THE EFFECT OF CORTISONE ON RENAL SUCCINODEHYDRASE ACTIVITY IN EXPERIMENTAL CYTOTOXIC NEPHRITIS

L. R. Polyantseva and A. A. Rogov

Department of General and Hospital Therapy of the Faculty of Hygiene (Head, Active Member AMN SSSR E. M. Tareev), and S. I. Chechulin Central Scientific Research Laboratory (Head, Candidate of Medical Sciences A. S. Chechulin) I. M. Sechenov I Moscow Medical Order of Lenin Institute (Presented by Active Member AMN SSSR I. V. Davydovskii)

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Modern histochemical methods of studying the activity of oxidizing enzymes concerned in the Krebs oxidation cycle of tricarbonic acid enable a sufficiently accurate determination to be made of the most functionally active structures. Normally, the activity of these enzymes also depends on the functional activity of the particular portion of the nephron concerned. Numerous histochemical investigations [7, 8, 12, 13, 15, 17, 18] have shown that the most active portions are the proximal sections of the convoluted and collecting tubules, i.e., those parts of the nephron concerned with active reabsorption. In other regions, enzymatic activity is much lower, and there is none in the glomeruli. This result is evidently due to the fact that the glomeruli are concerned only with passive filtration, which does not require any considerable energy expenditure.

In various pathological processes (for instance, disturbances of the circulation [14, 20]), radiation sickness [16], mercury poisoning [11, 19], and also in nephritis associated with nephrotic changes, succinodehydrase activity in the tubules is reduced [9].

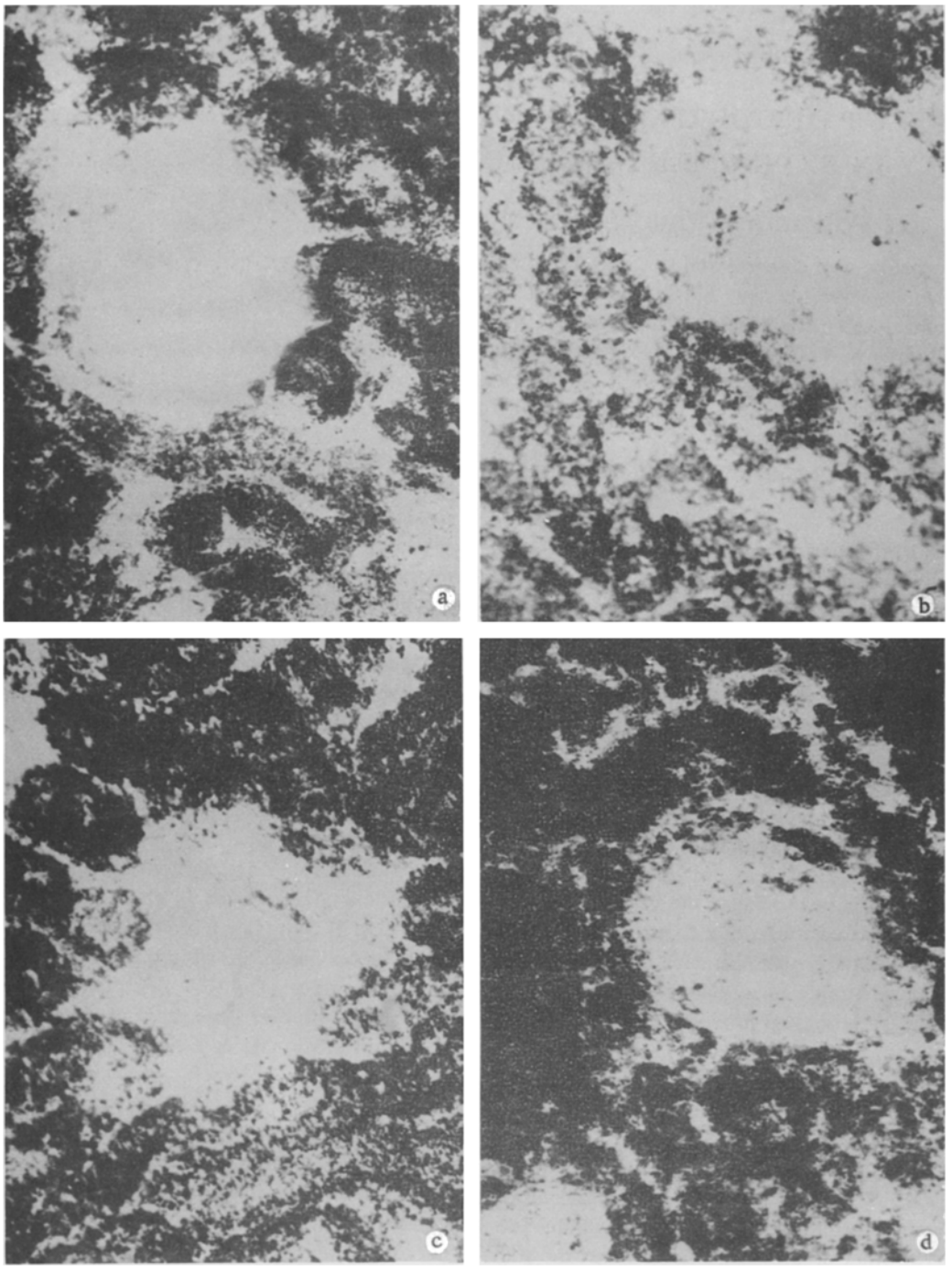
It has frequently been shown that cortisone and various other steroid hormones increase succinodehydrase activity in the epithelium of the convoluted tubules [5], and in several other organs. On this account, the aim of the present investigation has been to find whether cortisone increases the activity of the dehydrase of succinic acid in rats in which cytotoxic nephritis has been experimentally induced.

