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Physiological Functions of Glucocorticoids in Stress and Their Relation to Pharmacological Actions*

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Introduction and Background

Glucocorticoids and increased resistance to stress

Modern glucocorticoid endocrinology is a colorful, richly varied, but formless discipline—a profusion of cellular, physiological and pharmacological effects, seemingly unrelated through any central hormonal function. A current list of glucocorticoid effects might include such disparate items as stimulation of hepatic gluconeogenesis, inhibition of glucose uptake by peripheral tissues, suppression of inflammation, enhanced excretion of a water load, induction in various cells of tryptophan oxygenase and glutamine synthetase, suppression of numerous immune reactions, inhibition of secretion of several hormones and neuropeptides, and inhibition of activity of plasminogen activator and other neutral proteinases. Judging from recent writings on glucocorticoid physiology, an item that might be low on the list or missing altogether is "increased resistance to stress".

From the late 1930s into the 1950s, the topic of increased resistance to stress dominated many discussions of adrenocortical physiology. It provided the subject with an important unifying concept, particularly after it was found that stress, generated by almost any threat to homeostasis, stimulated a rapid increase in secretion of glucocorticoids.

Nowadays resistance to stress is rarely mentioned in the context of glucocorticoid physiology. However, its corollary that patients under treatment with glucocorticoids require extra amounts when stressed is usually strongly emphasized in relation to glucocorticoid therapy (1). Moreover, the importance of glucocorticoids for survival was brought out clearly in a survey by Dunlop (2) of the results of treatment of patients with Addison's disease from 1928 to 1962. Up until 1939, despite treatment with salt and cortical extract, most of the patients died within 2 years of diagnosis. When deoxycorticosterone acetate became available in 1939 survival increased significantly, but the lives of the patients remained very precarious. With the advent of cortisone in 1948, however, there was a dramatic improvement in survival, and Addisonian patients have since been able to lead nearly normal lives.

Reasons for the decline in the importance attributed to glucocorticoids in the response to stress are not hard to find. As we shall see, several emerge from a perusal of the literature from and about that period. Our main sources are a brief and charming personal account of this era by Gaunt (3), the monumental 1950 review by Sayers (4), and some of the books and articles of Selye (5–7) and Ingle (8–10).

By 1930 the extreme sensitivity of adrenal-deficient animals to stress from various sources (*e.g.* trauma, infections, strenuous exercise) was evident to many workers (3). With the cortical extracts that became available at that time, and the pure steroids that appeared later, it was gradually recognized that the adrenal cortex was responsible for at least two types of activity produced by two types of steroid: those that affect mainly salt and water metabolism, of which deoxycorticosterone was the prototype; and those that affect carbohydrate metabolism, of which cortisone was the prototype (3, 4). Selye dubbed them respectively mineralo-corticoids and glucocorticoids (6). Ironically, neither dexoycorticosterone nor cortisone, both of which were to play central roles in the rise and fall of Selye's theories on the relation of the adrenal cortex to stress and disease, have turned out to be important as hormones. Deoxycorticosterone has been superseded by aldosterone. Cortisone, shown to be dependent for glucocorticoid activity on its conversion to cortisol (11), was predicted (11), and subsequently demonstrated (12, 13), to have little or no affinity for glucocorticoid receptors.

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The general adaptation syndrome

The adrenocortical function of conferring resistance to stress was eventually ascribed to the glucocorticoids. In 1946 Selye (5) published an influential review with over 700 references. The review began by surveying studies on the responses to stress (many of them Selye's own) in terms of the General Adaptation Syndrome or G.A.S., defined as: the sum of all nonspecific, systemic reactions of the body which ensue upon long continued exposure to stress. The concept of the G.A.S. developed by Selye over the preceding decade, focused attention on the stereotyped aspects of the response to stress elicited by almost any stimulus. Important elements of the response, such as thymic involution, were considered to be due to elevated levels of sugar-active hormones or glucocorticoids. This part of the review appears to have been received by contemporary scientists with relative equanimity; it clarified, and codified within a theoretical framework, a view of responses to stress that was already widely shared. Nonetheless, an important debate did arise over the precise role of the glucocorticoids. We digress briefly to discuss this issue before returning to Selye's review.

"Permissive" or "normalizing" versus "regulatory" effects

During the 1940s, Ingle and other workers had begun to find evidence that glucocorticoids often functioned in a permissive or normalizing way (8, 9), which meant that their presence at basal levels was sufficient to permit normal expression of certain responses to stress and to other hormones. Permissive effects, therefore, in contrast to the "regulatory" effects postulated in Selye's theory of the role of glucocorticoids in stress, did not require increased levels of glucocorticoids in order to confer resistance to stress. The view that glucocorticoids played permissive roles thus came to be seen as an alternative to Selye's theory despite the fact that Ingle and others recognized that the severely stressed animal both required and secreted greater than normal amounts of adrenal cortical hormones (4, 8, 9, 14).

Although permissive actions probably manifest important functions of glucocorticoids at basal concentrations they have proved difficult to define and analyze and have sometimes been used as a catch-all for poorly understood glucocorticoid effects (*cf.* 15, 16). Nonetheless, whereas these actions are still discussed in texts on endocrine physiology, the G.A.S. is rarely mentioned.

Diseases of adaptation

The ideas on the G.A.S. in the first part of Selye's 1946 review seem to have foundered less under their own weight than because they were tied to the concept of "diseases of adaptation", the hotly controversial subject of the second part of the review. Such diseases, among which Selye listed diffuse collagen disease, allergy, and rheumatic diseases, were postulated to be caused by excessive or abnormal adaptive reactions to stress (5, 6), in other words by the G.A.S. gone awry. In experimental tests of this idea Selye had demonstrated, for example, that unilaterally nephrectomized animals kept on high salt diets and treated with large doses of deoxycorticosterone acetate developed lesions similar to some of those characteristic of naturally occurring diseases. The theory held that one of the principal causative agents of these diseases was excessive secretion of mineralocorticoids in response to stress.

Criticism of the theory was harsh and devastating (4, 8–10, 14). One among many telling objections leveled against it was the lack of evidence for involvement of high levels of mineralocorticoids in the etiology of most of the supposed diseases of adaptation. Another was the extreme artificiality of the conditions required by Selye to induce lesions experimentally.

Glucocorticoids and antiinflammatory effects

More than any direct criticism, however, what appears to have dealt the *coup de grâce* to the theory of diseases of adaptation was its failure to anticipate the most cataclysmic event in the history of glucocorticoid endocrinology. This, of course, was the discovery in the late 1940s of the antiinflammatory effects. Their first dramatic demonstration was the near-miraculous relief from symptoms of rheumatoid arthritis that followed treatment with cortisone or ACTH (17). The shock of this discovery and its effect on views of Selye's theory still echo in recollections of the time. To quote Gaunt (3): "The most unusual thing about this discovery was its unexpectedness." Shortly after the event Sayers (4) wrote as follows: "The findings are at odds with the thesis that the collagen diseases are induced by hyperactivity of the adrenal cortex." The thesis referred to was Selye's. In his scientific autobiography, Kendall (18), who shared the Nobel Prize in Medicine and Physiology of 1950 for his work on adrenal steroids that contributed to the discovery of the antiinflammatory effects, recalled that "The only reason for expecting that administration of ACTH might have another effect came from the hypothesis advanced by Dr. Hans Selye. For many years Selye had predicted that overactivity of the adrenal cortex is an etiologic factor in a large number of diseases. Among these diseases was rheumatoid arthritis. According to his prediction, the administration of ACTH would not relieve the symptoms of rheumatoid arthritis; rather, it would cause an exacerbation of symptoms."

By the 1960s the concept of diseases of adaptation, and with it the G.A.S., were largely ignored. The problem of how glucocorticoids protected against stress had also

ceased to attract the younger generation of glucocorticoid physiologists. For them, many new and fascinating areas, some of which we will discuss below, were beginning to open up.

