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.

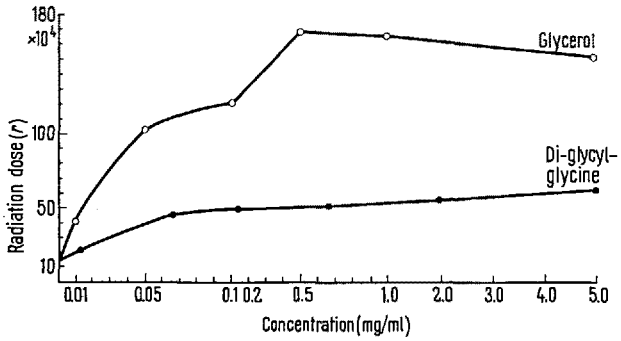

Fig. 2. 50%-dose values as function of the diglycyl-glycine concentration (values from Figure 1; note the interruption in the concentration scale). Glycerol values for comparison.

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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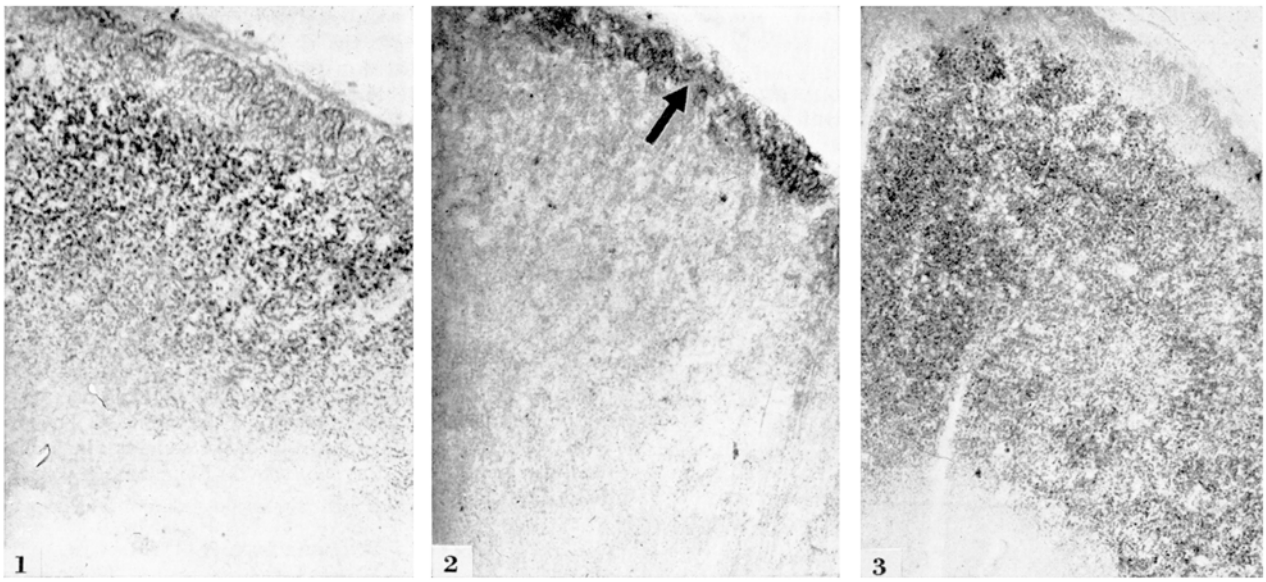

Fig. 1. 3β-hydroxysteroid dehydrogenase activity in adrenal cortex of normal Rhesus monkey (× 85.) Fig. 2. 3β-hydroxysteroid dehydrogenase activity in adrenal cortex of Rhesus monkey after cortisol treatment. Arrow indicates the outer border of the fascicular zone (× 85). Fig. 3. 3β-hydroxysteroid dehydrogenase activity in adrenal cortex of Rhesus monkey after ACTH stimulation (× 85).

cortical width, whereas cortisol and ACTH treatment led to marked changes of both.

Histologically, the adrenal cortex of the normal Rhesus monkey was found to be similar to the one of man, except the zona glomerulosa which was much wider than in man. The three zones were easily discernible and the fascicular zone showed a clear-cut separation into an outer clear-celled and an inner dark-celled layer. Histochemically, a strong 3β-hydroxysteroid dehydrogenase activity in the clear cells of the fascicular zone and a weak one in some cell groups of the glomerularis was found (Figure 1).

Surprisingly, aldosterone treatment failed to alter both histologically and histochemically the glomerular zone; actually, the normally present weak 3β-hydroxysteroid dehydrogenase activity was not clearly reduced.

Cortisol treatment led to a reduction of the cortical width mainly of the fascicular zone: a partial lipid depletion and atrophic changes of the clear cells of the outer fasciculata were observed. The dark cells were not increased in number. The activity of 3β-hydroxysteroid dehydrogenase was sharply reduced in the whole width of the fascicular zone and, unexpectedly, the enzyme activity was slightly increased in the zona glomerularis over the normal level (Figure 2), probably as a consequence of the global reduction in size of the gland.

After ACTH treatment the whole cortex – mainly the zona fasciculata – was increased in width; in the fascicular zone, dark cells appeared to be more numerous than in control cases. The 3β-hydroxysteroid dehydrogenase was found to spread to the whole of the zona fasciculata, leaving the glomerularis practically unaffected (Figure 3). In the fascicular zone the enzyme was found to be active not only in clear-cells, as normal, but also in dark, compact cells.

Colchicine administration caused the appearance of a high number of blocked mitoses in ACTH-stimulated glands, mainly in clear cells of the outer fascicular zone, while in adrenals of control animals and in those of animals treated with aldosterone and cortisol, the mitotic counts were extremely low or zero.

In the ACTH-treated monkeys 28 mitoses were counted in ten sections of the cortex, 3 in the untreated, 0 in aldosterone and 1 in cortisol-treated animals.

From the results outlined, the following conclusions may be drawn:

(1) Weight and histological changes after ACTH and cortisol treatment have been shown in the adrenal cortex of the Rhesus monkey, which are very similar to those observed in the cortex of other mammals and particularly of man.

(2) Histochemically, changes of the degree of activity and distribution of 3β-hydroxysteroid dehydrogenase of the cortex can be shown following hormonal treatment; while aldosterone does not affect either the histology or the histochemistry of the glomerular zone, cortisol decreases the width of the zona fasciculata as well as the degree of its enzyme activity, and ACTH leads to an increased width of zona fasciculata together with the appearance of higher activity of 3β-hydroxysteroid dehydrogenase both in clear and dark cells and of high numbers of colchicine mitoses in the outer fascicular zone.

(3) Owing to its similarity to the human gland, the adrenal cortex of the Rhesus monkey might be very valuable animal material for experimental studies[10].

Zusammenfassung. Es wird über mit Hydrocortisonacetat, ACTH und Aldosteronacetat induzierte morphologische und histochemische Veränderungen in der Nebennierenrinde des *M. rhesus* berichtet. Die bedeutende Verminderung der Steroid-3β-ol-dehydrogenasischen Tätigkeit nach Cortisol und deren Zunahme nach ACTH wird hervorgehoben.

C. CAVALLERO and G. CHIAPPINO

Istituto di Anatomia e Istologia Patologica della Università di Pavia (Italy), June 7, 1963.

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