METHOD AND RESULTS

The experiments were carried out on 50 white male rats which initially weighed 100-200 g; they were divided into three experimental groups of 12 animals each, and one control group of 14 animals. The first group received highly active cytotoxic serum subcutaneously and intravenously, and developed nephrotic nephritis. There was considerable edema of the subcutaneous tissue, in many cases hydrops developed, there was up to 3% protein in the urine and a deposition of hyaline cylinders and erythrocytes, and a marked increase in blood urea. The animals were killed by decapitation at various times from seven days to $1\frac{1}{2}$ months after developing nephritis. The kidneys were found to be engorged with blood and edematous, there were marked proliferative destructive changes in the glomeruli, and erythrocytes were present in Bowman's capsule. The epithelium of the convoluted tubules showed granular and fatty dystrophy, while in the lumen of the canals numerous hyaline bodies were frequently found.

After receiving the cytotoxic serum injections, the animals of the second group were then given a daily injection of 5 mg/100g of cortisone acetate injected subcutaneously. In this group, there was no edema, and less proteinuria (up to 0.6%). The rats were killed at the same time as were those of the first group. Histological examination of the kidneys showed that there were some nephritic changes, which included swelling of the glomeruli, small diapeditic hemorrhages into the cavity of Bowman's capsule, and a proliferation of the glomerular endothelium. Perivascular edema and round cell infiltration into the connective tissue were much less extensive. The epithelial cells of the convoluted tubules appeared swollen, and contained eosinophilic granules in the protoplasm. The lumens of the tubules were sometimes somewhat expanded, but never contained any hyaline bodies.

The third group received a prophylactic dose of 500 mg/100 g per 24 hours in addition to the cytotoxic serum. In these animals the nephritis was not accompanied by edema, there was only 0.3-0.2% of protein in the urine, and the amount of residual nitrogen in the blood was increased to 70 mg%. In these animals, the condition resembled a moderately severe destructive proliferative glomerulonephritis in which the loops of Henle were swollen. The epithelium of the tubules was somewhat flattened and there was a marked granularity of the protoplasm.



