STUDIES ON THE PATHOGENESIS OF ACUTE INFLAMMATION

II. THE ACTION OF CORTISONE ON THE INFLAMMATORY RESPONSE TO THERMAL INJURY*

BY FRED ALLISON, JR., M.D., MARY RUTH SMITH,§ AND W. BARRY WOOD, JR., M.D.

(From the Departments of Medicine and Preventive Medicine, Washington University School of Medicine, and the Barnes and Wohl Hospitals, St. Louis)

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(Received for publication, July 26, 1955)

It has been clearly established that cortisone, when given in sufficiently large doses, suppresses inflammation (1). In conditions involving acute inflammation, it delays the mobilization of the inflammatory exudate. This delay, in the case of certain acute bacterial infections, leads to lowered resistance of the host (2–5). It may cause dramatic relief of symptoms in certain disease states (1).

The exact manner in which the drug interferes with the formation of inflammatory exudate is not known. An effect upon any one of the three following stages of acute inflammation might theoretically be involved. First, the drug might block the earliest phase of the reaction—namely, the margination and endothelial sticking of leucocytes, which invariably precedes diapedesis (6). Secondly, it might interfere with the process of diapedesis itself. Or thirdly, it might suppress the extravascular migration of the cells, which follows diapedesis, and thus prevent accumulation of leucocytes at the site of the lesion.

The results of the present study indicate that cortisone suppresses acute inflammation by blocking the earliest phase of the response; namely, that involving the endothelial sticking of leucocytes. This finding appears to have important implications concerning the role of cell injury in the pathogenesis of inflammation.

Methods

The methods used in the present experiments were the same as those fully described in the preceding report (6). In no instance was a single rabbit ear chamber used for more

* These studies were supported by a grant from the Life Insurance Medical Research Fund.

† Present address: Department of Internal Medicine, University of Mississippi, Jackson.

§ Present address: Department of Microbiology, Johns Hopkins University School of Medicine and School of Hygiene and Public Health, Baltimore.

1 Cortisone (cortisone acetate) was generously supplied by Merck and Co., Inc., Rahway, New Jersey.

2 Schenley Laboratories, Inc., Lawrenceburg, Indiana, kindly provided the syncrobins (penicillin and streptomycin) used in this study.
RESULTS

Effect of Cortisone on Vasomotor Phenomena.—The preliminary treatment with cortisone substantially reduced the total volume of blood flow in the ear chambers (Figs. 1 to 4). An increase in arteriolar tone, as seen in Figs. 5 and 6, appeared to account for the diminished flow. Vasomotion, however, was not otherwise altered by the hormonal therapy during the pre-injury period.

Following the thermal insult, the circulation in the circle of tissue immediately surrounding the platinum wire remained permanently occluded (Fig. 7) exactly as in the untreated animals (6). The inflammatory reaction that subsequently evolved in the outlying tissue, however, was quantitatively different from that observed in rabbits receiving no cortisone (6).

In the peripheral vascular bed of the chamber the ischemia which followed the initial insult was prolonged from the usual 3 to 5 minutes to approximately 10 to 15 minutes. The blood flow gradually returned as the constriction subsided, but there was a marked delay, frequently for 2 to 3 hours, before vasodilatation set in. Eventually, dilatation of a mild degree developed but, as shown in Fig. 8, the caliber of vessels was often no greater even after 24 hours than that observed before the start of hormonal therapy. The increased pulsations, which accompanied vasodilatation in untreated animals, were also prevented. Thus by maintaining arterial tone after injury the cortisone tended to nullify the usual engorgement of the peripheral vasculature (Figs. 9 and 10). This effect, as will be described below, led to a suppression of the edema and hemorrhage regularly observed in untreated rabbits (6).

Failure of Hormone to Suppress Formation of Intravascular and Extravascular “Globules” Resulting from Thermal Injury.—Cortisone treatment had no influence on the occurrence of the globular bodies previously noted in the rabbit ear chamber following thermal injury (6). Their number and distribution appeared to be the same as those observed in untreated animals.

Post-Treatment Stasis, Edema, and Hemorrhage.—Early in the postinjury period, rouleaux formation of the red cells developed in vessels adjacent to the lesion; clumping and circulatory stagnation rapidly followed. The vascular stasis which became fully developed within 6 hours after injury was of the same distribution and extent as observed in untreated animals (6).

