Sensitization of Individual Vascular Territories to the Antiphlogistic Effect of Hydrocortisone
An Example of "Selective Conditioning"

By Hans Selye, M.D., Ph.D., D.Sc., F.R.S. (C)

Experimental interference with the circulation in a circumscribed tissue area, too slight to produce any manifest disturbance in itself, can strikingly alter topical reactivity to irritants and to the antiphlogistic effect of hydrocortisone. Thus, certain vascular territories can be "selectively conditioned" to the anti-inflammatory actions of blood-borne corticoids. Conversely, when tissue damage is severe, such "selective conditioning" may induce a seemingly paradoxical reversal of the hydrocortisone effect, so that the hormone enhances inflammation. Here, impairment of circulation and hydrocortisone facilitate the production by irritants of necrosis, more than of inflammation. The resulting widespread necrosis induces an even greater inflammatory response, despite hydrocortisone, than would have been observed without hormone treatment.

It has been amply substantiated, both by experimental and by clinical observations, that the so-called "gluco-corticoids" or "antiphlogistic corticoids," such as hydrocortisone, can inhibit inflammatory responses to a great variety of irritants. It has also been demonstrated that during stress, the endogenous production of antiphlogistic corticoids, by the adrenal glands, can rise considerably above the resting level. Indeed, an increase in the urinary excretion and the blood level of these hormones is considered to be a characteristic feature of the nonspecific response to systemic stress. At the same time, the inflammatory responses to topical injury are also greatly inhibited. It has been suspected, therefore, that exposure to systemic stress inhibits inflammation, at least in part, as a direct result of the increased secretion of antiphlogistic corticoids.

It is quite unlikely, however, that the pronounced antiphlogistic effect of the alarm reaction (for example, after hemorrhage, starvation, pyrogens) could be exclusively due to this mechanism. Quantitative analyses showed that, in general, systemic stress is much more effective in inhibiting inflammation than treatment with exogenous corticoids given in amounts causing a comparable rise in the blood level or urinary excretion of these hormones.

The intense antiphlogistic effect of exposure to stress can be duplicated by hydrocortisone, cortisone or corticotropin (ACTH), but the quantities required are so great that they almost invariably produce manifestations of overdosage (for instance, changes similar to those of Cushing's disease). Such are not commonly observed when a similar degree of anti-inflammatory effect is produced by stress itself.

The rather voluminous literature dealing with this field has recently been reviewed in a series of monographs. In an attempt to reconcile the above apparently conflicting data, we assumed that certain extra-adrenal manifestations of the stress response "condition" the organism to the antiphlogistic action of endogenous corticoids, thus potentiating their effects.

The possibility of such conditioning has been amply demonstrated by animal experiments. For instance, in an adrenalectomized rat, treated with small doses of a cortical extract, the blood-sugar remained essentially within normal limits, but it rose to very high levels upon exposure to traumatic shock. In untreated adrenalectomized animals, a similar traumatic injury caused hypoglycemia. Hence, we concluded that some extra-adrenal con-
sequences of shock, though in themselves unable to raise the blood-sugar, have sensitized the animal to the characteristic hyperglycemic effect of the glucocorticoids contained in our extract.\textsuperscript{6}

The so-called "prophlogistic corticoids," for instance, desoxycorticosterone, are also subject to such conditioning by nonhormonal agents. Thus, the experimental production of myocarditis, periarteritis nodosa and renal changes, by overdosage with desoxycorticosterone, depends upon the simultaneous intake of sufficient sodium.\textsuperscript{5}

These observations made it highly probable that, in directing the course of inflammation, near-physiologic doses of corticoids could be optimally effective only in individuals suitably conditioned for their anti- or prophlogistic actions. Since conditioning can be organ-specific, this kind of sensitization may even explain why corticoids often exhibit an abnormal affinity for certain tissues only. Thus, sodium enhances the effect of desoxycorticosterone upon the heart, vessels and kidney, but fails to affect the ability of this steroid to cause an involution of the adrenal cortex. Here, evidently, a conditioning factor has sensitized certain tissues selectively.

