12. D. Kranz, A. Hecht, and J. Furhmann, Exp. Pathol., 5, 38 (1971).
13. D. Kranz, A. Hecht, and J. Furhmann, Exp. Pathol., 11, 107 (1975).
14. P. P. Rumyantsev and A. M. Kassem, Virchows Arch. B, 20, 329 (1976).
15. S. Sasaki, T. Morishita, and S. Yamagata, Tohoku J. Exp. Med., 101, 147 (1970).

HISTOCHEMICAL AND MORPHOMETRIC CHANGES IN THE ADRENAL CORTEX DURING ACUTE VASCULAR INSUFFICIENCY

N. M. Kochubei

UDC 612-008.46-036.11-07:616.453-091-07

Artificial hypotension for 5 h produced in cats by intravenous injection of Arfonad is accompanied by depression of adrenal function. Insufficiency of the adrenals, especially of their glucocorticoid function, in established acute vascular insufficiency can be interpreted as adaptation aimed specifically at maintaining homeostasis.

KEY WORDS: adrenals; artificial hypotension; tissue enzyme profile.

The regulation of vascular tone is largely predetermined by the function of the adrenal glands [2, 4]. Hence, there is a need for studying the morphological and functional state of the adrenal cortex in acute vascular insufficiency accompanied by hypoxia during the first few hours [1, 6].

EXPERIMENTAL METHOD

Experiments were carried out on male cats weighing 3.5-4 kg. For morphological study the adrenals were removed immediately and again 24 h after the end of a 5-h period of artificial hypotension produced by injection of a 0.1% solution of Arfonad. For comparison the adrenals of intact and anesthetized animals were studied. The dimensions of the cells in all zones of the cortex were determined by drawing and weighing.

The material was fixed in 10% neutral formalin and paraffin sections were stained with hematoxylin–eosin and with gallocyanin and chrome alum by Einarson's method. The content of lipids and activity of various enzymes (glucose-6-phosphate dehydrogenase, G6PD; β-hydroxybutyrate dehydrogenase, β-HBD; succinate dehydrogenase, SD; lactate dehydrogenase, LD; NADH and NADPH dehydrogenases, NADH-D and NADPH-D; 3β-hydroxysteroid dehydrogenase, 3β-HSD) were determined in sections from unfixed tissue. The RNA content and activity of the enzymes were estimated cytophotometrically. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

Unlike in previous investigations of the adrenals of intact cats [7-9], their histochemical profile was studied. Predominance of direct oxidation of glucose in the hexose monophosphate shunt was found, as shown by the relatively high activity of G6PD, which participates in the initial stages of oxidation by this pathway (Fig. 1a).

Another no less important pathway for carbohydrate oxidation is anaerobic glycolysis, the importance of which in the adrenal cortex of cats can be assessed by the relatively high LD activity (Fig. 1c). The importance of other pathways of carbohydrate oxidation, especially the Krebs cycle, for the cat adrenals is evidently

Department of Pathological Anatomy, S. M. Kirov Leningrad Postgraduate Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 615-616, May, 1977. Original article submitted May 10, 1976.

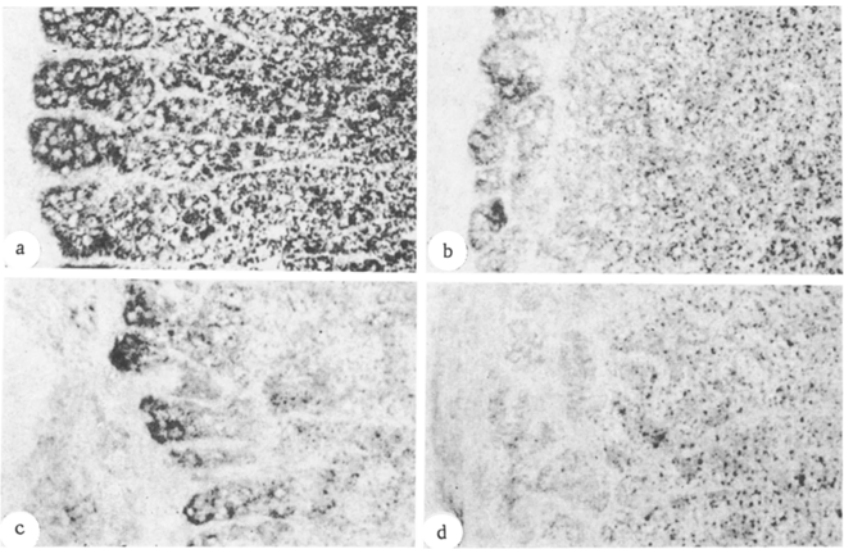

Fig. 1. G6PD and LD activity in the adrenal cortex: a, c) intact animals; b, d) after artificial hypotension for 5 h. Nitro-BT reaction, 112.5×.

small: SD activity did not reach a high level. The β-HBD activity in the cat adrenals was much lower than the activity of the other enzymes studied.

The NADH-D and NADPH-D activities, reflecting the intensity of terminal oxidation processes, were moderately high on the whole and differed when the activity of the enzymes was compared in the various zones of the adrenal cortex.