Edema formation, on the other hand, was found to be lessened. Hemorrhage about static vessels, although detectable within 3 to 6 hours after the insult
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(Figs. 11 and 12), also failed to extend rapidly during the period of observation. After 24 hours, in contrast to the findings in untreated animals, little or no hemorrhage was visible to the naked eye. As shown in Fig. 16, the perivascular accumulation of erythrocytes was minimal and did not obscure the microscopic details of the tissues.

Effect of Cortisone on Endothelial Sticking of Leucocytes.—In cortisone-treated animals before injury the number of circulating leucocytes appeared to be unchanged and there was no evidence of endothelial sticking. Immediately after injury a few white cells were seen to be sticking to the walls of vessels nearest to the damaged area but, with the passage of time, they continued to remain relatively scarce. Despite the progression of stasis during the first 30 minutes after the insult, there was little evidence of increased endothelial adhesiveness (Figs. 13 and 14). Appreciable leucocytic sticking was usually delayed for 2 to 3 hours, as seen in Fig. 15; at this time the unilateral phase of the reaction was often encountered. Thereafter, the adherence of white cells rapidly increased, but rarely did it attain either the intensity or the extent of that observed in untreated animals (6). Leucocytic thrombi resulting from the intercellular sticking of white cells (6) were occasionally noted during the most advanced stages of the reaction. Within 6 to 9 hours after injury the leucocytic sticking began to regress, and after 24 hours it was still detectable at only a few scattered points near the edge of the lesion.

The above findings indicate that cortisone had a pronounced suppressive effect upon the endothelial sticking of leucocytes. The onset, intensity, extent, and total duration of the reaction were all definitely influenced by the hormonal therapy.

Lack of Effect on Diapedesis of Leucocytes.—There was no demonstrable evidence that cortisone impaired in any way the passage of the leucocytes through the walls of the blood vessels once the cells had become adherent to the endothelium. No alteration of cellular motility was noted, and there was no increase in the incidence of incomplete diapedesis. Indirectly, however, the hormone did suppress diapedesis by preventing the endothelial sticking of the leucocytes. As emphasized previously (6), leucocytes do not undergo diapedesis until after they have become attached to the vascular endothelium.

Failure of Hormone to Influence Extravascular Migration of Leucocytes.—Once they had entered the tissue interstices, the leucocytes migrated at the usual speed and appeared to be unaffected by the hormone. As in the untreated

*The circulating white blood cells are well seen in the moving pictures but cannot be visualized in the still pictures because of their rapid motion in the blood stream.

* Shulman and coworkers (7) while studying the vascular effects of cortisone in the hamster cheek pouch noted endothelial sticking of leucocytes after prolonged administration of the hormone. They ascribed this effect to cortisone "poisoning." Although carefully looked for, no such effect was noted in the present experiments.
animals, the motion of the individual cells was predominantly random, but the resultant migration of all the cells eventually led to an accumulation about the site of injury (6). The number of leucocytes, however, which finally infiltrated the lesion in the cortisone-treated animals was significantly less than in the untreated controls (Figs. 17 and 18). This difference resulted from the suppressive action of the hormone upon the endothelial sticking of the leucocytes, preceding diapedesis; it was not due to an effect upon their extravascular migration in the tissues.

**Effect of Cortisone on Over-All Appearance of 24 Hour Lesion.**—Although the 24 hour lesions in the cortisone-treated rabbits exhibited the same four distinct zones previously described in untreated animals (6), the features of each area differed significantly from those in the untreated group (see Fig. 16). In the first or central zone, immediately surrounding the platinum wire, only a moderate number of motile leucocytes were noted and extravasated red cells were scarce. These findings were in marked contrast to those observed in the lesions of the untreated animals, in which the leucocytic infiltration was dense and extravasated erythrocytes were common. In the second zone, characterized by vascular stasis, far less perivascular hemorrhage and edema were present in the cortisone-treated group, although the amount of stasis appeared to be about the same as in the untreated controls. In the third zone, at the outer edge of the static area, only minimal signs of vasodilatation and leucocytic sticking could be detected in the treated rabbits. And in the fourth, or peripheral zone, the marked vasodilatation noted in the untreated animals was virtually absent in those treated with cortisone. The contrast between the 24 hour lesions in the two groups of animals is thus evident.