"Pharmacological effects" and glucocorticoid physiology

Selye was not alone in failing to predict the antiinflammatory actions of glucocorticoids. No one had predicted them. Nor were Selye's theories the only casualties of the discovery of antiinflammatory actions. The major casualty, in fact, may have been glucocorticoid physiology itself, at least as a unified discipline. Commenting on the antiinflammatory actions, Sayers (4) wrote: "Unfortunately, the adrenal physiologist is at a loss to give a rational basis for these empirical discoveries". Later he added: "It would appear that the therapeutic action of ACTH and cortisone in the collagen diseases is pharmacological rather than physiological in nature". Thirty-five years after the discoveries, this "bizarre pharmacological overdosage effect", as Gaunt (3) has called it, still lacks a rational basis in physiology. Unable to come to terms with the antiinflammatory effects, physiologists relegated them to a pharmacological limbo, and there they have remained.

The difficulties that the antiinflammatory effects posed for adrenal physiology, though less acute than those faced by Selye's bold and provocative theories, were of the same kind. If stress-induced levels of glucocorticoids increased resistance to stress, as physiologists had good reason to believe in 1949, what hope was there of incorporating into adrenal physiology an action of glucocorticoids at high doses that suppressed, rather than enhanced, a normal defense mechanism like inflammation? The recourse, a natural one in the circumstances, was to exclude these actions from physiology—quite unnecessarily as we hope to show. At the same time, the idea that glucocorticoids protect against stress began to lose its status as a unifying hypothesis—also unnecessarily, we believe.

Modern glucocorticoid endocrinology

The fragmentation of glucocorticoid endocrinology initiated by the schism between physiological and pharmacological actions accelerated in the decades that followed, and the mystery of how glucocorticoids protect against stress deepened. Already in the 1950s it was discovered that glucocorticoids rapidly induce certain liver enzymes such as tyrosine aminotransferase and tryptophan oxygenase (19). No connection could be convincingly established between these enzymes and glucocorticoid functions.

During the 1960s the study of the mechanism of action of glucocorticoids began to pick up speed. For the first time it became possible to elicit metabolic actions of glucocorticoids by adding the steroids at physiological concentrations directly to isolated tissues and cells (12). The initial studies, which identified inhibitory effects of glucocorticoids on glucose transport by several peripheral tissues, generally took as their points of departure the classic physiological observations of the 1930s and 1940s on regulation by glucocorticoids of carbohydrate metabolism in whole animals (20). Gradually, however, as glucocorticoids at physiological concentrations were found to produce more and more effects in isolated cells of all types (*cf.* 16), and as the interests of many workers focused on underlying molecular mechanisms, ties to physiology became looser, if not irrelevant.

Nowadays the study of glucocorticoid actions in isolated systems is almost a self-sufficient domain, with closer links to cell biology, immunology and molecular biology than to glucocorticoid physiology. This drift away from physiology was probably inevitable, and is seen throughout endocrinology. With the glucocorticoids, however, it was accentuated by the abundant variety of effects they exert in isolated systems, and by the failure of glucocorticoid physiology to provide a conceptual framework through which these effects could be related. These actions on isolated cells are not simply *in vitro* artifacts; in most cases the evidence is strong that they are mediated by glucocorticoid receptors (15, 16), and it can be inferred that similar cells in whole organisms would be similarly influenced by circulating glucocorticoids. Glucocorticoid physiology would be greatly enriched if it could be harmonized with these new phenomena.

The discovery of glucocorticoid receptors (12, 13, 21), and the gradual realization that a single, basic molecular mechanism may initiate most glucocorticoid actions (15, 16), has had a unifying influence on glucocorticoid endocrinology. Glucocorticoid receptors have been found in virtually every nucleated cell type in the body, and as far as we know, all glucocorticoid receptors are alike (16). Glucocorticoid effects can consequently be defined succinctly as those which are mediated by glucocorticoid receptors (22). The uniformity of glucocorticoid mechanisms has an important bearing on the antiinflammatory effects. As we have argued elsewhere (23), despite many suggestions to the contrary most evidence indicates that these effects are mediated by the same kinds of molecular mechanisms as the normal physiological effects of glucocorticoids. Already at the level of primary mechanisms of action, therefore, there is no justification for segregating the antiinflammatory effects as pharmacological.

In a review completed in 1976 (16), we concluded that among the several hundred reports of direct glucocorticoid effects on isolated systems that we catalogued, none gave any clues to a cellular basis for the functions of

glucocorticoids in stress. We were looking for evidence that glucocorticoids enhanced the body's normal defense mechanisms, just as physiologists had been doing since the 1930s. The evidence had been growing steadily more negative. An early suggestion that by lysing lymphocytes, glucocorticoids released antibodies and thereby enhanced the immune response, could not be confirmed by experiment (4). As time went on it became clear that glucocorticoids quite generally suppressed immune responses just as they suppressed inflammatory reactions. In fact, by far the majority of direct glucocorticoid effects that had been identified up to 1976 were inhibitory (16). Most of the stimulatory effects were inductions of enzymes. Those trends probably still continue.

Glucocorticoids and intercellular mediators

A series of observations from the mid-1970s up to the present has begun to reveal a general cellular mechanism by which glucocorticoids may exert many of these inhibitory actions. What has been found is that glucocorticoids inhibit the production, and sometimes the actions, of a variety of intercellular mediators such as the prostaglandins and the lymphokines (23). These and other observations which we review in the next section, have suggested to us that many fundamentally important glucocorticoid effects are not immediate consequences of direct primary actions of the hormones on their target cells, but are secondary effects carried by a network of intercellular mediators that are under glucocorticoid control (24).

We have furthermore been struck by the parallel between the generally inhibitory effects of glucocorticoids on the lymphokines, which appear to be proteins and peptides, and on ACTH, CRF, and ADH. The parallel takes on particular significance (24) now that synthesis and secretion of ACTH is known to be linked with that of other peptides such as β-endorphin, which may also be influenced by glucocorticoids. These substances: lymphokines, hormones, neuropeptides, prostaglandins, and other mediators that we mention later, share the characteristic of being important, and in many cases essential, elements of the body's normal defense mechanisms and of being secreted in response to various forms of stress.

Hypothesis on glucocorticoid functions in stress

These recent results, together with the older work we have discussed and other evidence dealt with later, present what to us is an overwhelming case that glucocorticoids generally suppress rather than enhance our normal defense mechanisms. We believe that instead of excluding these phenomena from glucocorticoid physiology, we should accept them as valid manifestations of the physiological functions of glucocorticoids in stress. If we also accept as valid the evidence that glucocorticoids confer protection in stress, the question that remains is, how can these functions be reconciled?

We propose that: (1) the physiological function of stress-induced increases in glucocorticoid levels is to protect not against the source of stress itself, but against the normal defense reactions that are activated by stress; and (2) the glucocorticoids accomplish this function by turning off those defense reactions, thus preventing them from overshooting and themselves threatening homeostasis.

After reviewing results on mediators, in the last section we survey glucocorticoid physiology as seen in the light of these new results and of this hypothesis. Our goal will be to show that our hypothesis not only removes the barriers that have excluded many "bizarre pharmacological overdosage" effects from glucocorticoid physiology, but brings unity to such disparate glucocorticoid effects as those on carbohydrate metabolism, immune reactions, water balance, shock, and levels of certain enzymes.

To our knowledge this general hypothesis is new. As we will point out, however, hints, and sometimes quite explicit statements, of the point of view it expresses have cropped up in the context of several specific glucocorticoid effects.

