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EFFECT OF HYDROCORTISONE ON BLOOD CLEARANCE OF INERT COLLOIDS BY CELLS OF THE RETICULOENDOTHELIAL SYSTEM

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Responses of the reticuloendothelial system (RES) and of its main component — the system of mononuclear resident phagocytes — to glucocorticoids have been studied in several investigations. However, different results have been obtained. This is evidently because the effects of hydrocortisone (HC) and of other glucocorticoids are dependent on the original functional state of the RES, which as a rule was not verified in these investigations. In addition, after administration of pharmacologic and physiologic doses of the same hormone, it is natural to expect both similar and diametrically opposite effects. After injection of HC in pharmacologic doses into cortisone-sensitive animals (rats and mice) the ability of the RES to cleanse the blood of inert colloids was most frequently observed [13]. Meanwhile, after injection of HC in a physiological dose (3 mg/kg) into adrenalectomized animals no change was found in the phagocytic activity of the RES [4]. According to some data, adrenalectomy itself causes depression [10], according to others — stimulation of the ingestive power of the RES [12]. It likewise is not yet clear how the behavior of the RES differs after single and repeated stimulation of HC.

It is important to study not only the response of the RES to HC, but also the course of recovery of functions of the system after the abrupt resetting of endocrine regulation in response to a large dose of the hormone.

EXPERIMENTAL METHOD

Experiments were carried out on 85 female Wistar rats weighing 240-280 g. HC was injected intraperitoneally into some rats in a dose of 125 mg/kg and blood clearance of inert colloid was tested after 2, 24, and 48 h and 1 and 3 weeks, and into other rats it was injected daily at 9-10 a.m. for 1, 2, and 3 weeks in a sessional dose of 12.5 mg/kg. Function of the RES was determined 24 h after the final injection of the hormone. In the control, 0.85% NaCl was injected at all times of the investigation. To study blood clearance, 2 ml of a suspension of colloidal carbon (from Günther Wagner, West Germany) was added to 3 ml of a

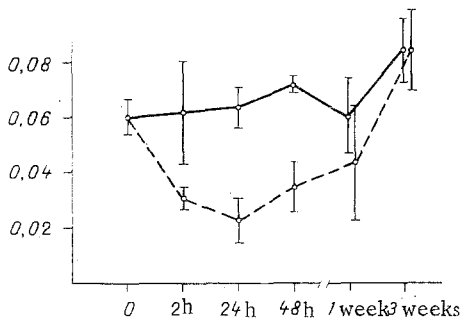

Fig. 1. K-indices after injection of hydrocortisone (125 mg/kg). Continuous line, control; broken line, experiment. Abscissa, K-indices; ordinate, time.

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TABLE 1. Half-Elimination Time of Colloid from Blood (in min) after Repeated Injections of HC in a Sessional Dose of 125 mg/kg (n = 5, M ± m)

Group of animals	Duration of continuous hormonal stimulation, weeks		
	1	2	3
Experimental	6,5±0,7	5,1±0,5	4,3±0,6
Control	4,5±0,6	5,0±0,3	4,6±0,3

3% solution of gelatin, and the resulting mixture was injected into the femoral vein in a dose of 0.16 ml/100 g body weight. The first portion of blood was taken from the retro-orbital sinus 10-20 sec after injection of the carbon particles, and later at intervals of 3 min for 30 min; 0.1 ml of blood was then diluted in 2.9 ml of 0.1% Na_2CO_3, containing 5 Units/ml of heparin, and the samples were subjected to photometry at 650 nm. The blood clearance rate (BCR) and K-indices were determined by Biozzi's method [5]. The total number of leukocytes was counted and their relative percentages calculated. Pieces of tissue from the central zones of the right lobe of the liver were fixed in neutral 10% formalin and in Carnoy's fluid. The number of Kupffer cells per 1000 hepatocytes was counted in sections stained with hematoxylin and eosin. At the same time, the percentage of these cells which had ingested particles of colloidal carbon ($\geq$ 50% of granules) was determined. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