**DISCUSSION**

Large doses of cortisone, when given in advance of thermal injury, exert an impressive inhibitory effect upon the ensuing inflammatory response. This effect results from a suppression of the initial stage of the reaction, namely that involving the adherence of leucocytes (and to a lesser extent the other cellular elements of the blood) to the endothelium of blood vessels surrounding the site of injury. Since diapedesis of leucocytes is invariably preceded by adherence of the cells to the endothelium (6), inhibition of this essential phase of the inflammatory reaction secondarily blocks diapedesis and in turn prevents the accumulation of extravascular exudate. In addition, the hormone, by maintaining arteriolar tone, suppresses the intense vasodilatation characteristic of acute inflammation and thus lessens the promotion of hemorrhage and edema. The circulatory stasis and thrombosis, on the other hand, which occur at the sites of maximum damage in the lesion are not demonstrably influenced by the treatment.

These findings are in essential agreement with earlier studies of Ebert and Barclay (8) dealing with somewhat slower inflammatory reactions caused by
tuberculous infections and serum sickness. They also confirm the recent observations of Zweifach (9) and Shulman et al. (10) concerning acute reactions to microtrauma in the rat’s mesoappendix and thermal injury in the hamster cheek pouch. The results of both of the latter investigations were reported while the present ones were in progress. In all four of these separate studies, which were conducted in vivo, cortisone was found to suppress the endothelial sticking of leucocytes and to inhibit arteriolar dilatation.

Shulman and his collaborators in a second publication (11) have suggested that the anti-inflammatory action of cortisone may result from its vasoconstrictive effect. It might be argued that the reduction in blood flow brought about by the hormone causes fewer leucocytes to be available for sticking and diapedesis. That this thesis must be questioned is indicated by the observation made in the present study that the number of circulating leucocytes in the lesion is not demonstrably decreased by cortisone. Only the number adhering to the vascular endothelium is affected. Furthermore, since cortisone may cause a delay of several hours in the sticking reaction, the total number of leucocytes passing through each patent vessel during this time must be so large as to make variations in white count of little consequence. The potential supply of leucocytes, therefore, does not appear to be significantly altered by vasoconstriction. Also vasodilatation has been shown not to be a prerequisite of leucocytic sticking and diapedesis (6, 9). Both may occur in undilated vessels. Thus the mere prevention of vasodilatation cannot alone account for the anti-inflammatory action of cortisone.

Ebert and Barclay (8) have postulated that cortisone in some way protects the endothelium from injury and thus blocks the inflammatory response. In support of this hypothesis are two important observations which have been made in vitro. Leahy and Morgan (14) in studying tuberculin-sensitive macrophages in tissue culture have demonstrated that treatment of the culture with cortisone will prevent the well known cytotoxic effect of added tuberculin. Of particular interest is the fact that the introduction of cortisone into the culture must be begun several days in advance, if the protective effect is to be obtained. The necessity for such preliminary treatment with the hormone has been repeatedly observed in vivo (15). Likewise, Kerby and Barrett (16) have reported that the white cells from patients undergoing treatment with cortisone, when tested in vitro, are relatively resistant to the damaging effect of bacterial endotoxin. To explain such direct protective action upon isolated cells, Szent-Györgyi has suggested (17) that adrenocortical steroids may affect cellular membranes in the same manner as steroids derived from cardiac

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*Reasonably accurate quantitative estimates relating to this important point were made possible by the continuous cinematographic recording of inflammatory reactions in individual blood vessels. These direct in vivo observations are also in agreement with results obtained by conventional methods of cellular counting (12). In fact, the relative increase in polymorphonuclear leucocytes noted in the differential counts (13) should provide more rather than fewer granulocytic cells for endothelial sticking.*
glycosides. In keeping with this attractive hypothesis is the observation of Martin et al. (18) that the mechanical disruption of leucocytes blocks the depressive effect of cortisone upon their production of lactic acid.

If the thesis is tentatively accepted that cortisone therapy alters cellular surfaces in a manner which protects them against noxious stimuli, it might be assumed that the hormone would also exert a similar protective action upon the tissue cells subjected to the original injury. Such an action at the primary site of injury would be expected to protect the cells of surrounding blood vessels secondarily; for it would spare them from the injurious products of tissue breakdown which otherwise would reach them by diffusion (19). There is no experimental evidence, however, to support the view that cortisone lessens the cellular damage caused directly by the inciting stimulus. In the present studies, for example, the portion of the lesion produced directly by the burn itself appeared exactly the same, whether in untreated or cortisone-treated rabbits. The failure of cortisone to influence the primary tissue response may be due to its inability to alter cellular reactions to relatively strong stimuli. It is well known that such stimuli will often “break through” the anti-inflammatory effect of the hormone (20). Although cortisone may eventually be shown to block production of the tissue factors which cause inflammation, the evidence that it does so is at present unconvincing.

