Effect of Cortisone upon Chondroitin Sulfate Synthesis by Animal Tissues.* (18571)

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The symptoms and the pathological picture in arthritis and certain other of the so-called "collagen diseases" appear to be closely related to an abnormal increase in the amount of extracellular connective tissue substance in the lesions. Responses to cortisone therapy are usually marked by a decrease in the amount of ground substance in the lesions. Ragan and co-workers(1) have shown that cortisone inhibits the formation of granulation tissue in wounds. We have shown(2,3) that wound granulation tissue *in vitro* and *in vivo* exhibits high capacity for the incorporation of inorganic sulfate into the chondroitin sulfate of the tissue. Considering these facts, it appeared to us that cortisone might exert its action through the inhibition of the synthesis of certain mucopolysaccharides by connective tissue cells. If this should be the case, one might expect cortisone to cause a decrease in the synthesis of chondroitin sulfate from inorganic sulfate. We have found(4) that the labeled inorganic sulfate fixed by tissues maintained *in vitro*(2,3) or *in vivo*(5) is bound in the chondroitin sulfate of the connective tissue. Using a method similar to that of Meyer and Rapport(6), it was found that the action of hyaluronidase upon the labeled sulfate containing material was very similar to its action upon the chondroitin sulfate from bovine connective tissue. It appeared to us that the labeled sulfate was bound in the chondroitin sulfate of the connective tissue ground substance.

Since the *in vitro* method of studying anabolic sulfate metabolism lends itself to quantitative determinations, it was considered desirable to use the method to study the influence of cortisone upon synthesis of chondroitin sulfate by tissues.

Experimental. Preliminary experiments with chickens had indicated that dosages of cortisone acetate approximating 25 mg per day per kilo of body weight had no effect upon wound healing. This was in marked contrast to the effect in rats where we found that 8 mg per day per kilo almost completely suppressed the formation of granulation tissue. Since most of our quantitative data were for chicken tissues, it was considered desirable to continue with this species. The experimental procedure used in this investigation has been described in detail in previous papers(2,5,7). Briefly, it is as follows: Replicate samples of tissue weighing approximately 3.0 mg each were prepared from the heart, skeletal muscle, and liver of chick embryos on the sixteenth day of incubation. Samples were also prepared from the regenerating tissue of healing muscle wounds of young adult chickens. The granulation tissue was removed from sterile wounds on the fifth day following partial section of *M. pectoralis* major. Each sample was placed in 2 ml of sterile medium† containing sodium sulfate and graded concentrations of recrystallized cortisone alcohol.

* The technical assistance of Gladys Friedler, Angela Congiundi and Doris Frankel is acknowledged. The radioactive sulfur used in this investigation was supplied free by the United States Atomic Energy Commission. This investigation was supported by grants from the American Cancer Society upon recommendation of The Committee on Growth of The National Research Council. The cortisone was supplied by Merck and Co.

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6. Meyer, K., and Rapport, M. M., *Arch. Biochem.*, 1950, v27, 287.

† Tyrode's solution modified to contain 4.8 mg of sulfate ion per liter of solution. The sulfate was labeled with radioactive sulfur, S^{35}, to give a specific activity of 6.5×10^7 counts per mg of sulfate ion as determined by a method described earlier(5).

TABLE I. Effect of Cortisone Alcohol Upon Chondroitin Sulfate Synthesis and Sulfate Esterification by Embryonic Chick Tissues *in Vitro*.

Tissue	Conc. of cortisone in medium, mg/l	Sulfate fixed by tissues (μg/100 mg tissue): Calculated as SO_4 ion	Sulfate fixed by tissues (μg/100 mg tissue): Calculated as chondroitin sulfate synthesized	Wt of SO_4 ion esterified (μg/100 mg tissue)
Skeletal muscle	0	1.3	5.85	.45
" "	35	.32	1.4	.28
" "	70	.15	0.68	.20
Heart ventricle	0	1.41	6.3	.53
" "	35	.45	2.0	.33
" "	70	.22	1.0	.23
" "	120	.10	0.45	.10

TABLE II. Effect of Cortisone Acetate and Cortisone Alcohol Upon Chondroitin Sulfate Synthesis and Sulfate Esterification by Tissue from Healing Wounds in Chickens.

Conc. of cortisone in medium	mg/l	Sulfate fixed by tissues (μg/100 mg tissue): Calculated as SO_4 ion	Sulfate fixed by tissues (μg/100 mg tissue): Calculated as chondroitin sulfate synthesized	Wt of SO_4 ion esterified (μg/100 mg tissue)
Controls, none	0	1.4	6.3	.70
Susp. of cortisone acetate	200	1.2	5.4	.60
Cortisone alcohol	25	1.2	5.4	.60
" "	50	.50	2.3	.40
" "	75	.30	1.4	.20
" "	120	.08	0.36	.07

Tissues were removed on the fifth day after wounding.