Glucocorticoid Regulation of Lymphokines, Arachidonic Acid Metabolites, Hormones, Neuropeptides and Other "Mediators" of Stress-Induced Defense Mechanisms

General considerations

Of the mediators we discuss in this section, arachidonic acid metabolites such as the prostaglandins and leukotrienes have become familiar enough to endocrinologists to require little introduction. The lymphokines (25, 26), on the other hand, have yet to find a place in the endocrine sun, though they qualify as hormones at least as well as the prostaglandins. They share many properties with the brain peptides. Those lymphokines that have been characterized are proteins or peptides, act through high-affinity membrane receptors, and are secreted in response to stress in the form of infection or tissue damage. Many are regulated by glucocorticoids. Some lymphokines may even be brain peptides, and *vice-versa*. For example, lymphocyte activating factor (LAF, or interleukin 1[1]) appears to be identical to endogenous

[1] Abbreviations, synonyms, and trivial names used: IFN-γ, immune interferon, γ-interferon; MAF, macrophage activating factor; FRAF, Fc-receptor augmenting factor; LAF, lymphocyte activating factor; IL-1, interleukin 1; EP, endogenous pyrogen; MCF, mononuclear cell factor; TCGF, T-cell growth factor; IL-2, interleukin 2; CSF, colony stimulating factor; NK, natural killer; CRF, corticotropin releasing factor; ACTH, adrenocorticotropin, corticotropin; ADH, antidiuretic hormone, vasopressin; POMC, pro-opiomelanocortin; serotonin, 5-hydroxytryptamine, 5HT; dexamethasone, 9α-fluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione

pyrogen (EP) which acts on the hypothalamus; the opioid peptide β-endorphin has been reported to enhance lymphocyte proliferative responses. Like the brain peptides and other hormones, lymphokines have in recent years spawned major growth industries, in both a commercial and a metaphorical scientific sense (27). For reasons familiar to endocrinologists, a single lymphokine will often appear under several aliases associated with biological activities that at one time were ascribed to separate lymphokines. As lymphokines are purified, characterized, and synthesized by recombinant DNA techniques, their biological actions are being more sharply defined and their nomenclatures are being simplified.

Glucocorticoid effects on mediators have mostly been studied in culture systems with the techniques of modern biochemistry and cellular immunology. A few general points should be made. Wherever these effects have been carefully investigated from an endocrinological standpoint, they have been found to exhibit steroid specificity and concentration dependence consistent with the effects being mediated by glucocorticoid receptors (16). Typically what that means is that synthetic glucocorticoids like dexamethasone are ten-to-twenty times more active than cortisol and corticosterone, and that a half-maximal effect with dexamethasone, for example, is obtained at concentrations around 5 nM. We will disregard any effects requiring concentrations higher than 1000 nM, since there are strong reasons (16, 23) for believing that such nonspecific steroid effects have neither physiological nor pharmacological significance. Where tested, the effects have also been blocked by inhibitors of protein and RNA synthesis. The mechanisms through which glucocorticoids regulate mediators, including those involved in inflammation (23), appear similar to those through which they exert many other effects (16).

In the survey that follows we make no attempt to give exhaustive accounts of the biochemistry and physiology of mediators but simply refer to recent reviews and articles. Our main purpose is to document with results of the last decade the ubiquity of these glucocorticoid effects, and to provide sufficient background on each mediator so that we can later consider it in relation to glucocorticoid physiology as a whole. We will use the term mediator in a broad sense, so that we can include under this heading substances that functionally, at least, are comparable to conventional mediators.

Immune interferon (IFN-γ): Fc-receptor augmenting (FRAF), macrophage activating (MAF), and natural killer (NK) stimulating activities. Colony stimulating factor (CSF).

Interferons are a family of soluble protein factors first recognized almost 30 years ago by their anti-viral activities (28, 29). Immune or γ-interferon (IFN-γ), which we consider here, seems to be a product of antigen- and lectin-stimulated T lymphocytes. Human immune interferon consists of 146 amino acid residues (28). A form of human immune interferon that has been produced by DNA recombinant techniques is referred to as IFN-γ_1 (28). In connection with glucocorticoid effects, we will discuss three important activities of immune interferon: augmentation of monocyte and macrophage Fc-receptors, activation of macrophages, and enhancement of natural killer (NK) activity (28).

Mononuclear phagocytes (monocytes and macrophages) have surface receptors called Fc receptors that specifically bind the Fc portion of immunoglobulin G (IgG) (30). These receptors are important for recognition by macrophages of particulate antigens that have been antibody-tagged or opsonized, and are thus involved in clearance of immune complexes, bacterial pathogens, and, during the course of autoimmune disease, of antibody-tagged host cells. They may also guide mononuclear phagocytes in the destruction of tumor cells (31), in stimulation of immunoglobulin production (32) and in the release of inflammatory mediators (33).

Some years ago it was found that a lymphokine activity produced by activated human lymphocytes caused a pronounced increase (as much as 10-fold) in the number of Fc receptors on normal human monocytes and on the human leukocyte cell lines HL-60 and U-937 (34). This lymphokine was referred to as Fc-receptor augmenting factor (FRAF). FRAF production in cultures of mixed allogeneic human cells was specifically blocked by glucocorticoids (35). The dose-response relationship for this effect is illustrated in Fig. 1. Recently it was shown that immune interferon has the same effect on Fc-receptors as FRAF (36). Probably all of the FRAF activity detected in culture supernatants such as those assayed in Fig. 1 is due to immune interferon. Consequently, we can conclude that glucocorticoids inhibit production of immune interferon.

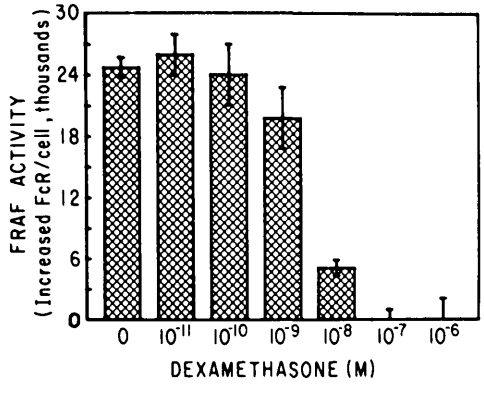

FIG. 1. Effect of dexamethasone on Fc receptor augmenting factor (FRAF) production. Dexamethasone was added at the start of FRAF production in an allogeneic mixed cell culture of human mononuclear cells. The supernatants were removed after 48 h at 37 C for assay of FRAF activity. Results are the means of triplicate Fc-receptor determinations. (From Ref. 35).

This demonstration of a direct glucocorticoid effect on isolated cells is consistent with early results indicating suppression by glucocorticoids of interferon formation in mice (37). It also suggests that glucocorticoids should inhibit other activities mediated by immune interferon, which they do.

Macrophages can be activated *in vivo* and in culture to lyse tumor cells (*cf.* 38). Activation in culture can be accomplished without the aid of antigen by supernatants from stimulated T lymphocytes which contain a lymphokine called MAF (38). MAF activity, assayed by the capacity to activate macrophages to lyse tumor cells under standard conditions, has been suspected for some time to be due to immune interferon (38) and it has recently been shown that MAF probably is immune interferon (39).

With cloned mouse T lymphocytes it has been found that several lymphokines are produced by the same cell (38). These lymphokines are: immune interferon, assayed by both antiviral and MAF activity; T cell growth factor (TCGF), which we discuss later, and colony stimulating factor (CSF), a growth factor (or factors) that stimulates production of granulocytes and macrophages from immature progenitor cells in culture (40).

When these T cell clones are treated with glucocorticoids at physiological concentrations, production of all the lymphokines is inhibited (41). The inhibition depends on steroid concentration in a manner similar to that shown for FRAF activity in Fig. 1 and is specific for glucocorticoids. Proliferation of the clones is also inhibited but much more slowly than lymphokine production. Consequently, inhibition of lymphokine production is not caused by lymphocyte death. The glucocorticoids therefore inhibit the rate of production per cell of each lymphokine. Such apparently coordinated inhibition of production of several lymphokines has interesting implications for underlying molecular mechanisms.

