, W. BOLLAG,
M. KOFLER, and H. LÜTHY

Forschungsabteilung der F. Hoffmann-La Roche & Cie. AG, Basel, and Laboratorium für Medizinische Strahlenphysik, Bürgerspital, Basel (Switzerland), March 17, 1965.

[9] J. WEISS, in *Organic Peroxides in Radiobiology* (Eds., R. LATARJET and M. HAISSINSKY; Pergamon Press, London 1958), p. 42.
[10] J. A. V. BUTLER, in *Organic Peroxides in Radiobiology* (Eds., R. LATARJET and M. HAISSINSKY; Pergamon Press, London 1958), p. 36. – B. E. CONWAY and J. A. V. BUTLER, J. chem. Soc. *1952*, 834.
[11] G. SCHOLES, J. WEISS, and C. M. WHEELER, Nature *178*, 157 (1956).
[12] J. L. BOLLAND, Quart. Rev. London *3*, 6 (1949).
[13] K. BERNEIS, M. KOFLER, and W. BOLLAG, Helv. chim. Acta *47*, 1903 (1964). – K. BERNEIS, M. KOFLER, and W. BOLLAG, Exper. *20*, 73 (1964).

Determination of the Dry Mass of Nervous and Glial Cell Nuclei by Interference Microscopy[1]

BRATTGARD and HYDEN[2,3] and HYDEN[4] measured the dry mass concentration of nerve cells by means of X-ray microscopy and calculated indirectly the total dry mass of the same cells on the basis of the area.

This method is laborious and does not seem well suited for the determination of the dry mass of the nuclei. Interference microscopy seems to show marked advantages for this purpose, and in this paper the values obtained in the measurements of the dry mass of nervous and glial cell nuclei of albino rats are described.

Samples were taken from white matter (corona radiata, lumbar enlargement of the spinal cord) and from grey matter (lumbar enlargement of the spinal cord).

The tissue (5 mg) was dispersed in anhydrous glycerol (Merk) after previous freeze-drying, or directly in BARNES, ESNOUF, and STOCKEN fluid[5]. For preparation of the dry nuclei, the tissue was dropped immediately after dissection into semifrozen Freon 12 and rapidly transferred to the histological freeze-drying apparatus (TD2, Edwards Crowley). After completion of the drying, the tissue was dispersed in anhydrous glycerol by Potter-Elvejhem apparatus.

In the second case, the nuclei were dispersed directly in BARNES, ESNOUF, and STOCKEN[5] solution and the nuclear fraction was separated from the homogenate by centrifugation in a refrigerated centrifuge (Eispirouette, Phywe), which was rapidly accelerated to 3000 *g* and then left to decelerate until it stopped.

The nuclei prepared according to this method undergo a notable loss of the soluble proteins and their dry mass values correspond substantially to the content of insoluble proteins, whereas the nuclei prepared in anhydrous glycerol possess their full complement of proteins. The difference in the values obtained with the two methods gives an indication of the nuclear content of soluble proteins[6].

Nuclei were measured in a chamber about 40 μ thick (obtained by interposing thin mica foils between slide and coverslip) in order to maintain their spherical shape, which is altered by compression when they are placed directly between slide and coverslip.

Dry mass measurements were made with a Smith-Baker interference microscope using a × 100 'shearing' objective and 'half-shade' eyepiece. The formula used for the calculation of the total dry mass was:

$$M = KQA/100\alpha,$$

where M is the total dry mass, K is a constant (calculated by PELLEGRINO et al.[7]), Q is the optical path difference,

[1] This investigation was supported by a grant to this Department from the Consiglio Nazionale delle Ricerche (No. 04/76/4/3482).
[2] S. O. BRATTGARD and H. HYDEN, Acta radiol., Suppl. 94 (1952).
[3] S. O. BRATTGARD and H. HYDEN, Int. Rev. Cytol. *3*, 455 (1954).
[4] H. HYDEN, in *The Cell* (Ed. G. BRACHET; Academic Press Inc., New York 1960), vol. 4, p. 215.
[5] D. W. H. BARNES, M. P. ESNOUF, and L. A. STOCKEN, in *Advances in Radiobiology* (Oliver and Boyd, Edinburgh and London 1957), p. 211.
[6] C. PELLEGRINO, R. TONGIANI, and M. P. VIOLA, Exp. Cell Res. *53*, 419 (1964).
[7] C. PELLEGRINO, P. D. RICCI, and R. TONGIANI, Exp. Cell Res. *31*, 167 (1963).