On the other hand, even this type of sensitization fails to furnish an adequate explanation for the frequently observed regional differences in the hormone-sensitivity of structurally identical tissues. For example, systemic treatment with corticotropin or hydrocortisone may exert pronounced antiphlogistic effects upon the connective tissue of one region, while elsewhere in the patient, the same tissue proves to be relatively insensitive. A case in point is the occasional hormone refractoriness of certain joints in patients with rheumatoid arthritis who otherwise respond very favorably to this therapy. Furthermore, in rats receiving a constant daily overdosage with desoxycorticosterone, a "migrating polyarthritis" may develop. This often affects one joint first, then disappears there only to affect other articulations, while, at a later time, it may return to the joint which first suffered.\textsuperscript{6} Here again, we are forced to assume that, apart from systemic conditioning factors—which necessarily affect all tissues of the same kind more or less uniformly, irrespective of their position in the body—there are means by which tissues, in certain regions, can selectively alter their sensitivity to topical stress and to the hormones which influence inflammation.

The purpose of the experiments to be reported here was to explore the possibility that local vascular reactions may be responsible for the selective conditioning of certain body-regions to the antiphlogistic effects of hormones.

**Experimental Materials and Technics**

*Experimental Animals and Their Subdivision Into Groups.* Thirty young female hooded rats, weighing 45 to 60 Gm., have been subdivided into three groups of 10, each group having an initial average body weight of 54 Gm.

As indicated in tables 1 and 2, the rats of group I received no hormone treatment, those of group II were given hydrocortisone by the subcutaneous route, at a distance from the paws, and those of group III received this same hormone topically into both hind paws. (For details of hormone treatment, see below.)

The animals of all three groups were given 0.1 ml. of a "Combiotic" (Pfizer) solution, twice daily subcutaneously. They were fed with "Purina Fox Chow," occasionally supplemented by bread.

*Surgical Procedure for the Production of a Limited Vascular Deficiency.* In order to induce a vascular deficiency, selectively in one hind paw, a complete ligature of the left common iliac artery was performed through a longitudinal suprapubic incision on the first day of the experiment, in the rats of all three groups. As judged by the rapid normalization of skin color in the hind paws, this operation is soon followed by compensatory vascular changes; hence, on the sixteenth day, a second operation was performed, again in the animals of all three groups, in order to interfere further with the blood-supply. At this time, the abdomen was opened by a transverse incision to avoid the necessity of cutting through the previously formed scar. Through this approach, it was readily possible to ligate, or sever by mere tearing, all the collaterals arising from the left side of the abdominal aorta between the left renal artery and the (now ligated) left common iliac. At the same time, the caudal artery, which emerges from the aorta at its bifurcation into the two common iliacs, was also ligated.

Following this more extensive second operation, the color of the skin remained somewhat paler on the left than on the right side but, again, only during four to five days. After that, even the motility of the vasoligated limb proved to be
essentially normal; when the rats were forced to walk hanging upside-down from a grill, they used both hind paws with equal facility.

Treatment with Hydrocortisone. Hydrocortisone acetate was used in the form of Merck's microcrystal suspension. For subcutaneous injection, in group II, the original suspension of 25 mg. per milliliter was diluted by the addition of water, with a few drops of Tween 80, as a suspending agent. Of the resulting preparation, containing 10 mg. per milliliter, we administered 0.1 ml. subcutaneously on the back, every day, to all rats of the second group, between the first and the fourteenth day of the experiment. Since this dosage caused a very considerable decrease in the normal growth rate, it was subsequently diminished to 0.1 ml. every second day.

In group III, 0.1 ml. of the undiluted original suspension, that is the equivalent of 2.5 mg. of hydrocortisone acetate, was injected directly into each hind paw, half of this amount being deposited under the skin of the dorsum pedis, the other half under the plantar aponeurosis.