In the case of murine MAF activity, glucocorticoids inhibit not only production of the lymphokine but also the activation of macrophages by the lymphokine, and the tumoricidal activity of activated macrophages (42; Kelso, A. and Munck, A., unpublished data). The glucocorticoids thus inhibit at three levels. As we show later, by inhibiting LAF and TCGF production they can also decrease proliferation of T cells and thus may reduce the population of T cells that produces immune interferon.

NK activity is an activity that appears to be associated with a subpopulation of normal lymphocytes. It is measured by the ability of cells to spontaneously lyse certain tumor cell targets. There is considerable evidence that NK activity is involved in resistance to tumor growth *in vivo* and is a primary mechanism of immune surveillance (43).

Glucocorticoids administered *in vivo* are known to depress NK activity of human peripheral blood. Interferons, on the other hand, enhance NK activity in mice and rats, and in culture systems (43–45). It has now been shown that glucocorticoids, added at physiological concentrations directly to human peripheral blood leukocytes, inhibit NK activity (45). The effect is specific for glucocorticoids, and as shown in Fig. 2, gives a typical dose-response curve. Various types of interferons, including cloned immune interferon, enhance NK activity even in the presence of glucocorticoids, but in their presence the levels of enhancement are lower. For this reason it seems likely that although the inhibition of NK activity by glucocorticoids may be partly due to a block in endogenous production of interferon, other mechanisms operate as well (45).

Lymphocyte activating factor (LAF): endogenous pyrogen (EP), mononuclear cell factor (MCF)

This factor, also known as interleukin 1 (IL-1), is a product of macrophages, and therefore technically a monokine rather than a lymphokine. Murine LAF, which has only been partly purified, is a protein with molecular weight of 12,000 to 15,000. Physiologically, it accounts partly for the role of macrophages in the regulation of proliferation of T cells since it stimulates T cells to produce TCGF (46). An intriguing associated property of LAF is that of acting on the fever center of the hypothalamus, and it appears to be identical to EP (46, 47). In addition, it may be the trigger molecule that initiates synthesis of acute phase proteins that follows tissue injury, inflammation or infection (46). Finally, it

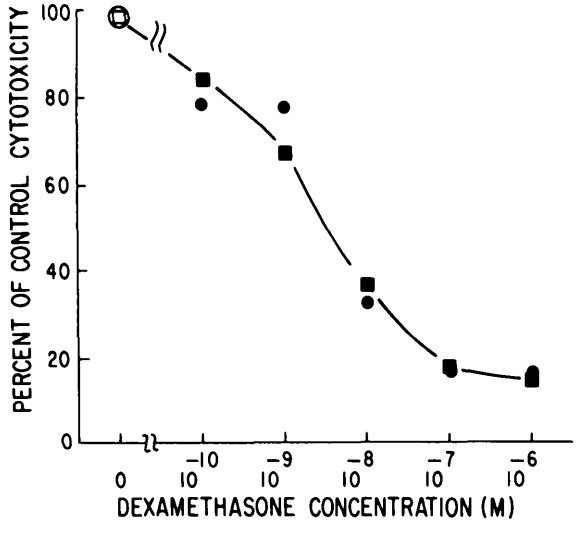

FIG. 2. Dose-response relationship for suppression by dexamethasone of natural killer (NK) activity of human peripheral blood leukocytes. Cells were treated with various concentrations of dexamethasone for 18 h prior to a 4-h assay for cytotoxicity against K562 target cells. Data are expressed as percentage of control cytotoxicity (untreated cells) at effector:target ratios of 50:1 (■) and 25:1 (●). The values are means of triplicates, with SD less than 10% of the mean in each case. (From Ref. 45).

may be identical to a MCF that increases the production of collagenase and prostaglandins by isolated rheumatoid synovial cells, raising the possibility that LAF acts on fibroblasts as well as lymphocytes (46).

Cortisol at physiological concentrations has recently been found to inhibit LAF production by murine macrophages (48). This effect, together with that on TCGF which we consider next, can account in large part for the suppression by glucocorticoids of immune responses that are mediated by T cells. The fever-suppressing effects of glucocorticoids (49, 50) can perhaps also be ascribed to inhibition of LAF (*i.e.* endogenous pyrogen) production.

T cell growth factor (TCGF)

TCGF, a product of T lymphocytes, is also known as interleukin 2 (IL-2) (26). Human TCGF has been purified to homogeneity and has an apparent molecular weight of around 15,000 (51–53). A cDNA from human TCGF has been cloned and used to determine the amino acid sequence of the protein (54). Including a putative signal sequence, it consists of 153 amino acids. The human TCGF gene has just been cloned and sequenced (55). Receptors for TCGF have been identified on intact antigen- and lectin-activated T lymphocytes (52), and on membranes from such cells (26). Binding to the receptors shows high affinity (K_d around 10 pM), and specificity for target cells and lymphokines in agreement with the biological activity of purified TCGF (52). For example, no binding of TCGF to B lymphocytes can be detected. TCGF appears to provide the key signal for proliferation of antigen-activated T cells, and thus is essential for the clonal expansion that follows the initial antigen–recognition phase of the normal immune response (26, 53). It has also been reported that TCGF stimulates NK activity (56–57) and simultaneously induces production of immune interferon (57). Whether the enhancement of NK activity is due to a direct effect of TCGF or is caused by the induced interferon is not known (57).

The discovery that glucocorticoids inhibit TCGF production by activated normal human lymphocytes (58, 59) has greatly clarified the understanding of how glucocorticoids suppress primary immune responses. Fig. 3 shows the dose-response relationship for inhibition by dexamethasone of TCGF production and [^{3}H]thymidine incorporation (a measure of cell proliferation) by lectin-stimulated human lymphocytes. Both are inhibited in parallel, therefore the experiment leaves open the question of whether cell proliferation is inhibited for lack of TCGF production, or *vice versa*. The result in Fig. 4 demonstrates that the fundamental effect of dexamethasone in this case is to inhibit TCGF production since replenishment of the cultures with exogenous TCGF stimulates the cells to almost normal rates of proliferation. The original experiments (58, 59) did not deal with the question of whether the inhibition of TCGF was secondary to inhibition of production of LAF, which, as we pointed out earlier, stimulates TCGF production. Subsequent experiments with cloned cells such as those described above have demonstrated that there is a direct effect on TCGF production independent of that on LAF.

Glucocorticoids have been known for many years to inhibit T cell mitogenesis (61), an effect generally ascribed to suppression of proliferation of lymphocytes in a glucocorticoid-sensitive stage of differentiation (cf. 62).

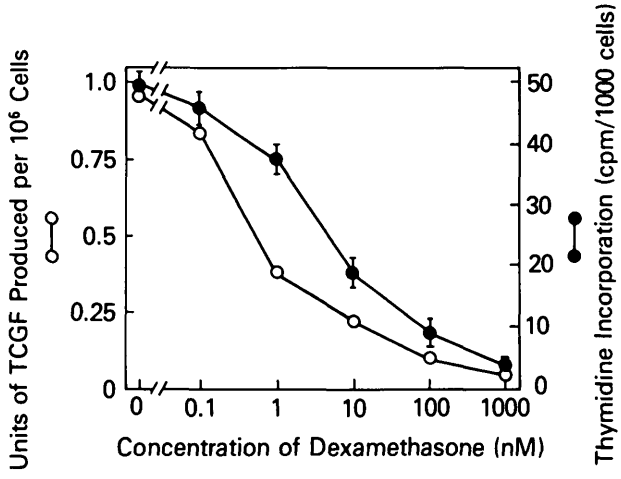

FIG. 3. T cell growth factor production and [^{3}H]thymidine incorporation by human peripheral lymphocytes stimulated for 48 h by phytohemagglutinin in the presence of varying concentrations of dexamethasone. T cell growth factor was assayed in the supernatants by using a bioassay which measures the ability of the sample to maintain the proliferation of a growth factor-dependent T cell line. In separate experiments the cell line used to measure T cell growth factor was found to be relatively resistant to the effects of dexamethasone. (From Ref. 60).

