REGENERATION OF THE RABBIT SPLEEN AFTER HYDROCORTISONE INJECTION

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The action of hydrocortisone on the rabbit spleen and the regenerative processes arising after cessation of administration of the hormone were studied. During prolonged administration of hydrocortisone the absorptive function of the reticuloendothelial system is depressed and atrophy of the spleen develops, as shown by a reduction in the weight and size of the organ. Most of the splenic follicles are converted into random clusters of lymphocytes. Pyroninophilic cells disappear from the white pulp. After cessation of the action of the hormone the animal's general condition improves, the development of the atrophic changes in the spleen is halted, the splenic follicles are restored, and the number of pyroninophilic cells in them increases. The use of a regeneration stimulator under these conditions accelerates recovery of the body weight of the animal, reversal of the atrophy of the spleen, and restoration of its normal structure.

KEY WORDS: spleen; hydrocortisone; stimulation of regeneration

After resection the rabbit spleen not only does not regenerate, but it even shows signs of atrophy [4]. The writer showed previously that injection of ethanolamine after hemiresection of the spleen causes the development of a special form of regeneration hypertrophy, characterized by an increase in length of the organ [6].

It was important to discover how the ability of the rabbit spleen to regenerate is manifested after changes caused by the action of pathological factors, both under ordinary conditions and during the action of a regeneration stimulator [5, 7].

Of the factors causing a pathological state of the lymphoid organs, the choice fell on hydrocortisone. According to data in the literature, this substance causes atrophy of lymphoid organs [2, 3] and leads to a decrease in the RNA concentration in the mitochondria of the spleen [1].

The study of regenerative processes in the rabbit spleen after atrophy induced by hydrocortisone is of definite interest because, whereas the absence of regeneration after resection could be to some extent explained by the high compensatory powers of the other lymphoid organs, after injection of hydrocortisone these powers are limited, so that new conditions are created for the development of regeneration of the spleen and for the associated use of a regeneration stimulator.

EXPERIMENTAL METHOD

Experiments were carried out on 45 rabbits of the Rex breed, strictly selected by weight (1500.0 ± 65.0 g).

On alternate days all the experimental animals received injections of hydrocortisone (from Richter) in a dose of 2 mg/kg. The rabbits of group 1 were killed on the 30th day of administration of the hormone and the rabbits of group 2 30 days after the last injection of the hormone. The animals of group 3, starting from the 30th day after the last injection of hydrocortisone, were given intraperitoneal injections of colamine hydrochloride (ethanolamine) in a dose of 10 mg/kg on alternate days for 30 days. Intact rabbits served as the control for each group. In the course of the observations on the animals the Congo Red index was determined periodically. This test consists essentially of colorimetric calculations of the rate of disappearance of intravenously injected Congo Red from the blood stream, which depends on the functional state of the reticuloendothelial system (RES). The material was subjected to the usual histological analysis, some sections were

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TABLE 1. Changes in Weight and Length of Spleen, Diameter of Splenic Lymphatic Follicles, and Number of Pyroninophilic Cells in Them (M ± m)

Group of animals	Weight of spleen, mg	Length of spleen, mm	Diameter of splenic follicles, μ	Number of pyroninophilic cells in reactive centers, %			Lymphoid follicles without reactive centers, %
				under 65 cells per field of vision	under 30 cells per field of vision	under 15 cells per field of vision	
Control	1710,0±0,62	55,0±0,12	346,8±12,1	39,3±0,63	22,2±0,45	17,3±0,34	21,2±0,24
30th day of hydrocortisone administration	536,0±6,41	45,0±2,11	184,7±2,92	6,1±0,36	14,3±0,84	18,5±0,68	61,1±1,4
30th day after end of hydrocortisone administration	1359,0±15,5	50,8±0,87	210,6±12,3	8,5±0,11	21,5±0,12	39,5±0,69	30,5±0,48
30th day of stimulation of regeneration after end of hydrocortisone administration	1755,0±14,6	56,0±0,13	288,9±13,4	20,7±0,99	30,7±0,56	25,5±0,67	23,1±1,15