Distribution of succindehydrase activity in the rat adrenal cortex as revealed by the neotetrazol reaction using the method of Shelton and Schneider. a) Distribution of succinodehydrase in the normal rat kidney; b) two weeks after injecting cytotoxic serum there is a considerable reduction in the activity of the enzyme in the epithelium of the tubules, and an activation in the glomeruli; c) repeated injections of cortisone beginning on the second day after injecting the cytotoxic serum prevents the reduction of activity of the enzyme; d) when cortisone is injected repeatedly, starting on the day before the injection of the cytotoxic serum, succinodehydrase activity in the cortex of the kidney is greater than in the control animals. Magnification 10 × 20.

In the fourth, or control group, eight rats were untreated and remained healthy, while six received a daily injection of cortisone acetate.

For histochemical investigation of the activity of succinodehydrase in the kidneys, sections 20μ thick were cut from freshly frozen portions, and Shelton and Schneider's neotetrazol reaction applied. The sections were incubated at 37° for 15 minutes in a medium consisting of equal volumes of distilled water, a 0.1 M phosphate buffer (pH 7.5), a 0.1% solution of neotetrazol hydrochloride, and a 0.1 M solution of sodium succinate. As a result of the reaction, the water soluble neotetrazol was reduced to mono- and diformazan, which were deposited as insoluble red and blue granules respectively at the site of the enzymatic activity.* The results of the experiments were as follows.

Rats with nephrotic nephritis differed from normal animals in showing activation of the enzyme in the loops of some of the glomeruli, a result which may be associated with an active proliferative reaction; at the same time, there was a considerable depression in the succinodehydrase activity in the epithelium of the convoluted tubules. This latter effect was particularly well shown two weeks after the onset of nephritis. Because of the very small amount of formazan deposited, the outlines of the tubules became very indistinct. In this group there was a very clear relationship between the inactivation of succinodehydrase and the severity of the nephritis.

In healthy animals receiving cortisone, there was an increased activity of the enzyme in the epithelium of the convoluted tubules.

In the group receiving cortisone after the injection of cytotoxic serum, the reaction of activating the succinodehydrase in the glomeruli was preserved, but by contrast with the first group, the reaction in the epithelium of the convoluted tubules was increased.

Finally, in the animals receiving cytotoxic serum after a prophylactic dose of hormone, the activity of the enzyme was increased (to a level greatly above normal) in the epithelium of the convoluted tubules and in the collecting tubules. Cortisone had no effect on the activity of succinodehydrase in the glomeruli. Its activity was the same as in untreated nephritis (see figure a, b, c, and d).

Our results agree completely with those of other authors who found an activation of oxidative enzymes in the tubules after treatment with the glucocorticoid type of cortisone [5]. Because, in our experiments, there was at the same time a considerable reduction in the proteinuria, it is probable that cortisone causes an increased reabsorption of protein by the epithelium of the tubules.

It is important to note the increased succinodehydrase activity in the glomeruli when there is inflammation. Under normal conditions, in all the collective tissue elements (ground substance, fibrous structures and cells) the activity of the oxidized enzymes is very low [8, 12, 17].

The increased activity in the connective tissue cells during inflammation may possibly be due to one of three causes:

1) An increased metabolic rate in the connective tissue cells;

2) Increased permeability of the cell membranes which are normally inaccessible to the relatively high-molecular-weight tetrazol salts;

3) The effect of a changed metabolism in inflammation may be to cause a binding of the oxidative enzymes, which may be present in large amounts in the connective tissue cells.

SUMMARY

A study was made of succinodehydrase activity in the kidneys of rats suffering from experimental cytotoxic nephritis and treated with cortisone. In untreated nephritis, the activity of the enzyme in the epithelium of the convoluted tubules decreased considerably, and was intensified in some of the glomeruli.

Cortisone administration before or two days after the injection of the cytotoxic serum caused activation of succinodehydrase in the tubular epithelium without having any effect upon its activity in the glomeruli.

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