The Production of Inflammation. In order to produce inflammation, we used two fundamentally different irritants: dextran and croton oil. Dextran elicits a transient, purely serous type of inflammation, and acts quite selectively on certain regions (paws, snout), even when it is introduced by the intraperitoneal or intravenous route. It had first been seen in rats treated with egg-white, and since its morphologic features are reminiscent of anaphylactic or angioneurotic tissue reactions, it was designated—for lack of a better name—as the "anaphylactoid reaction" of the rat. Subsequent investigations have shown that this type of reaction is, by no means, specific for egg-white; it can also be elicited by dextran, hyaluronidase and many other substances. It is readily inhibited by antiphlogistic hormones.

Croton oil acts only where applied. It evokes a long-lasting, often hemorrhagic proliferative inflammatory response, which tends to proceed towards necrosis. For the purpose of this investigation, the commercial 6 per cent dextran solution was diluted 1:10 with physiologic saline. Of this, 2 ml. was injected intraperitoneally in all rats on the twenty-fifth day of the experiment, that is, nine days after the second operation.

The resulting anaphylactoid reaction was assessed by the appraisal of the swelling and hyperemia in the hind paws two hours after the dextran injection. The response was graded in a scale from 0 to ++++. This type of grading is not as accurate as could be desired, but its great advantage for the assessment of such transient reactions is the rapidity with which a reading can be made. In order to avoid any subjectivity in the grading, the degree of inflammation was graded by an assistant, experienced in such tests, but unaware of the arrangement and purpose of this particular experiment. The mean swelling was calculated, for the right and the left hind paws separately, by adding the total number of plusses and dividing their sum by the number of individuals in each group (see table 1). In addition, a characteristic representative of each group was selected for a photographic illustration of the response.

After a rest of 48 hours, that is, on the twenty-seventh day of the experiment, a so-called "topical irritation arthritis" was produced in both hind paws of the rats, in all three experimental groups, by the injection of 0.1 ml. of an 0.1 per cent croton oil solution (in corn oil). The immediate response was assessed two hours later, using the same scale as in the dextran experiment. In addition, a second reading was taken 10 days later, that is, on the thirty-seventh day of the experiment, to appraise the more slowly developing chronic inflammation and necrosis.

RESULTS

The results of the first experimental series are summarized in table 1 and are also illustrated by figure 1.

Our data clearly indicate that, in the rats of group I, which received no hydrocortisone, the anaphylactoid response to dextran was much more pronounced than in the animals which were given either subcutaneous or topical hormone treatment. Although the

Table 1.—Effect of Interference with the Arterial Blood Supply upon the Ability of Hydrocortisone to Inhibit the Phlogistic Effect of Dextran

<table>
<thead>
<tr>
<th>Group</th>
<th>Arterial Ligature</th>
<th>Hydrocortisone</th>
<th>Dextran</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>Mean</th>
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<tbody>
<tr>
<td>I</td>
<td>Left: yes</td>
<td>no</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>1.4</td>
</tr>
<tr>
<td>II</td>
<td>Right: no</td>
<td>subcutaneous</td>
<td>trace</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>2.2</td>
</tr>
<tr>
<td>III</td>
<td>Left: yes</td>
<td>subcutaneous</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Right: no</td>
<td>topical</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>
degree of reaction varied from animal to animal, in each case it was less pronounced on the left (vasoligated) side. Hence, it may be concluded that even a deficiency in arterial blood-supply, which causes no visible pallor, nor any noticeable impairment in the motility of the extremity, suffices to diminish the ability to respond with inflammation.

Parenthetically, it should also be mentioned that a certain percentage of the rats in our colony (such as animal number 10 in group I) are nonreactors to dextran or egg-white. But since this is rare it does not significantly vitiate the results of such assays.

After subcutaneous treatment with hydrocortisone in group II, the response is diminished on the normally vascularized (right) side. On the other hand, the vasoligated paw showed complete inhibition in all but one rat (animal number 1).

Topical treatment with hydrocortisone, as applied in group III, yielded essentially similar results. Here, with one exception, the inhibition was somewhat more pronounced on the right side. In any event, all 10 animals showed complete inhibition of the dextran reaction on the vasoligated (left) side.