Activity of 3β-HSD, an enzyme concerned in steroid production, was detected in the cat adrenals in the zona glomerulosa and zona fasciculata: The intensity of the reaction in the zona fasciculata was almost twice that found in the zona glomerulosa (Fig. 3a).

The study of the distribution of the sudanophilic lipids showed that the zona glomerulosa of the cat adrenals contained virtually no sudanophilic material. The intermediate or so-called sudanophobic zone was absent and cells loaded with lipids bordered directly on the zona glomerulosa. The lipids were distributed irregularly between the cells of the zona fasciculata, and their total content fell gradually in the direction toward the zona reticularis (Fig. 2).

During artificial hypotension there was a significant decrease in the size of the cells and their nuclei in all zones of the adrenal cortex which began after administration of the hexobarbital and continued during the 5 h of artificial hypotension ($P < 0.1$); a statistically significant increase in the size of the cells was found 24 h after the end of the experiments only in the outer layer of the zona fasciculata ($P < 0.01$). On visual estimation no difference from the control was found in the distribution of lipids among the zones of the adrenal cortex. The content of lipids increased significantly in the zona glomerulosa and zona reticularis ($P < 0.01$) but was virtually unchanged in the zona fasciculata ($P > 0.05$).

After artificial hypotension for 5 h the DNA content fell sharply compared with that in the adrenal cells of both the control and the intact animals ($P < 0.05$). This tendency still continued 24 h after the end of artificial hypotension. The clearest changes in the tissue enzyme profile were observed in the zona fasciculata of the adrenal cortex. The SD activity, reduced after administration of hexobarbital, reached its initial level during artificial hypotension. The role of glycolysis in the adrenal tissue of cats is much greater than the role of aerobic oxidation. Under the experimental conditions LD activity was reduced in the zona fasciculata of the adrenal cortex ($P < 0.1$; Fig. 1d).

Activity of NADH-D and NADPH-D increased after administration of hexobarbital, but during artificial hypotension it was at the initial levels. After 5 h of artificial hypotension activity of G6PD was significantly reduced in the zona fasciculata of the adrenal cortex ($P < 0.1$; Fig. 1b), but 24 h after the end of the experiment the G6PD activity was at or even higher than its initial level in all parts of the cortex. The β-HBD activity in both layers of the zona fasciculata 5 and 24 h after the end of artificial hypotension remained at half its initial level.

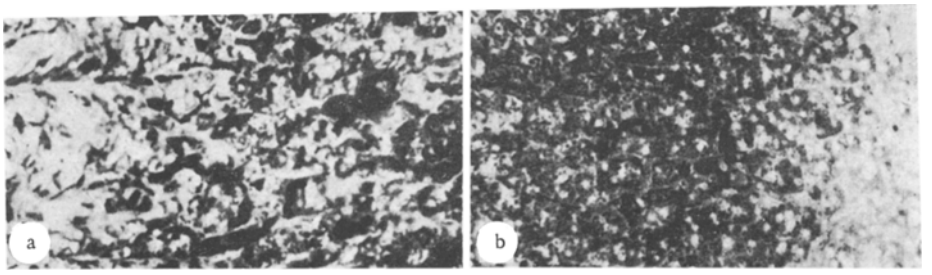

Fig. 2. Distribution of sudanophilic lipids in adrenal cortex: a) in zona glomerulosa of adrenals of intact animal; b) in deep parts of zona fasciculata. Sudan black B, 112.5×.

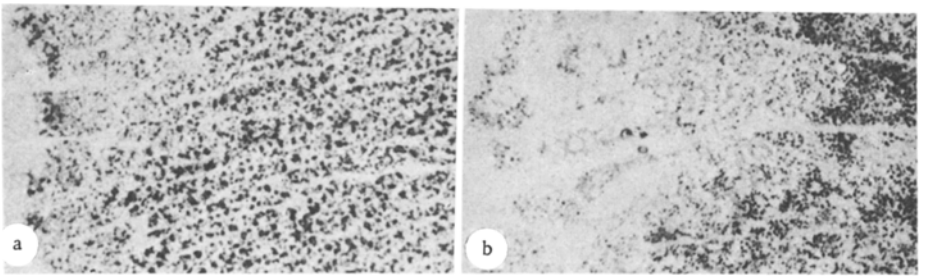

Fig. 3. 3β-HSD activity in adrenal cortex: a) intact animal; b) after artificial hypotension for 5 h. Nitro-BT reaction, 112.5×.

Activity of 3β-HSD, a specific enzyme of steroid formation, was reduced 1 h after administration of hexobarbital ($P < 0.1$), and it remained low 24 h after the end of artificial hypotension (Fig. 3b).

The distribution of all the enzymes studied in the cat' adrenals was characterized by distinct zonality. High G6PD, LD, and 3β-HSD activity in the adrenals of the male cats was combined with moderate NADH-D and NADPH-D activity and with low SD and β-HBD activity.

