their analogues XVI–XXIII) are completely devoid of antimicrobial activity. Further increase in ring size causes a fall in biological activity of the depsipeptide molecule. Thus, the cyclooctadepsipeptide (XII) built up similarly to enniatin B (I), has a narrower antimicrobial spectrum than the latter. The activity is completely lost when the *L*-N-methylvaline residues of this compound are replaced by *L*-valine residues (compound XIV) or when their configuration is changed. One of the closest cyclopolymer homologues of compound (XII), namely cyclododecadepsipeptide (XV) is also without antimicrobial activity.

It is noteworthy that all linear depsipeptides corresponding to the cyclodepsipeptides (I)–(XXIII) are entirely inactive.

We have thus found that of the depsipeptide cyclopolymer homologues we have studied, the highest activity is manifested by cyclohexadepsipeptides with regularly alternating hydroxy and amino acid residues. This is apparently due to the fact that such cyclohexadepsipeptides possess the most appropriate conformation, sterically complementary to the active centre of the corresponding enzymes.

Zusammenfassung. Die Beziehungen zwischen Struktur und antimikrobieller Aktivität in der Reihe der Depsipeptide wurden untersucht. Cyclotetradepsipeptide sind praktisch inaktiv. Die grösste Aktivität besitzen die Cyclohexadepsipeptide mit regulärer Oxy- und Aminosäurensequenz. Eine weitere Vergrösserung des Depsipeptidringes führt zu einem Abfall der biologischen Aktivität. Cyclohexadepsipeptide mit irregulärer Oxy- und Aminosäurensequenz sind völlig inaktiv.

M. M. SHEMYAKIN, YU. A. OVCHINNIKOV,
V. T. IVANOV, A. A. KIRYUSHKIN,
G. L. ZHDANOV, and I. D. RYABOVA

Institute for Chemistry of Natural Products, USSR Academy of Sciences, Moscow (USSR),
July 22, 1963.

Influence of Diglycyl-glycine on the Radiation Sensitivity of Catalase

Recently we have shown[1] that glycerol added to catalase solution prior to irradiation provides excellent protection to the enzyme. A maximum protective effect was obtained with very small amounts of glycerol (0.0004 vol %). The protective factor was about 13 as compared with controls containing no glycerol, and at higher glycerol concentrations the protective effect was found to decrease slightly. In order to explain this protective effect, we proposed a chelating mechanism by which glycerol forms a complex with the metal-ions present in the catalase molecule. These complexed metal-ions consequently inhibit the decomposition of radiation produced H_2O_2 into reactive radicals. Metal-ions, such as Cu^{2+} or Fe^{3+}, catalyse this destruction as is well known[2].

There is also additional radiation damage produced by radicals which originate from the decomposition of radiation produced H_2O_2 in solution. Because of their short lifetime, they will, preferably, undergo competitive reactions such as radical combinations and reactions with H_2O_2 rather than reactions with the enzyme molecules. This depends, however, on the degree of dilution[3].

If this mechanism is correct, a modification of the radiation response should be expected when other organic substances, acting as a radical scavenger, are added to the catalase solution.

In our investigations we used diglycyl-glycine as a radical scavenging agent. Diglycyl-glycine (Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved in the enzyme solution before irradiation. Concentrations which were used are mentioned in Figure 1 and are expressed in mg/ml. Catalase solution ($8 \times 10^{-8} M$) was prepared by dissolving 2 mg of lyophilized beef liver catalase (Worthington Biochemical Corp., Freehold, New Jersey) in 100 ml of a 0.05 *M* phosphate buffer, pH 7.0. Hydrogen peroxide ($5 \times 10^{-3} M$) was prepared by diluting 0.15 ml of a 30% H_2O_2 solution (Superoxol, Merck & Co., Rahway, New Jersey) with 25 ml of 0.05 *M* phosphate buffer, pH 7.0.

The catalase activity was determined spectrophotometrically using the decrease in optical density (of H_2O_2) at 240 mμ as function of time after mixing enzyme and substrate (method of BEERS and SIZER[4]). A Cary 14 Spectrophotometer (Applied Physics Corp., Monrovia, California) was used for the determination. 1 ml of H_2O_2 was rapidly injected into 2 ml of catalase solution. All measurements and irradiations were done at room temperature. 2 ml samples of catalase solution were irradiated in Lucite containers with different doses (0 to 2.7×10^6 *r*). The irradiations were done with a beryllium-window X-ray tube (100 kV, 12 mA, HVL 0.064 mm Al; Philips Electronics Inc., Mount Vernon, New York). The dose rate was about 9×10^4 *r*/min. The X-ray tube was calibrated with an air-wall ionization chamber.

The modification of the radiation effect on catalase by diglycyl-glycine is shown in Figure 1. As can be seen, diglycyl-glycine protects catalase very well. The 50%-

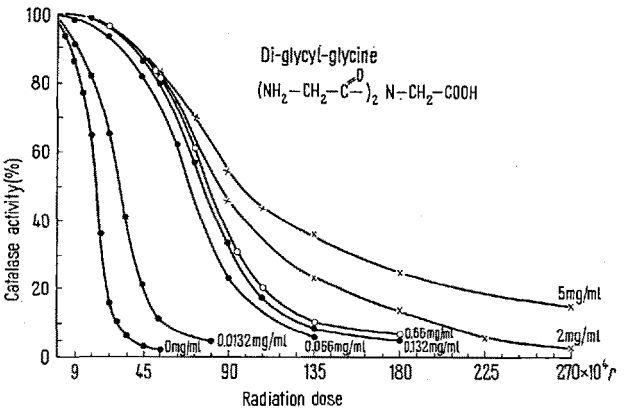

Fig. 1. The influence of different concentrations of diglycyl-glycine (mg/ml) on the radiation sensitivity of catalase.