The acute inflammatory reaction to croton oil assessed two hours after the injection, yielded results almost exactly identical with those of the dextran test. There were no spon-

<table>
<thead>
<tr>
<th>Group</th>
<th>Arterial Ligation</th>
<th>Hydrocortisone</th>
<th>Croton Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Left: yes</td>
<td>no</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
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<td>0 0 0 0 0</td>
</tr>
<tr>
<td>II</td>
<td>Left: yes</td>
<td>subcutaneous</td>
<td>++ ++ ++ ++</td>
</tr>
<tr>
<td></td>
<td>Right: no</td>
<td>subcutaneous</td>
<td>+ 0 0 0 0</td>
</tr>
<tr>
<td>III</td>
<td>Left: yes</td>
<td>topical</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>Right: no</td>
<td>topical</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

* The degree of inflammation is listed in the upper line with the necrosis (in brackets) underneath.
taneous nonreactors, and, in group I, the reaction was virtually equal on the right and left. This is presumably due to the fact that croton oil overcomes slight inhibitory influences more readily than dextran.

A second reading on these same animals, on the tenth day after croton oil injection (thirty-seventh and last day of the experiment), on the other hand, gave us important supplementary data, summarized in table 2.

In the animals of group I, inflammation had virtually disappeared by the tenth day after the croton oil injection, and no necrosis had developed, on either side. Evidently, the vasoligature in itself was not sufficient to alter tissue reactivity so as to induce any extensive cellular death upon contact with the irritant.

Conversely in both hydrocortisone treated groups, the most striking phenomenon was the frequent occurrence of necrosis, usually accompanied by some inflammation, on either side. In both these groups, the degree of necrosis was considerably higher on the left (vasoligated) than on the right side. Furthermore, in these two groups, inflammation was less pronounced than necrosis on the vasoligated side, while the two types of change were approximately balanced in the contralateral paw, whose blood-supply remained intact. However, to us, the most striking finding was that, under the conditions of this experiment, hydrocortisone, an antiphlogistic hormone, actually stimulated inflammation, this effect being further enhanced by the vasoligature, which in itself also diminishes inflammatory reactions.

Discussion

The data derived from the dextran experiment revealed that diminution of the arterial blood-supply decreases the ability of tissues to respond with an acute serous inflammation, at least to an irritant which reaches them through the blood. Thus, a normally latent circulatory deficiency can become manifest at times when the tissues are exposed to an irritant, which creates an increased requirement of blood, for the normal phlogistic response. (Compare right and left paws in group I of table 1.)

Subcutaneous treatment with hydrocorti-
evident in many of the croton-oil treated rats—inflammation was enhanced by hydrocortisone, alone or in combination with vasoligature.

Apparently, the hormone proved adequate in suppressing acute inflammatory phenomena following croton-oil injection, just as in the dextran tests. However, at a later stage, the results became complicated by the fact that hydrocortisone also enhances the susceptibility of tissues to necrosis in contact with the croton oil. Necrotic tissue is in itself a potent phlogistic stimulus and the irritation, resulting from the combined effect of the croton oil and the necrosis, apparently stimulated inflammation so much that the hydrocortisone no longer sufficed to prevent the phlogistic response. This would explain the otherwise apparently paradoxical and yet quite manifest, finding that hydrocortisone alone, and especially in combination with the vasoligature, actually stimulated the development of a chronic inflammatory response to a chemical irritant.

Published findings concerning the effect of experimental interference with the blood-supply upon inflammation are rather conflicting. For instance the latest report along these lines yielded entirely negative results. The iliac artery and vein of the rat were ligated on one side, and abscesses were then produced in both thighs by the injection of turpentine. “Despite the undoubted restriction of blood-supply of the operated side, no significant difference was found in the granulation tissue as compared with the control side.” Injection of cortisone, directly into the turpentine abscesses, inhibited granuloma formation, but again, to the same extent on the side of the vasoligature and in the contralateral thigh. As the authors themselves state, the development of a collateral circulation may have vitiated their results.