In order that we might observe the effect of cortisone upon growth and survival of tissues *in vitro*, control cultures of embryonic heart tissues were prepared in Carrel flasks. The tissue fragments were placed in plasma clots and covered with bovine serum ultrafiltrate containing embryo extract and concentrations of cortisone alcohol equivalent to those used for the chemical studies. Heart, skeletal muscle, and wound tissues were incubated at 37°C for 45 hours. Liver tissue samples were incubated at 21°C for 70 hours in order to obtain maximum conjugation of the sulfate in the medium(7). At the end of the incubation period the tissues were removed from the medium and soaked for 20 minutes in cold tap water. The washings were combined with the culture medium, and the soluble labeled organic sulfate in the medium was determined by the method of Layton and Frankel(7). The labeled sulfate fixed in the tissue was determined by the author's method (5).

Results. By observing the tissues in Carrel flasks it was found that high concentrations of cortisone did not affect the migration of fibroblasts during the first 72 hours. Rapid degeneration of fibroblasts occurred after the third day. Pulsation of heart tissues continued for more than 2 weeks in the presence of cortisone. It would appear that the tissues were capable of carrying on essential metabolic activities while maintained in concentrations of cortisone which suppressed chondroitin synthesis.

It was found that cortisone caused a graded inhibitory response in the fixation of labeled sulfate by embryonic tissues. The sulfate fixation was almost completely suppressed by cortisone concentrations in excess of 100 mg per liter. The esterification of sulfate by heart and skeletal muscle was inhibited to

7. Layton, L. L., and Frankel, D. R., *Arch. Biochem.*, in press.

TABLE III. Effect of Cortisone Alcohol Upon Sulfate Conjugation by Embryonic Chick Liver Tissue *in Vitro*.*

Conc. of cortisone in medium, mg/l	No. of samples	Wt of sulfate ion esterified (μg/100 mg tissue)
0	12	4.4
50	12	5.2
100	12	4.5

* No phenol was added to the medium (7).

approximately the same degree as was the fixation. Data from a representative experiment are shown in Table I.

From the data of Table II it will be seen that tissue from healing wounds was similar to embryonic tissue in its response to cortisone. Conjugation of sulfate by liver tissue was not affected by concentrations of cortisone which completely suppressed sulfate fixation and synthesis of soluble organic sulfate by heart and skeletal muscle (Table III).

Sulfate conjugation by the liver tissue is probably a detoxication mechanism (7-9) and hence a phase of the catabolic metabolism. The fact that cortisone did not affect the synthesis of ester sulfate by liver tissue, while it did inhibit esterification of sulfate by heart and skeletal muscle tissues may indicate that different mechanisms are involved. It is possible that the soluble organic sulfates synthesized by heart and muscle are related to the connective tissue and plasma mucoids and to heparin. The decrease in the sedimentation rate of the blood of patients receiving cortisone therapy may be due to a decreased synthesis of soluble plasma mucoids by the connective tissue cells.

It is possible that the system affected by cortisone therapy is the one which synthesizes the chondroitin sulfate moiety of the mucoids. These investigations have been extended to a preliminary study of tissues *in vivo*. Similar results were obtained. These will be reported in a subsequent paper.

Summary. 1. The experiments described indicated that cortisone inhibits the synthesis of chondroitin sulfate by embryonic and wound tissues maintained *in vitro*. Graded responses were obtained with graded concentrations of cortisone in the medium. Cortisone was shown to inhibit the synthesis of soluble organic sulfate by heart and skeletal muscle; it had no such effect upon the synthesis of soluble ester sulfate by liver tissue. 2. Concentrations of cortisone which suppressed sulfate fixation, had no apparent effect upon the initial migration of fibroblasts. Cortisone had no effect upon the pulsations of heart tissue. 3. It was suggested that the palliative action of cortisone in the connective tissue diseases may be due to its inhibitory effect upon the synthesis of the chondroitin sulfate moiety of the connective tissue ground substance.

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Received February 20, 1951. P.S.E.B.M., 1951, v76.

On the Existence of a Cell Granule in a Thermophilic Bacterium.* (18572)

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In a study of a thermophilic bacterium† (1-3) we showed that most of the enzymes

* Aided by a grant from the Division of Research Grants and Fellowships, U. S. Public Health Service.

† Thermophile No. 2184 obtained from the National Canners Association. This organism has tentatively been identified by us as *Bacillus stearothermophilus* (Donk).

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