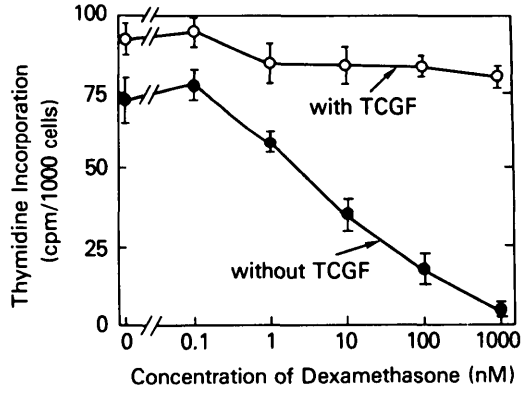

FIG. 4. The ability of T cell growth factor to overcome dexamethasone-induced inhibition of mitogenesis in phytohemagglutinin-stimulated human peripheral blood lymphocytes. [^{3}H]thymidine incorporation was measured after 72 h exposure to phytohemagglutinin and several concentrations of dexamethasone with and without T cell growth factor at 1 U/ml. (From Ref. 60).

The results we have just described, however, show that a major part of the glucocorticoid effect can be accounted for by the inhibition of production of TCGF and the consequent lack of clonal expansion of antigen-sensitive lymphocytes for which TCGF is essential. This mechanism provides a plausible explanation for the well-known fact that glucocorticoids are much more effective in suppressing an immune response, *in vivo* or in culture, when they are present early rather than late in the response. When present early, the glucocorticoids prevent the TCGF-dependent clonal expansion from taking place; when present late, they are ineffective because clonal expansion has already occurred (60). With this explanation there is no need to postulate changes in glucocorticoid sensitivity with stage of lymphocyte differentiation. As we have discussed elsewhere (62), the evidence for such changes is not strong.

Not all effects of glucocorticoids on lymphocyte proliferation are necessarily due to lack of TCGF. There is ample evidence that glucocorticoids can kill tumor cells under conditions where it is unlikely that TCGF plays any role (63). Furthermore, proliferation of some mouse T cell clones can be partly inhibited by dexamethasone even in the presence of excess amounts of TCGF (41).

Prostaglandins, thromboxanes and leukotrienes

The physiology and pharmacology of the arachidonic acid metabolites have been reviewed extensively in the last few years (64–66). Emphasis has recently been on the contribution of these substances to inflammatory and allergic processes but they are produced in, and can affect, virtually every mammalian organ system. Relatively little is known about their physiological roles under normal conditions, however. Reports of inhibition of prostaglandin production by glucocorticoids at physiological concentrations began to appear around 1975 (for review, see Ref. 23). The glucocorticoids block the release of arachidonic acid from cellular phospholipids by inhibiting the activity of phospholipase A_2. The molecular mechanisms through which the glucocorticoids act involve glucocorticoid receptors and appear to require RNA and protein synthesis (67, 68) as in most physiological effects of the hormones. Inhibition of phospholipase is apparently due to protein mediators, the secretion and synthesis of which are stimulated by glucocorticoids (68, 69).

With the discovery of the leukotrienes and their involvement as mediators of allergic and inflammatory reactions (66), the glucocorticoids have acquired new significance as antiinflammatory agents of wider scope than the aspirin-like compounds. Leukotriene synthesis would be expected to be blocked by glucocorticoids since arachidonic acid is an essential precursor, but not by the aspirin-like drugs which block the cyclooxygenase reaction that is not on the biosynthetic pathway to leukotrienes (66).

An aspect of glucocorticoid actions of potential significance for their role in stress is the protection that glucocorticoids provide against arachidonate-induced toxicity and death (70, 71). The mechanisms are unknown but from studies on rabbits (70) there is evidence that glucocorticoids decrease the plasma levels of thromboxane B_2 achieved after administration of arachidonate and increase the rate of clearance of arachidonate.

Bradykinin

Bradykinin, a nonapeptide of the kinin family, is released during tissue damage and causes inflammation through vasodilation, increased vascular permeability and other effects on the vascular sysem (23, 72). It induces release of arachidonic acid, with formation of prostaglandins and other arachidonic acid metabolites; these metabolites may be responsible for many of its effects (23).

Cortisol at physiological concentrations inhibits activation by bradykinin of cultured human synovial fibroblasts, and simultaneously blocks formation of bradykinin-induced arachidonic acid metabolites (73). Footpad edema induced locally by bradykinin in mice was reported to be inhibited by prior intraperitoneal injection of dexamethasone with a latent period of over 1 h. This antiinflammatory effect of dexamethasone *in vivo* was blocked by antiglucocorticoids, actinomycin D and cycloheximide, consistent with conventional glucocorticoid receptor-mediated mechanisms (74).

Serotonin

Serotonin (5-hydroxytryptamine) is an inflammatory agent, though physiologically it is probably more important as a neurotransmitter. Its inflammatory actions are elicited through increased vascular permeability (72).

In experiments very similar to those just described on footpad edema by bradykinin, dexamethasone has been shown to inhibit footpad edema caused by serotonin. The dexamethasone effect was again blocked by antiglucocorticoids, actinomycin D and cycloheximide (75).

Histamine

Histamine, like serotonin, may have dual functions as inflammatory agent and neurotransmitter (23, 76). It is released from mast cells and basophils in response to immediate hypersensitivity reactions, and causes vascular changes leading to inflammation (23, 72). Its levels in the hypothalamus are raised by stress (76). Glucocorticoids decrease release of histamine from rodent mast cells (23, 77) and human basophils (78), but apparently not from human lung mast cells (79).

Neutral proteases:plasminogen activator and collagenase

Here we deliberately begin to extend usage of the term of mediator beyond its usual boundaries to allow us to consider glucocorticoid effects in a wider context.

Neutral proteases are enzymes that are released in response to a variety of inflammatory stimuli. They are found at inflammatory sites where they apparently contribute to initiation and progression of normal inflammatory processes, but they are also potentially destructive.

Plasminogen activator, a serine proteinase produced by macrophages and other cells, converts plasminogen to plasmin, which initiates fibrinolysis, and participates in normal processes of tissue remodeling, kinin formation, complement production and cell migration. Excessive production of plasminogen activator can lead to tissue damage, and can cause blood vessels to leak and produce serious hemorrhage (23, 80). Collagenase is a major secretion product of adherent rheumatoid synovial cells and stimulated normal rat synovial cells. Its secretion by these cells, as well as by macrophages and chondrocytes, is stimulated by a variety of agents including lymphokines. By degrading collagen in cartilage, tendon, ligaments and bone, it may contribute to destructive lesions found in rheumatoid arthritis (23). Many of the peptides resulting from activity of these proteases, such as the kinins, are mediators of chemotactic and other activites (80–82). The neutral proteases themselves can therefore legitimately be regarded as links in chains or cascades of mediators.

Glucocorticoids have been shown to reduce dramatically the activity of plasminogen activator and collagenase (as well as elastase) induced in a variety of cell types by many different agents (83–89). The mechanism of inhibition of plasminogen activator has been studied with rat hepatoma (HTC) cells (90–92) and human fibroblasts (93). Inhibition has been shown to be due to induction by glucocorticoids of a cellular product that specifically inhibits plasminogen activator (90, 92, 93). Anucleate hepatoma cells treated with glucocorticoids do not produce the inhibitor although they have glucocorticoid receptors, which leads to the conclusion that induction of the inhibitor is probably due to transcriptional activation of the gene for the inhibitor (91).