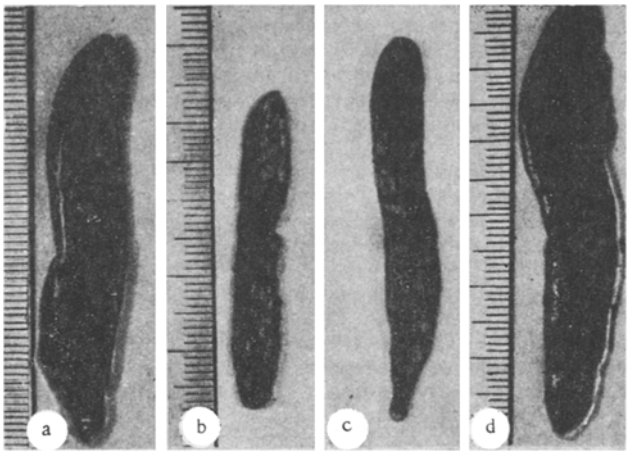

Fig. 1. External appearance of rabbit spleen: a) control; b) on 30th day after beginning of hydrocortisone injections; c) 30 days after end of hydrocortisone injections, d) 30th day after end of hydrocortisone injections and beginning of administration of regeneration stimulator.

stained by Brachet's method, the number of splenic lymphatic follicles in a field of vision was counted, their diameter was measured, and the presence of reactive centers in them and the number of pyroninophilic cells in these centers were determined. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Throughout the period of the experiments no appreciable variation was found in the Congo Red index of the control animals (90.8 ± 0.87 to 89.3 ± 0.44). Under the influence of hydrocortisone it fell significantly, to 73.6 ± 0.01 by the 30th day. The index rose 1 month after the end of administration of the hormone (83.2 ± 2.08), but did not reach the control level. Stimulation of regeneration after administration of the hormone increased this index and brought it close to the control level. Prolonged administration of hydrocortisone caused emaciation of the animals. They became apathetic and their fur became rough and lost its sheen. However, on the 30th day of the experiment, the animals receiving hydrocortisone had increased in weight by 380.0 ± 33.6 g, and 1 month after the end of its administration by 750.0 ± 52.1 g (compared with 980.0 ± 12.5 g in the control). The results obtained by stimulation of the animals differed considerably from those in the groups mentioned above (1140.0 ± 26.0).

Under the influence of the hormone the weight of the spleen on the 30th day was reduced to 536.0 ± 6.4 mg and its length to 45.0 ± 0.1 mm, compared with 1710.0 ± 0.62 mg and 55.0 ± 0.2 mm respectively in the control (Table 1). The weight of the spleen showed some recovery 1 month after the end of hydrocortisone injections to reach 1350.0 ± 15.5 mg and its length 50.8 ± 0.87 mm (Fig. 1). A significant change was observed when

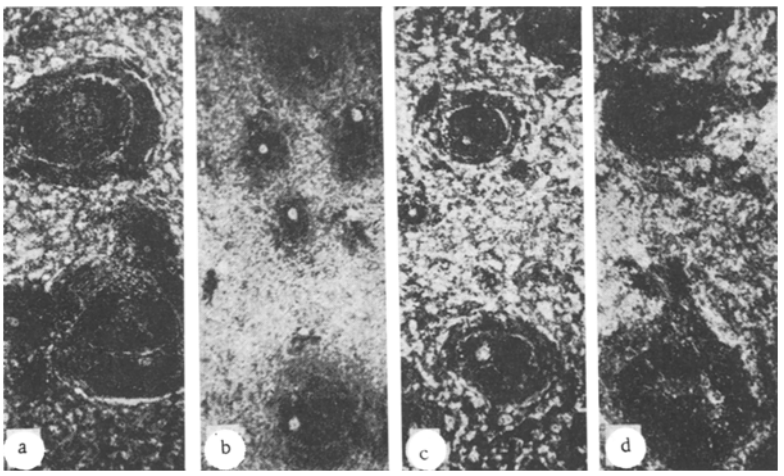

Fig. 2. Section through spleen of rabbits: a) control (intact spleen); b) on 30th day after beginning of hydrocortisone injections; c) 30 days after end of hydrocortisone injections; d) on 30th day after end of hydrocortisone injections and beginning of administration of regeneration stimulator. 125 ×.

regeneration was stimulated after the end of hydrocortisone injections. After 1 month the weight of the spleen was fully restored, to reach 1755.0 ± 14.6 mg and its length was 56.0 ± 0.13 mm.