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values plotted in Figure 2 (values from Figure 1 were used) show that the protection is about 5 times greater for samples containing 5 mg/ml of the peptide. The increase in protection is, however, not so great as in the case of glycerol[1]. Moreover, no maximum in protection can be observed; the 50%-value curve was observed to continue increasing at the highest concentration (5 mg/ml) used. From this result it can be concluded that the diglycyl-glycine effect can be explained by a radical scavenging mechanism which, in turn, modifies the radiation effect. For comparison, the glycerol values, published previously[1], were included in Figure 2. Glycerol, as a chelating substance, protects, at the same concentration, much more than diglycyl-glycine, a radical scavenger.

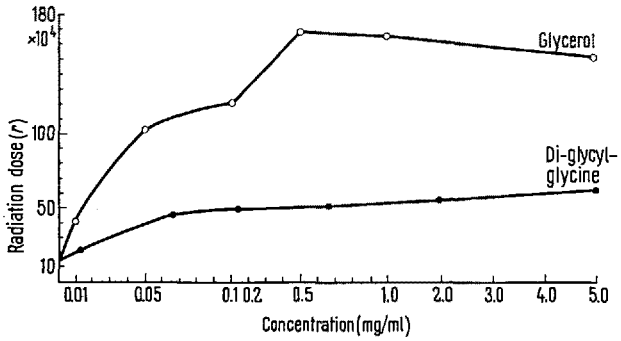

Fig. 2. 50%-dose values as function of the diglycyl-glycine concentration (values from Figure 1; note the interruption in the concentration scale). Glycerol values for comparison.

From this it can be concluded that the greatest radiation protective effect will be obtained if the metal-ions in the biological system are complexed by some chelating agents. Thus, the number of highly reactive radicals being derived from the decomposition of hydrogen peroxide is reduced.

Zusammenfassung. Die Veränderung der Strahlenempfindlichkeit von Katalase durch Diglycyl-glycin wurde spektralphotometrisch untersucht. Die Bestrahlungsdosen variierten zwischen $0-2{,}7 \times 10^6$ *r*. Der Schutzeffekt lässt sich durch einen Radikal-abfangenden Mechanismus erklären. Strahlenbiologische Folgerungen werden diskutiert.

W. Lohmann, A. J. Moss jr.,
W. H. Perkins, and C. F. Fowler

Departments of Physiology and Biophysics, Medicine, and Radiology, University of Arkansas Medical Center; and Radioisotope Service, VA Hospital, Little Rock (Arkansas, U.S.A.), July 22, 1963.

Histochemistry of 3β-Hydroxysteroid Dehydrogenase in Monkey Adrenal Cortex

Enzyme histochemical studies on adrenal cortex of man and mammals have focused the attention on a particular enzyme activity, the 3β-hydroxysteroid dehydrogenase, which converts C_{19} and C_{21} steroids with Δ^5-3β-hydroxyl structure to those with the Δ^4-3 ketone grouping characteristic of most of the active steroid hormones[1-3]. Thus, this enzyme activity appears to be indispensable for the biosynthesis of all the cortical hormones[2,5]. In previous histochemical researches the behaviour of this enzymatic system in the adrenal cortex of mammals and man, both under normal and pathological conditions, has been investigated[6-9]; the present paper deals with the 3β-hydroxysteroid dehydrogenase of the adrenal cortex of Rhesus monkey whose activity, distribution and reactivity apparently show a behaviour very similar to the ones of the human adrenal cortex.

Twelve adult male monkeys (*Macacus rhesus*) weighing 1.8–2.1 kg were used. Three untreated animals were taken as controls, the remaining divided into groups of three animals and treated with the following hormones at the indicated daily doses: aldosterone acetate 100 μg/kg for 15 days, cortisol acetate 20 mg/kg for 15 days and ACTH 50 I.U./kg for 3 days. One animal of each group was injected subcutaneously with colchicine (1.5 mg/kg) 9 h before sacrifice.

After sacrifice the glands were removed, weighed and rapidly chilled with dry ice to −7° C. 5 μ cryostat sections were used for the demonstration of the 3β-hydroxysteroid dehydrogenase according to the method of Wattenberg (1958) and adjacent sections were fixed and stained with the usual methods. In stained sections corresponding to the major axis of the gland the width of the cortex was measured with a graduated eyepiece; the values reported in the Table are the means of ten measurements. In colchicine-treated animals the mitoses count was carried out on ten 5 μ sections corresponding to the major axis of the gland and stained with hematoxylin and eosin.

The mean adrenal weights and the mean values of the width of the adrenal cortex are reported in the Table.

From the Table it appears that aldosterone treatment did not change consistently both the gland weight and the

Groups of animals	Weight of glands mg/100 g body weight	Cortical width mm
Control	34.3	0.960
Aldosterone	32.5	0.890
Cortisol	19	0.525
ACTH	68	2.115

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