

FUNCTIONAL STATE OF THE ADRENAL CORTEX IN EXPERIMENTAL HEMOLYTIC ANEMIA DUE TO PHENYLHYDRAZINE

N. A. Grigorovich and A. F. Fiks

Department of Pathological Physiology (Head—Prof. N. N. Zaiko) of the N. I. Pirogov
Odessa Medical Institute (Director—Honored Scientist Prof. I. Ya. Deineka)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*

Vol. 49, No. 5, pp. 54-57, May, 1960

Original article submitted May 6, 1959

Steroid hormones from the adrenal cortex have been widely used in recent years in the treatment of the acquired hemolytic anemias [1, 2, 3, 5]. It has been suggested that the therapeutic action of the adrenal steroid hormones in this group of diseases is the result of depression of the functions of the reticuloendothelial system, where anti-red cell antibodies are produced. We know from the literature, however, that the steroid therapy of the hemolytic anemias is not always accompanied by obvious improvement. When administration of the adrenal hormones is discontinued, recurrence of the disease is common [3]. Satisfactory results from the treatment of the acquired hemolytic anemias with steroid hormones, just as in the course of the disease before treatment is discontinued, are to a large extent dependent on the reactivity of the patient and, in particular, on the functional state of the adrenal glands.

In the literature known to us, we were unable to find data concerning the function of the adrenal cortex at various periods in the course of the acquired hemolytic anemias. We therefore decided to make an experimental study of the functional state of the adrenal cortex in animals with hemolytic anemia due to phenylhydrazine. There are reports in the literature that the development of anemia in animals poisoned with phenylhydrazine, as in cases of acquired hemolytic anemia, is based upon autoimmune mechanisms [6, 7].

EXPERIMENTAL METHOD

Experiments were carried out on 84 white rats of both sexes, weighing 110-280 g. The animals were kept on a balanced diet. Anemia was induced by a single subcutaneous injection of a 2% solution of phenylhydrazine hydrochloride in a dose of 40 mg/kg. The blood picture and the functional state of the adrenal cortex were investigated after 3, 6, 12, and 24 hr and on the 2nd, 4th, 6th, 9th, and 13th days after injection of phenylhydrazine. Estimations were made of the red cell count, the blood hemoglobin concentration, and the red blood picture in films. The hematological investigations were made by the usual methods. In order to define the functional state of the adrenal cortex the following tests were used: the

eosinophil reaction of the blood, the change in weight of the adrenal glands and the estimation of the free ascorbic acid in the adrenals. Histological studies of the adrenals were also carried out. The eosinophils were counted by Dunger's method. The content of free ascorbic acid in the adrenal tissues was estimated by titration of an extract of the adrenals with 2,6-dichlorophenolindophenol. For the histological study of the adrenals, films stained with hematoxylin and eosin were used.

EXPERIMENTAL RESULTS

As the figures in the table show, the phenylhydrazine anemia reached its maximum on the fourth day. At this time the red cell count fell to 59.5 %, and the hemoglobin concentration to 63.2 % of the mean blood level in the intact animals. The red blood picture in the film showed severe anisocytosis, macromicrocytosis, severe polychromatophilia and the presence of solitary normoblasts and red cells with Jolly bodies.

TABLE. Composition of the Peripheral Blood and Indices of the Functional State of the Adrenal Cortex at Various Stages of Hemolytic Anemia Due to Phenylhydrazine.

No. of exptl. animals	Time elapsing after injection of phenylhydrazine	Mean values of indices studied (as % of controls)				
		red cell count	hemo-globin concn.	eosino-phil count	weight of adrenals	ascorbic acid content
5	3 hours	—	—	79.5	—	—
5	6 "	—	—	23.1	—	—
7	12 "	—	—	208.5	—	—
7	24 "	—	—	121	—	—
10	2 days	77.2	60.0	192	100	114
10	4 "	59.5	63.2	198	100	106
10	6 "	68	63	139	120	86.5
10	9 "	77.9	89.5	75.4	135	85.4
10	13 "	84.5	93.8	62.5	110	99

Starting on the sixth day of the observations, signs of regeneration of the red blood were observed. On the 13th day after injection of phenylhydrazine, the red cell count reached 84.5 % and the hemoglobin concentration in the blood 93.8 % of that in the controls. By the ninth day of the experiment, no abnormalities could be detected in the red blood picture in the films of 5 of the 10 experimental animals. On the 13th day no abnormalities in the morphological structure of the cells were observed in the blood films of all the experimental animals.

In the course of the first six hours after the injection of phenylhydrazine, when the signs of hemolysis of the red cells were still slight [4], the character of the eosinophil reaction of the blood gave evidence of the participation of the adrenal cortex in the pathogenesis of the developing anemia. Two phases were discerned in the reaction of the eosinophils to the injection of phenylhydrazine. In the course of the first six hours, to judge by the developing eosinopenia, the functional activity of the adrenal cortex was increased. It may be considered that this was the normal reaction of the adrenals to an inadequate stimulus, in this case the procedure of injection of phenylhydrazine. Twelve hours after injection, the increase in the functional activity of the adrenal cortex was replaced by a decrease. The number of eosinophils in the peripheral blood rose to 208.5 %. At the end of the first 24 hours of the experiment a further increase in the activity of these glands was observed, but to a lesser degree than during the first six hours. The number of eosinophils in the peripheral blood at this time fell nearly to the level observed in the blood of the intact animals (121 %).

As the table shows, the development of hemolytic anemia was accompanied by depression of the activity of the adrenal cortex. At the height of the anemia a marked eosinophilia was observed in the peripheral blood: the eosinophil count in the blood was 192-198 % by comparison with that in the intact animals. The free ascorbic acid content of the adrenal tissue was increased by 6-14 %. No changes in the weight of the adrenals could be detected at this period.

With the commencing regeneration of the red blood, an increase in the activity of the adrenal cortex was observed, and this continued while normalization of the composition of the red blood took place. On the sixth day of the experiment the eosinophil count in the blood

fell from 198 to 139 % and continued to fall further, so that on the ninth day it was 75.4 %, and on the 13th day, 62.5 % of the level in the blood of the intact animals. The increase in the functional activity of the adrenal cortex was accompanied by an increase in the weight of the glands. Six days after injection of phenylhydrazine, the weight of the adrenals in the experimental animals was, on the average, 20 % higher than that in the intact animals, and after nine days it was 35 % higher. The ascorbic acid content of the adrenal glands on the sixth to the ninth day after injection of phenylhydrazine was increased by 14-15 % by comparison with its content in the adrenal glands of intact animals. On the 13th day of the experiment the ascorbic acid content in the adrenals of the experimental animals was the same as in the intact animals. However, the observed increase in weight of the adrenals and the eosinopenia of peripheral blood were evidence of the increased activity of the adrenal cortex.

Microscopic examination of adrenal preparations taken on the second, fourth and sixth days of the experiment showed well-marked signs of cloudy swelling of the cells of the zona fasciculata of the adrenal cortex. The microscopic picture of the adrenal glands on the ninth and 13th days showed no essential abnormalities by comparison with the morphological state of the adrenal glands of the intact animals.

SUMMARY

A study was conducted of the functional condition of the adrenal cortex in rats at various stages of experimental hemolytic anemia. With the development of anemia the functional activity of the adrenal cortex was depressed. Restoration of the morphological composition of the blood was accompanied by a rise of the functional activity of the adrenal cortex.

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