The changes observed in the adrenals must be regarded as the results of the action of acute hypoxia accompanied by acute cardiovascular failure. In these experiments, when the action of the ganglion blocker led to circulatory hypoxia, and the animals were under the influence of a general anesthetic, the changes in the adrenals ought best to be regarded as the result of mixed hypoxia [1].

Morphometric indices of the cells of the endocrine glands are known to reflect their functional activity to a certain degree [5]. Accordingly, the significant decrease in size of the adrenal cortical cells of the cat during hypotension for 5 h can be interpreted, it seems, as the result of depressed adrenal function under the experimental conditions studied. This is confirmed also by the unchanged lipid content in the zona fasciculata of the adrenal cortex and the tendency toward an accumulation of lipids in the zona glomerulosa and zona reticularis.

The results of the cytophotometric investigations showing a significant decrease in the RNA content and in the activity of the oxidoreductases during mixed hypoxia were particularly distinct in the zona fasciculata of the adrenal cortex; taking into account the results of the morphometric and histochemical investigations, it can be concluded that the glucocorticoid function of the adrenal cortex is depressed under conditions of artificial hypotension. Indices of depressed adrenocortical function obtained by morphological methods of investigation do not contradict the results of the present experiments showing virtually no change in the total plasma 11-hydroxycorticosteroid concentration, if allowance is made for the possible disturbance of its utilization by the tissues when circulatory disturbances are present [3].

LITERATURE CITED

1. N. F. Kan'shina, Arkh. Pat., No. 7, 82 (1973).
2. G. P. Konradi, The Regulation of Vascular Tone [in Russian], Leningrad (1973).
3. V. K. Kulagin and V. V. Davydov, in: Stress and its Pathogenetic Mechanisms [in Russian], Kishinev (1973), pp. 28-31.

4. V. A. Levtov and D. I. Parolla, in: Problems in Regulation of the Regional Circulation [in Russian], Leningrad (1969), pp. 96-105.
5. Yu. A. Medvedev, in: Problems in Pathological Anatomy [in Russian], Leningrad (1970), pp. 203-218.
6. V. I. Osipov, Artificial Hypotension [in Russian], Moscow (1967).
7. H. S. Bennett, Am. J. Anat., 67, 151 (1940).
8. S. Davies, Q. J. Microscop. Sci., 80, 81 (1937).
9. L. Nicander, Acta Anat. Suppl., No. 16 (1952).

MICROSPECTROPHOTOMETRIC CHARACTERISTICS OF RENAL ENZYMES IN ACUTE CIRCULATORY FAILURE

A. P. Kurilin

UDC 616.8-009.831-06:616.61-008.64-07:616.61-008.931-074

Activity of lactate, succinate, glucose-6-phosphate, β-hydroxybutyrate, NADH, and NADPH dehydrogenases, and of alkaline and acid phosphatases in the kidneys was investigated in intact cats, during hexobarbital anesthesia, after hypotension produced by injection of a ganglion blocker (Arfonad) for 5 h, 24 h after the end of artificial hypotension, and after massive blood loss. All parts of the nephron responded by differential changes of enzyme activity. A sharp decrease in the role of the principal pathway of oxidation (the Krebs cycle) in energy metabolism took place, and under the conditions of circulatory hypoxia the glycolytic pathway and pentose shunt became predominant.

KEY WORDS: acute vascular failure; kidneys; enzyme activity.

Acute systemic circulatory failure (collapse), of varied origin, frequently leads to acute renal insufficiency [4, 6], which is based on circulatory hypoxia of the kidney and, it has been suggested, a disturbance of the function of energy-forming systems, especially of oxidoreductases and hydrolases [1-4, 6].

In this investigation the dynamics of activity of various oxidoreductases and hydrolases in the kidney was studied in the early stages of acute circulatory failure and after blood loss.

EXPERIMENTAL METHOD

Oxidative and hydrolytic enzymes of the kidneys were investigated in 50 male cats divided into four groups: 1) intact (8), 2) anesthetized (10), 3) killed immediately after artificial hypotension for 5 h, and 24 h after its end (25), and 4) killed after hypotension for 4 h, caused by blood loss (6). The general anesthetic, a 10% solution of hexobarbital, was injected intraperitoneally (0.1 g/kg). Artificial hypotension was produced in the anesthetized animals by intravenous drip injection of a 0.1% solution of Arfonad or by repeated bleeding from the femoral artery. The blood pressure fell to 50% of its initial level, at which it was maintained throughout the experiments.

To assess the dynamics of activity of oxidoreductases and hydrolases, a series of eight enzymes was chosen for testing: lactate dehydrogenase (LD), the most important enzyme of glycolysis; glucose-6-phosphate dehydrogenase (G6PD), an indicator of the intensity of glucose oxidation in the pentose shunt; succinate dehydrogenase (SD), a histochemical indicator of the Krebs cycle; β-hydroxybutyrate dehydrogenase (β-HBD),

Department of Pathological Anatomy, S. M. Kirov Leningrad Postgraduate Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 617-619, May, 1977. Original article submitted May 25, 1976.