Insulin and glucagon

These and other classical hormones which we will take up later appear to be influenced by glucocorticoids in ways similar to some of the mediators we have already considered. The physiological actions of insulin and glucagon in relation to those of the glucocorticoids will be dealt with in the last section. There is evidence from experiments both *in vivo* and with isolated pancreas that glucocorticoids inhibit insulin secretion. In man, prednisolone appears to inhibit the early insulin response to glucose (94). With isolated perfused rat pancreas, corticosterone at 100 nM inhibits acutely and strongly the secretion of insulin induced by both glucose and arginine (95). Glucagon secretion in this system is increased by corticosterone (95). These corticosterone effects in the perfused pancreas begin in less than 2 min from the time steroid infusion is started, which is extraordinarily rapid in comparison with rates of most known actions of glucocorticoids (16). The effects have not been shown to be specific for glucocorticoids, but if they are, their rapidity would seem to preclude their initiation through molecular mechanisms involving gene transcription such as can account for most glucocorticoid actions (15, 16).

Neural and pituitary peptides:corticotropin-releasing factor (CRF), antidiuretic hormone (ADH), corticotropin (ACTH), β-endorphin

The major research developments of the last few years in the field of brain and pituitary peptides have opened new vistas on the functions of these substances, and at the same time have revealed a broader role than contemplated in classic glucocorticoid physiology for the inhibitory action of glucocorticoids on the hypothalamus and pituitary. Various aspects of these subjects have been covered in numerous recent reviews. Here we cite only a few (96–100).

At the level of the pituitary, the most remarkable recent discovery is that the region on the chromosome that codes for ACTH is part of a gene that also codes for several other biologically active peptides including β-endorphin. When this gene is transcribed and translated it gives rise to a protein called proopiomelanocortin (POMC) that is processed by proteolytic cleavage to ACTH, β-endorphin, β-lipotropin and other peptides. The end products vary with the cells in which processing takes place (*cf.* 100).

At the level of the hypothalamus, the quest for CRF seems finally to have reached its goal with the characterization of a 41-residue peptide that fulfills most physiological and biochemical expectations for this hormone (101). Recently the cDNA for the ovine CRF precursor has been cloned and sequenced (102).

With these and other developments, the physiological significance of the actions of glucocorticoids on the hypothalamus and pituitary has broadened in several ways. One of these is that glucocorticoids appear to regulate the levels of β-endorphin and thereby its actions. Synthesis and/or secretion of β-endorphin and POMC are stimulated by CRF or stress, and inhibited by glucocorticoids acting both directly on the pituitary and indirectly *via* inhibition of CRF production (100, 101, 103–110).

(Whereas glucocorticoids suppress plasma ACTH and β-endorphin concomitantly in rats (103), it has been reported (111) that in humans and rhesus monkeys plasma β-endorphin is not suppressed). Furthermore, stress-induced analgesia, an effect ascribed partially to β-endorphin, is diminished in hypophysectomized animals (*cf.* 112), enhanced by adrenalectomy (112), and suppressed by glucocorticoids (112–114). β-endorphin is found not only in the brain but in many peripheral sites (115) and its actions may extend to tissues besides the brain. For example, it has recently been shown that β-endorphin enhances lymphocyte proliferation (116) and NK activity (117). Whether any glucocorticoid effects on the immune system are mediated through β-endorphin is an open question, however.

The potential scope of glucocorticoid actions has also been enlarged by observations that CRF and ACTH, classically thought to serve exclusively in the regulation of glucocorticoid levels, have direct effects on the brain that may modify behavior and other traits (97, 98, 118). ADH, classically considered to serve mainly in the regulation of water metabolism, also appears to affect brain functions such as memory and behavior (97, 99). ADH can in addition potentiate stimulation of ACTH release from the anterior pituitary by CRF (96, 119). Since CRF, ACTH and ADH levels are all suppressed by glucocorticoids (22), it is conceivable that these hormones mediate some glucocorticoid effects on the brain.

Glucocorticoid physiology:extension through mediators

According to the conventional view of glucocorticoid physiology, outlined in Fig. 5, glucocorticoids act on peripheral target cells to give rise directly to their observed effects in whole organisms. They increase blood glucose by stimulating gluconeogenesis in liver cells, they modulate the immune system by killing lymphocytes, and so on. Only rather tenuous physiological connections have been suggested to exist between these primary actions. For example, from a metabolic standpoint the function of lymphocyte killing and other catabolic actions of the glucocorticoids can be thought of as providing substrates necessary for gluconeogenesis (*cf.* 20). Independently of these peripheral actions, the glucocorticoids control their own plasma concentrations through a negative feedback loop involving CRF and ACTH. The loop, together with CRF and ACTH, is considered to have the sole function of regulating glucocorticoid concentrations. Superimposed on this control system and also acting through CRF and ACTH is the influence of stress, which raises glucocorticoid levels and thereby enhances in some unknown way the organism's resistance to stress.

With the results we have just reviewed on glucocorticoid modulation of mediators, we begin to perceive a range of potential secondary influences of the glucocorticoids that extends far beyond the primary effects they exert directly on their target cells. Transmitted, and perhaps amplified, through the complex intercellular network constituted by the mediators, these secondary effects can probably exercise far more subtle and varied control over cellular processes than such primary actions as cell killing.

We are thus led to the broader scheme of glucocorticoid physiology outlined in Fig. 6, which includes both primary glucocorticoid effects and secondary effects transmitted through various mediators. Also indicated are some ways in which negative feedback suppression of CRF and ACTH by glucocorticoids can be viewed as part

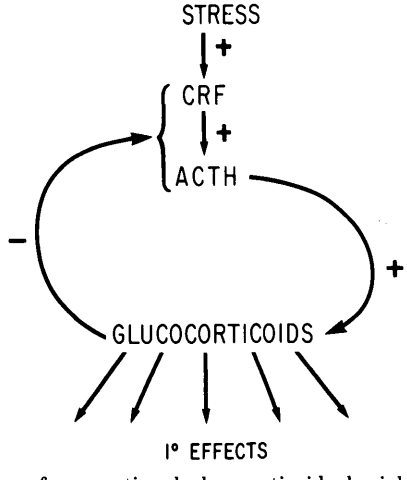

FIG. 5. Outline of conventional glucocorticoid physiology. Observed physiological effects are assumed to be primary effects, *i.e.* direct consequences of the actions of glucocorticoids on their target cells. The negative feedback actions on corticotropin releasing factor (CRF) and ACTH function only to regulate glucocorticoid levels.

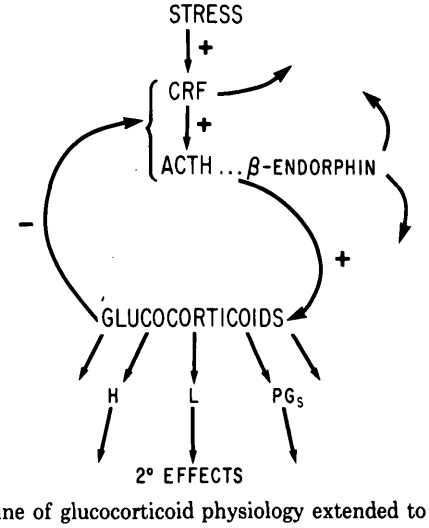

FIG. 6. Outline of glucocorticoid physiology extended to include secondary effects transmitted through the mediators that are regulated by glucocorticoids. The negative feedback actions on CRF and ACTH regulate glucocorticoid levels, but also have the potential for influencing brain functions mediated by CRF, ACTH and β-endorphin. H, classical hormones such as insulin; L, lymphokines such as immune interferon; PGs, prostaglandins and other arachidonic acid metabolites.

of a more extensive set of suppressive actions on peptides such as β-endorphin that influence the brain. Formally, the inhibitory actions on CRF and β-endorphin are similar to those on the lymphokines, which are also peptides.

