CHANGES IN SUBCUTANEOUS CONNECTIVE TISSUE OF RATS AFTER INJECTION OF CORTISONE

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During the past 10 years there have been obtained numerous data concerning the potent antiinflammatory action of cortisone. It was shown that cortisone retarded such components of the inflammatory process as vascular dilation, exudation, marginal standing of leucocytes, fibroblast proliferation and the development of granular tissue [7, 11, 13, 14, 15, 18, 19, 20, 21]. The antiinflammatory properties of cortisone have promoted its wide utilization in clinical practice.

Considerably less work has been devoted to the study of the cortisone action on connective tissue found under normal conditions. Investigation in this direction touched mainly upon the action of cortisone on the ground substance and on the fat cells of the connective tissue. A number of authors have presented results concerning the influence of cortisone on the lowering of hyaluronic acid on the ground substance of connective tissue [8, 9, 12, 16] and on the decrease in number and degranulation of fat cells [8, 9, 10, 12, 17].

The data are quite scanty concerning the action of cortisone on other cellular components of normal connective tissue. Of interest are the investigations of I. A. Alov [1], who observed that under the influence of cortisone there were stimulations of the fibroblast differentiation process and of formation of the ground substance in subcutaneous connective tissue of mice.

The problem of this study is to investigate the action of cortisone on various components of rat subcutaneous connective tissue and to establish the character of the changes produced by this hormone.

EXPERIMENTAL METHODS

Rat subcutaneous connective tissue was studied in film preparations, prepared by the method of G. Yasvoin For a more accurate evaluation of changes in the cellular composition of connective tissue there were counted the percentage ratios among various cellular elements [3-6]. The following cellular forms were differentiated: 1) young fibroblasts with one or two offshoots; 2) mature fibroblasts with a clearly defined ectoplasmic layer and numerous offshoots; 3) fibrocytes– differentiated fibroblasts with strongly elongated and sometimes deformed nucleus and sharply reduced cytoplasm; 4) macrophages; 5) lymphoid cells; 6) polymorphonuclear leucocytes; 7) degenerating deformed cells, whose origin was difficult to ascertain. One thousand cells were counted in each preparation.

The author has also utilized various histochemical methods for the study of connective tissue: vital staining with neutral red; staining with Sudan black for lipids; staining with toluidine blue with phosphotungstic acid fixation [2] for ground substance; staining with toluidine blue after fixation for chromotropic mucopolysaccharides; staining according to van Geison for collagen; reaction with periodate fuchsin sulfurous acid (PAS reaction) for polysaccharides.

The experiments were conducted on rats weighing 50-60g; Cortisone was injected intraperitonially. In the first series of experiments, two groups of rats (5 rats per group) were injected one time with doses of 5 and 12.5 mg, in the control experiments, 3 rats were injected with 0.5 ml of physiological solution. The animals were killed with ether on the following day.

In the second experimental series rats were injected with 12.5 mg of cortisone for a day (two injections per day); a control group of 3 rats was given physiological solution. The animals were killed five days after the last injection. From subcutaneous connective tissue of each rat's back there were prepared three film preparations and the cytograms were counted.

EXPERIMENTAL RESULTS

In the table are presented data obtained in the experiments.

As is evident from the table, the percent of fibrocytes in the subcutaneous connective tissue increased sharply after a single injection of cortisone. With respect to other cells, particular changes in comparison with the control were not noticed. There were no differences in experimental results from a single injection of 5 and 12.5 mg of cortisone.

Repeated cortisone injection produced a more clearly expressed action on connective tissue. Leucocytes and

Changes in the Percentage Relation of the Cellular Composition of Rat Subcutaneous Connective Tissue Following Single and Repeated Injections of Cortisone