SUMMARY

The anti-inflammatory action of cortisone upon the acute cellular response to thermal injury has been systematically studied in the rabbit ear chamber. The hormone has been shown to suppress the reaction of acute inflammation in its earliest recognizable phase; i.e., that involving vasodilatation and the adherence of leucocytes to the vascular endothelium. Evidence has been presented that the anti-inflammatory effect of the hormone cannot be explained on the basis of its vasoconstrictive properties alone. The experimental observations support the hypothesis that cortisone exerts a direct protective action upon endothelial cells and leucocytes, and that in so doing, it renders them refractory to the tissue products which initiate inflammation.

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EXPLANATION OF PLATES

Hoffman Laboratories, Inc., New York, generously processed all still photographic film using the special fine grain developer, PRA. All photographic prints were made by Mr. K. Cramer Lewis, Department of Illustration, Washington University School of Medicine.

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Figs. 1 and 2. The status of the vascular bed of an uninjured ear-chamber before institution of cortisone treatment. Note the degree of filling of the vascular channels. The dark central area in Fig. 1 is the shadow of the platinum wire. Fig. 2 depicts vessels in a peripheral zone of the chamber. × 30.

Figs. 3 and 4. Same fields shown in Figs. 1 and 2 after 3 days of cortisone therapy. The reduction in volume of blood flow is apparent. × 30.

Fig. 5. Arteriole (A) and venule (V) uninjured ear chamber prior to cortisone treatment. Note the calibers of the two vessels. A lymphatic channel (L) lies adjacent to lower side of arteriole. × 250.

Fig. 6. The effect of 3 days of cortisone treatment on vascular tone. When compared to Fig. 5, the calibers of the arteriole (A) and the vein (V) are reduced. × 250.

Fig. 7. Five minutes after injury. Cortisone has failed to affect the primary lesion. Compare with size of primary lesion in untreated rabbit. See Fig. 3 of previous paper (6). × 40.

Fig. 8. Suppression of vasodilatation. 24 hours after injury the caliber of the arteriole (A) is still smaller than before the start of cortisone treatment (See Fig. 5). × 250.

Fig. 9. The peripheral vascular bed of an uninjured ear chamber before cortisone. Note the calibers of the arteriole (A), the venule (V), and the capillaries (C). × 65.

Fig. 10. Suppression of vascular dilatation by cortisone. The same field as in Fig. 9 but 24 hours after injury. The arteriole (A), venule (V), and capillaries (C) are not dilated; in fact, they are relatively constricted. Compare Figs. 9 and 10 with Figs. 4 and 5 of previous report (6). × 65.
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Fig. 11. The onset of hemorrhage about static vessels (arrows) 3 hours after injury. The area of injury is to the right. × 65.

Fig. 12. Suppression of hemorrhage by cortisone. The same area as in Fig. 11 but 24 hours after injury. Note that hemorrhage has progressed very little. Compare with Figs. 10 and 11 of previous report (6). × 65.

Fig. 13. A venule (V) and an arteriole (A) in a cortisone-treated rabbit before injury. Note the absence of leucocytic sticking. × 250.

Fig. 14. No sticking of white cells, in same venule (V) 30 minutes after injury. Compare with finding in untreated rabbit (Fig. 14, previous report (6)). Note that arteriole (A) has become slightly dilated following injury in spite of cortisone therapy. × 250.

Fig. 15. Absence of intense leucocytic sticking in the same venule (V) 3 hours after injury. Only a few adherent leucocytes are present. (Compare with Fig. 15 of previous report (6)). × 250.

Fig. 16. Appearance of ear chamber 24 hours after injury during continued cortisone administration. The four zones (1, 2, 3, 4) correspond qualitatively with those in the untreated animal. See Fig. 18 of previous paper (6). Quantitatively there is much less hemorrhage in the chamber of the treated animal. × 40.

Fig. 17. Tissue of central area of ear chamber during cortisone treatment but preceding injury. There are practically no cells visible in the extravascular tissue. × 250.

Fig. 18. Suppression of inflammatory exudate by cortisone. Same field as in Fig. 17. Although vascular stasis has developed, few cells have entered the extravascular tissue. Compare with heavy exudate seen in untreated animal (Fig. 28 of previous report (6)). × 250.
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