As we have discussed elsewhere (24), the outline in Fig. 6 immediately suggests the possibility of applying lymphokines to control certain unwanted side effects of glucocorticoid therapy such as increased susceptibility to infection. To the extent that a side effect is due primarily to inhibition of production of a particular lymphokine, administration of that lymphokine along with the glucocorticoid may selectively overcome the effect. Although such ideas are still speculative, it should be possible soon to test them when lymphokines such as immune interferon and TCGF are synthesized in quantity by DNA recombinant techniques. From the standpoint of glucocorticoid physiology, perhaps the most significant property of the mediators we have examined is that they are components of normal physiological defense mechanisms and are secreted in response to particular derangements of homeostasis. For example, insulin is secreted in response to certain metabolic disturbances, endorphins and other neuropeptides are secreted in response to pain, lymphokines are secreted in response to infections, prostaglandins in response to tissue damage, vasopressin in response to hemorrhage, and so on. The functions of the mediators, though not always fully understood, are usually clearly aimed at restoring homeostasis. The mediators are thus important links in primary defenses against stress.

CRF and ACTH are also secreted in response to stress but their main function in those circumstances appears to be to raise levels of glucocorticoids. The glucocorticoids in turn suppress the synthesis, secretion, or actions of almost every mediator we have dealt with. In fact, suppression of mediator action is probably the most general cellular effect of glucocorticoids that is known at present. By suppressing the mediators, the glucocorticoids suppress the defense mechanism in which the mediators participate.

The difficulty of reconciling these observations on glucocorticoids with the traditional view that elevated levels of glucocorticoids enhance our defenses against stress has forced us to consider the obvious alternative, namely, that the physiological function of stress-induced levels of glucocorticoids is in fact to suppress those defenses.

With the antiinflammatory actions of the glucocorticoids before us as a model, it is obvious what the glucocorticoids may accomplish by suppressing primary defense mechanisms. As we have already pointed out, the mediators, and more generally the defense mechanisms in which the mediators participate, can themselves cause damage and endanger survival of the organism if they are activated for too long. The glucocorticoids can be regarded as a means by which the defense mechanisms are damped or switched off after they have accomplished their purpose. In a real sense, therefore, the glucocorticoids can be thought of as protecting us against our own defense mechanisms.

Glucocorticoid Functions in Stress: Suppression of Normal Defense Reactions

Restatement of the hypothesis

Our point of departure for this section is the hypothesis formulated at the close of the Introduction. We were led to it through a consideration of the widespread influences that glucocorticoids have recently been found to exert on a variety of mediators. Now we return to glucocorticoid endocrinology as a whole, in order to demonstrate how the hypothesis can be used to reinterpret some of the major actions of the glucocorticoids within a unified framework.

To contrast our hypothesis with the traditional view, we illustrate them in Fig. 7 in highly schematic form. The *top panel* depicts a number of ways in which stress can impinge on the organism and threaten homeostasis through tissue damage, metabolic and neural disturbances, *etc.* Normal physiological defense reactions, represented by the ovals, are shown as restoring homeostasis by specifically counteracting each threat. These defense reactions are assumed to involve many mechanisms in addition to stress-induced secretion of lymphokines, hormones, neuropeptides and other mediators.

The *middle panel* of Fig. 7 presents the traditional view that glucocorticoids increase resistance to stress by enhancing our defense mechanisms. This view has appeared in many forms. For example Selye, in his 1946 review (5), suggested that stress increased the need for sugar, hence the sugar-active glucocorticoids were essential for resistance to stress. Another example is the frequent appeal to the permissive enhancement of vascular and other responses to catecholamines (120) in order to explain the beneficial effects of glucocorticoids in the treatment of shock. The clearest example, perhaps, one we have already mentioned, is the suggestion that by causing lymphocytolysis the glucocorticoids release preformed antibodies and thereby enhance the immune response to infection (see Ref. 4).

This last suggestion was rejected long ago (4). It is possible, indeed likely, that the permissive actions and those on blood glucose confer some resistance to stress, particularly where glucocorticoids are lacking as in an adrenalectomized animal or a patient with severe adrenocortical insufficiency. What has been clear since the 1950s, however, is that neither the permissive actions nor those on glucose are adequate to explain the resist-

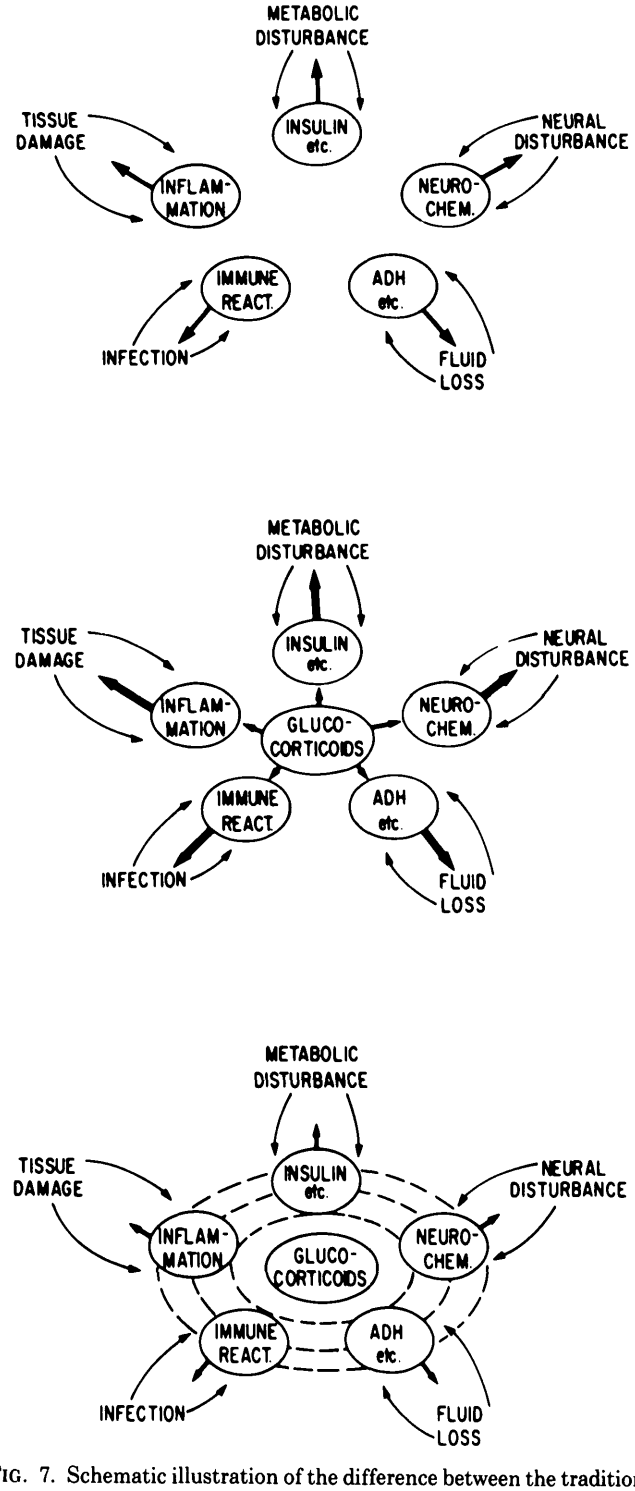

FIG. 7. Schematic illustration of the difference between the traditional view of the role of glucocorticoids in stress and the proposed new hypothesis. *Top panel*: various stress-induced disturbances of homeostasis are shown to elicit specific defense reactions. *Middle panel*: illustration of the traditional view that glucocorticoids enhance defense reactions. *Bottom panel*: illustration of the new hypothesis that glucocorticoids suppress defense reactions in order to prevent them from overshooting. The suppression indicated by the dashed curves is assumed not to take effect until the initial defense reactions have been activated.

ance to stress provided by high levels of glucocorticoids (8, 14).