Connective tissue cells	Control	Experiment		
		single injection		repeated injection
		5 mg	12.5 mg	12.5 mg
	6 rats	5 rats 12.5 mg	5 rats	4 rats
Polynuclear cells	0,51±0,17	0,36±0,18	0,66±0,25	—
Lymphoid cells	1 45±0,19	0,73±0,05	1,04±0,1	0,8±0,04
Macrophages	26,95±4,5	18,16±0,59	16,92±0,98	13,83±0,16
Young fibroblasts	1,36±0,25	1,03±0,52	2,02±0,66	—
Mature fibroblasts	69,3±4,49	71,1 ±0,52	78,58±1,59	85,27±0,16
Fibrocytes, within the above number.	9,93±2,36	40,6 ±11,67	39,1 ±8,08	65,27±4,03
Degenerating cells	0,41±0,15	3,6 ±0,7	0,78±0,19	0,1 ±0,04

young fibroblasts disappeared. The percent of fibrocytes increased even more; many of the cells belonging to this group had a sharply deformed pycnotic nucleus, strongly reduced cytoplasm, and resembled degenerating cells. The cytoplasm of many mature fibroblasts contained large vacuoles. Reduced cytoplasm was encountered in many macrophages.

In the third series of experiments we studied, by means of histochemical methods, the action of repeated cortisone injection on the connective tissue. Cortisone injection and preparation of connective tissue films was done by the same method as in the second experimental series. Thirty-six rats, divided into six equal groups, were used in the experiments; three rats from each group served as controls. Connective tissue films were stained with one of the previously described histochemical methods; control films were stained together with the experimental.

In the case of vital staining with neutral red, in the residual cytoplasm of the fibrocytes, especially numerous in experimental animals, there was noted a small number of fine red granules. Macrophages contained a considerable number of more coarse granules. In the control and in the experimental there were not encountered any diffusely stained cells.

After staining with Sudan black the cytoplasm of control and experimental polynuclear cells was filled with fine lipid granules, quite monotypic in diameter; in the fibroblasts the endoplasm was stained, whereupon more intensely stained portions were situated closer to the nucleus. The nuclei were not stained. The cytoplasm of control and experimental fibrocytes was stained more intensely than the cytoplasm of fibroblasts. In the connective tissue cells of control and experimental animals granular fat deposits were not observed.

With other staining methods (according to van Gieson; toluidine blue with phosphotungstic acid fixation; toluidine blue for metachromatic compounds; periodate fuchsin sulfurous acid for polysaccharides) there could not be established any changes after cortisone injection as compared with the control.

As is evident from the obtained data, the increase in the number of fibrocytes was most characteristic of the action of cortisone on the subcutaneous connective tissue of the rat. Young monoplasmic fibroblasts disappeared from subcutaneous connective tissue after repeated injection of cortisone; together with this there was an increase in the number of highly differentiated fibroblasts. These data are in agreement with the results of I. A. Alov's investigations on mice [1]. During the differentiation process the fibroblast goes through the stage of a diplasmotic vacuolar cell and into a fibrocytic, with a sharply reduced cytoplasm and a deformed nucleus. However, as follows from the present experiments, cortisone did not produce cellular injury, because even fibroblasts in the last stages of differentiation did not show signs of degeneration.

I. A. Alov assumes that the process of intensified fibroblastic differentiation is accompanied by the formation of ground substance. This takes place, as he assumes, at the expense of fibroblastic ectoplasm dissolution by growing vacuoles or via fragmentation of ectoplasmic offshoots. During the action of cortisone there were not observed any increase in the quantity or changes in the morphological peculiarities of the ground substance. Staining for collagen, mucoids and ground substance did not reveal any differences in connective tissue from control and experimental animals, consequently, the process of fibroblast differentiation from a mature fibroblast to a fibrocyte was not accompanied by an increase in the amount of ground substance. The formation of the latter, evidently, is the function of the young cells of the fibroblastic series.

It is of interest to compare the present results with the data of V. V. Shikhodyrov [6], who, by subjecting dogs to x-ray irradiation, has also obtained stimulation of the fibroblast differentiation process in subcutaneous connective tissue. In the given case x-rays produced an effect on connective tissue similar to the influence of cortisone.

SUMMARY

The author studied the effect of cortisone on the relative quantities of different cell forms on the histochemical characteristics of cells and of ground substance in the subcutaneous tissue of normal rats. Cortisone stimulated the process of fibroblast differentiation, causing a striking increase in the mean percentage of the most differentiated fibroblasts (fibrocytes). Cortisone provoked no degeneration of fibroblasts or any changes of histochemical peculiarities in the ground substance or in the connective tissue cells. No increase in the amount of the ground substance was noted during differentiation of fibroblasts into fibrocytes.

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