An extensive clinical literature emphasizes the frequency of various inflammatory manifestations in areas inadequately supplied by blood. It is very unlikely that, in such cases, interference with the blood-supply, as such, could have directly enhanced the reactivity of the tissues in the sense of a prophlogistic effect. It is much more probable that an inadequate blood-supply so interferes with the formation of a granulomatous barricade that even normally well-tolerated, mild stimuli (infections, mechanical or chemical irritants) cause widespread necrobiosis or even frank necrosis. Apparently an inflammatory granuloma formed in undernourished tissue is subnormal in its ability to delimit the damage from the healthy tissue. Hence, the injury tends to propagate itself over a wide area, forming an extensive but, functionally speaking, inferior inflammatory tissue.

Antopol noted that, in mice, overdosage with cortisone can produce “granulomatous nodules” in various tissues and, from these, he isolated Corynebacterium pseudotuberculosis murium. We have made similar observations, but found that if necrosis and infection are inhibited by simultaneous treatment with somatotrophic hormone, then the granulomatous nodules (which usually develop around the infected necrotic foci) do not appear. This is another apparently paradoxical condition, since the anti-inflammatory hormone (cortisone) seems to have produced, and the pro-inflammatory (somatotrophic) hormone inhibited, granuloma formation. All this becomes understandable now, for inflammation largely depends upon two factors: the degree of irritation and the ability to respond. Whenever conditions are such that the initial inhibition of inflammation permits rapid spread of tissue damage, the antiphlogistic stimulus itself can augment the area which is predisposed eventually to undergo inflammatory changes.

When we decided to undertake these experiments, it seemed almost self-evident that the effect of combining two essentially antiphlogistic agents—interference with blood-supply and hydrocortisone—would have to be an even greater inhibition of inflammation than could be obtained by either of them alone. However, such summation does not always occur in biologic reactions. In the present experiments, the necrosis facilitating effects of hydrocortisone and of vasoligature were additive. The antiphlogistic effect of the two compounds, however, proved to be mutually
synergistic only under conditions of mild irritation unaccompanied by necrosis.

The observations reported here clearly show that, the manifold, interacting mechanisms governing the phenomena of selective conditioning are very complex. Hence, synergisms and antagonisms between factors thus affecting tissue responses cannot be predicted with any degree of reliability on theoretic grounds and must be subjected to experimental verification in each instance.

SUMMARY

Experiments on the rat revealed that ligation of one common iliac artery with its chief collaterals causes a lasting, though latent, circulatory disturbance in the corresponding limb. If, after this operation, dextran is injected intraperitoneally, a serous inflammation develops in all four paws, but least in that with the latent vascular deficiency.

Hydrocortisone (subcutaneously in the back or topically in the paw) also inhibits this anaphylactoid response, but again most markedly in the region with diminished blood supply.

The synergism between the antiphlogistic effect of hydrocortisone and the vasoligature is particularly striking when the hormone is given subcutaneously and dextran intraperitoneally. Although both the antiphlogistic steroid and the irritant are brought to the paw through the blood-stream, a reduction in blood supply increases the effect of the former and diminishes that of the latter.

A synergism between the antiphlogistic effect of hydrocortisone and of the vasoligature could also be demonstrated when croton oil was injected directly into the paw. Hence, the selective sensitization to the antiphlogistic action of hydrocortisone by a reduction in blood supply is possible not only against a blood-borne irritant—like dextran—which causes anaphylactoid inflammation in hypersensitive tissues, but also against topical treatment with a substance which evokes inflammation wherever applied.

In rats not receiving hormone treatment, the inflammatory response vanished, without leaving any visible sequelae, either on the intact, or on the vasoligated side. This same dose of croton oil, however, frequently caused necrosis after pretreatment with hydrocortisone, especially on the vasoligated side. The areas of necrosis were usually surrounded by inflammation, despite the hormonal treatment, and that, even in the vasoligated extremities.

The fact that, under these circumstances, a potent antiphlogistic hormone, hydrocortisone, can actually stimulate inflammation, is apparently paradoxic. It is tentatively assumed that both vasoligation and hydrocortisone facilitate the breakdown of tissue by croton oil; the irritation of the resulting necrotic tissue being then added to that of the injected irritant, inflammation is stimulated to such an extent that the circulatory disturbance and the hormone fail to inhibit it.

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