The splenic lymphoid follicles in the spleen of the rabbits receiving hydrocortisone for 30 days were indistinctly demarcated from the red pulp (Fig. 2). In most cases they consisted of random collections of lymphocytes, forming small islets. Reactive centers disappeared from many of the splenic follicles. The number of follices per field of vision remained almost unchanged under the different experimental conditions compared with the control, but their diameter changed appreciably depending on the experimental conditions.

For instance, on the 30th day of hydrocortisone injections the mean diameter of the splenic follicles was 184.7 ± 2.9 μ, rising to 210.6 ± 12.3 μ 1 month after the end of injection of the hormone compared with 346.8 ± 12.1 μ in the control. When a regeneration stimulator was used the increase in the diameter of the splenic follicles was greatest, and reached 288.9 ± 13.4 μ at the same period of observation, although it was still below the diameter observed in the control animals.

After 4 weeks of hormone injections the capsule and connective-tissue septa of the spleen had become thinner and, after impregnation with silver nitrate, the argyrophilic fibers in them were clearly visible. The walls of the central arteries were thickened and often obliterated. In such cases the splenic lymphoid follicles were either grossly atrophied or had lost their outlines and changed into random clusters of deformed lymphocytes, staining dark blue with hematoxylin. Usually the reactive center was absent in such follicles. The number of these follicles was 61.1 ± 1.4%.

As these experiments showed, under the influence of hydrocortisone the number of splenic lymphoid follicles containing pyroninophilic cells after 1 month of observation had fallen to 38.9% compared with 78.8% in the control. Impregnation with silver showed a clearer and denser network or argyrophilic fibers at the periphery of the splenic follicles. Some increase in the thickness of the capsule and connective-tissue septa was observed. A lumen was found in nearly all the vessels. Most of the splenic lymphoid follicles were clearly demarcated from the red pulp, but at the same time there were other follicles still consisting of random clusters of lymphocytes. The number of splenic follicles without reactive centers was 30.5 ± 0.48%.

No signs of obliteration were found after injection of colamine hydrochloride. The splenic follicles were better formed than in the previous group. The number of splenic follicles without reactive centers was reduced, whereas the number of follicles containing many pyroninophilic cells per field of vision was appreciably increased, although not up to the control level.

The results thus indicate that during prolonged administration of hydrocortisone the Congo Red index falls, reflecting the state of the absorptive function of the RES. Under the influence of the hormone atrophy of the spleen develops, as shown by a decrease in the weight and size of the organ, in the diameter of the splenic

lymphoid follicles, and in the number of lymphocytes in the red pulp. The splenic follicles lose their configuration and change into random clusters of lymphocytes, and most of them lose their reactive center. Condensation of the capsule and connective-tissue stroma is observed.

After the end of hormone injections the absorptive function of the RES increases again, the general condition of the animals improves, the atrophic changes in the spleen cease, the number of lymphocytes in the organ increases, and the normal splenic lymphoid follicles are restored (reappearance of the reactive centers and an increase in the number of pyroninophilic cells in them).

Administration of a regeneration stimulator after the end of hormone injections appreciably accelerates both the recovery of the animal's general condition and restoration of the atrophied spleen.

One month after the end of administration of hydrocortisone, the spleen which had partially atrophied under the influence of the hormone is almost completely restored. If colamine hydrochloride is given under these conditions, regeneration of the organ takes place more rapidly and the weight of the spleen regains the control level during the same period of observation.

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HUMORAL MECHANISMS OF REGULATION OF REPARATIVE OSTEOGENESIS

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The effect of the blood serum from animals with active osteogenesis on the biosynthesis of nucleic acids and protein and on mineralization of regenerating bone tissue was studied in experiments in vivo and in vitro. Incorporation of labeled precursors of DNA ([^{3}H]thymidine) and protein ([^{14}C]proline) in the recipients was intensified and mineralization of bony callus (incorporation of ^{85}Sr) was accelerated. Comparison of the order of stimulation of nucleic acid and protein synthesis suggests that the active principle of the serum promotes more rapid cell proliferation in the fracture zone.

KEY WORDS: reparative osteogenesis; humoral regulation; radioactive isotopes

One of the aspects of the problem of the regulation of repair processes that has received the least study is the control of the natural course of reparative osteogenesis. The only information available is concerned with the stimulating effect of breakdown products of bone [4] or of the blood serum of animals with fractures [1, 8] on this process. The present writers [7] have also found that during the period of intensive osteogenesis the blood serum of animals acquired the property of stimulating fracture healing.

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