A single injection of a large dose of HC led to involution of the lymphoid system. The weight of the thymus began to fall after 24 h and the greatest decrease (by 4-5 times) was observed one week after injection of HC, when the ingestive function of the RES began to be restored and partial normalization had occurred three weeks after the single injection of the hormone. Similar changes took place in the weight of the spleen: One week after injection of HC it was reduced almost by half compared with the control. One of the most characteristic primary changes in the peripheral blood was a reduction in the number of lymphocytes, accompanied by an increase in the relative number of neutrophils. These changes were recorded for one week after hormonal stimulation. The total number of leukocytes in the peripheral blood was reduced by more than half compared with the control 2 h after injection of HC, recovery began after 24 h, and the number was indistinguishable from the control after 48 h. The number of monocytes in the blood fell after 24 h by one third, but after 48 h, on the other hand, it was a little larger than in the control.

By 2 h after injection of HC in a dose of 125 mg/kg BCR from the inert colloid was reduced by more than half. This decrease continued to be observed for 24-48 h after injection of HC (Fig. 1). BCR was close to the control level again one week after injection of the hormone, but still remained low; after three weeks it was completely restored. The greatest decrease in BCR 24 h after injection of HC correlates with a decrease in ingestion of the colloid by Kupffer cells: In the control, particles of carbon were ingested by 74.0 ± 2.5 Kupffer cells, compared with 52.4 ± 3.5 Kupffer cells 24 h after injection of HC. The relative number of these cells in the liver at this time did not differ significantly from the control.

Daily injection of HC for one week led to a very small decrease in BCR, but injections for 2-3 weeks had no effect on the half-clearance time of colloid from the blood (Table 1).

In response to injection of HC in a dose of 125 mg/kg, BCR from inert colloids thus fell immediately. The main contribution to clearance of the blood from corpuscular inert particles is made by the Kupffer cells of the liver [3]. Depression of the ingestive power of these cells during the first few hours after injection of the hormone is evidently associated with a sharp rise in the HC concentration in the blood and RES cells up to the level at which HC begins to behave as a membrane stabilizer of the phagocytes and an inhibitor of endocytosis [2]. Meanwhile, 24-48 h after injection of the hormone, depression of the ingestive function of the RES and of its main component — the Kupffer cells of the liver — may be associated not so much with the direct effect of high blood HC levels as with secondary changes in the endo-

crine status in response to the high HC level. In particular, the reaction to HC may be manifested as hypersecretion of insulin (an antagonist of HC) and as lowering of the blood glucose level. In turn, hypoglycemia leads to a decrease in BCR from the inert colloid [8]. Finally, the possibility cannot be ruled out that after injection of HC the level of plasma opsonins and, in particular, of fibronectin, may be lowered. In this sense the situation can be likened to the different variants of acute stress, in which the plasma fibronectin concentration falls and, at the same time, persistent depression of the ingestive function of the RES develops [11]. All these three working hypotheses require experimental proof.

At the time of maximal depression of RES the number of monocytes in the blood was reduced by one third. This was due most probably to inhibition of proliferation and differentiation of monocyte precursors in the bone marrow, on the one hand [6], and their delayed release from the bone marrow into the peripheral blood, on the other hand [7]. Restoration of the ingestive function of the RES correlates in time with the increase in the number of monocytes in the blood after the previous monocytopenia and with marked involution of lymphoid tissue.

The sharpest changes in ingestive function of the RES cells thus took place after a single injection of HC. When HC was injected daily for one week a phenomenon of "escape" of the RES from hormonal control was observed. This phenomenon was even more evident if HC was injected for two or three weeks. In the latter case, by the end of hormone injections, no depression of the ingestive capacity of the RES whatever was observed. The possibility cannot be ruled out that tolerance of the RES to HC has the same molecular basis as the decrease in sensitivity of hepatocytes to glucocorticoids, when injected repeatedly for three weeks [1]. During repeated hormonal stimulation the receptors for the hormone in the hepatocytes are exhausted and it loses its ability to induce HC-dependent syntheses of the enzymes of gluconeogenesis. It is perfectly possible that receptors for HC may also be present in Kupffer and endothelial cells, and that these are "used up" during repeated hormonal stimulation. Receptors for HC have been found in the lung macrophages [9], but Kupffer cells have not yet been studied from this point of view. The reactivity of cells of the hepatic RES to the hormone, manifested as resistance of its function to prolonged hormonal overloading, is of great importance in processes of adaptation.

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