As we discussed earlier, the role of glucocorticoids depicted in the *middle panel* of Fig. 7, implying as it does a stimulation by glucocorticoids of inflammatory reactions, was completely incompatible with the antiinflammatory effects discovered in the 1940s. That incompatibility was dealt with by categorizing those effects as pharmacological and excluding them from physiology.

The *bottom panel* in Fig. 7 illustrates our hypothesis that the physiological function of glucocorticoids in stress is to suppress the primary defense reactions. Dashed lines are intended to represent a gradual, general suppressive influence of glucocorticoids on all activated defense mechanisms. This influence is assumed to be sufficiently delayed in relation to the initial stress stimulus to allow the appropriate defense mechanisms to become activated. Most glucocorticoid effects already have a built-in delay of hours in their time of onset. There may also be more subtle control of timing, as we shall see in the case of the immunosuppressive actions.

An immediate advantage of our hypothesis is that, in contrast to the traditional view, it is entirely compatible with the antiinflammatory and immunosuppressive effects, which become natural consequences of the physiological functions of glucocorticoids in stress. What we will show is that in addition, the hypothesis provides a new perspective on many other familiar but often puzzling glucocorticoid actions and restores to glucocorticoid physiology a measure of the unity it has long lacked. Our discussion of glucocorticoid actions will be quite brief; endocrinologists are already well acquainted with the main facts and the groundwork for less familiar material has been laid in the last section.

Before taking up these topics we must make some important qualifications regarding the scope of our hypothesis. It is designed to account for the body's requirement for elevated, regulatory, levels of glucocorticoids in stress. It is not designed to account for the body's requirement for basal, permissive or normalizing (8) levels of glucocorticoids in the absence of stress. We accept the distinction made by Selye, Ingle and others (4–9) between the regulatory functions of the glucocorticoids in stress and their normalizing or permissive functions (*see above*). As we have explained elsewhere (16), we find no reason to think that these two functions are exerted through fundamentally different cellular processes; in fact there may be a continuous gradation between the two. The high levels of glucocorticoids that exert regulatory functions are necessary, according to our hypothesis, to temporarily occupy most glucocorticoid receptors in the body and influence maximally the cellular processes involved in defense mechanisms. Normalizing lev-

els, by modulating the same processes more subtly over long periods, could have qualitatively very different effects. Developmental influences may fall into this category. It has been suggested, for example, that tonic suppression of lymphoid cells by normal levels of glucocorticoids are beneficial for survival (121) and selective proliferation (63) of these cells. Also related to normalizing effects may be the stimulation of proliferation sometimes seen when glucocorticoids are added to cultured cells (122); this phenomenon has been ascribed partly to modulation of receptors and actions of epidermal growth factor (EGF) (123).

Our hypothesis is not designed to account for pathological changes, such as osteoporosis and redistribution of fat caused by chronically elevated levels of glucocorticoids. These changes may begin with glucocorticoid effects similar to those we postulate in the physiological response to stress, *i.e.* suppression of various defense mechanisms, but they obviously proceed far beyond physiological needs. In fact, once primary defense reactions have coped with a stress-induced disturbance and glucocorticoids have suppressed the defense reactions, the most important physiological need is to bring down the levels of glucocorticoids. Presumably that is accomplished when control of glucocorticoid levels returns to the negative feedback system.

Regarding responses to elevated levels of glucocorticoids, a puzzling observation often remarked on in the literature (8, 14) is that doses of glucocorticoids that under normal conditions are toxic, may be well tolerated during stress. Our hypothesis offers a plausible explanation. If we suppose that the toxic effects begin with excessive suppression of normal defense mechanisms, then it is easy to understand that since stress-activated mechanisms are likely to be more resistant to such suppression than unactivated mechanisms, stress will confer some protection against the toxic effects.

Antiinflammatory actions

We have already pointed out that our hypothesis on the functions of glucocorticoids in stress brings the antiinflammatory actions and the closely related immunosuppressive actions under the aegis of normal glucocorticoid physiology, obviating the need for treating them separately as pharmacological actions. We return to this subject in our discussion of immunosuppressive actions. Here we deal briefly with cellular mechanisms and touch on the matter of the pharmacological doses required to produce antiinflammatory effects.

Cellular mechanisms of the antiinflammatory and immunosuppressive actions of glucocorticoids have been the subject of several recent reviews (23, 124). A major role can undoubtedly be attributed to suppression by glucocorticoids of the various mediators of inflammation and immune reactions that we have described. These include immune interferon, LAF, TCGF, prostaglandins and leukotrienes, histamine, serotonin, bradykinin, the neutral proteinases, and perhaps even β-endorphin. It is striking that out of 13 substances listed in a recent survey of mediators of inflammation (72), 8 are among those we mentioned because their production or actions are suppressed by glucocorticoids. Other mediators, like macrophage inhibiting factor (MIF), have also been invoked in this context (*cf.* 23). Aside from regulating mediators, the glucocorticoids also regulate cell traffic (124), reverse macrophage activation (125), and as we have already noted, suppress NK activity (45).

One reason the antiinflammatory effects have been branded as pharmacological is that they require high, pharmacological, doses. For many years these effects were thought to be produced through molecular mechanisms different from those of physiological effects. For example, it was suggested in the 1960s that glucocorticoids could give rise to antiinflammatory effects by intercalating into membranes of lysosomes and thereby stabilizing these organelles. Despite the weak experimental basis for this mechanism (*cf.* 126) it is still frequently referred to.

Running counter to these ideas has been the evidence that steroid specificity for antiinflammatory actions *in vivo* (127) is typical of physiological effects mediated by glucocorticoid receptors. Furthermore, as we described in the previous section, numerous results indicate that glucocorticoid actions on inflammatory mediators are produced through normal glucocorticoid receptors and mechanisms. Why pharmacological doses are necessary is therefore not clear. The same question arises in connection with the controversial treatment of hemorrhagic and septic shock with huge bolus doses of glucocorticoids. Here again, the steroid specificity is in accord with effects mediated by glucocorticoid receptors (128). There is no evidence for the involvement or existence of special glucocorticoid receptors with low affinity such as could explain the need for high steroid concentrations. In these circumstances, a reasonable working hypothesis would seem to be that glucocorticoids are required in very high doses when used for treating certain inflammatory conditions because tissue perfusion at the sites where their actions are needed is very poor, so the only way to raise substantially the local hormone concentrations for prolonged periods of time is to maintain extremely high concentrations in the peripheral circulation. The same explanation could apply to glucocorticoid treatment of shock since poor tissue perfusion is a recognized phenomenon in the advanced stages of this condition (*cf.* 129).

Immunosuppressive actions

Possible cellular mechanisms of the immunosuppressive actions have been described in our discussion of the antiinflammatory actions. We have begun to explain why both these actions should be regarded as manifestations of physiological functions of glucocorticoids in stress. Now we develop this subject further by considering some important results and ideas that have emanated from the field of immunology.

Sorkin, Besedovsky, del Ray and colleagues (130–136) have explored extensively the relation between the immune and endocrine systems with particular emphasis on immune-neuroendocrine connections. In the course of their work they have made the following observations and suggestions that are relevant to the role of the glucocorticoids. They found that injection of antigens into rats or mice led to a 2- to 5-fold increase in blood corticosterone levels after about 6 days (131, 132). At about this time the antigenic response, measured by number of plaque-forming cells, also reached a peak (132). Subsequently they showed that adrenalectomy almost abolished the so-called antigenic competition phenomenon in which injection of one antigen inhibits the immune response to a non-cross-reactive antigen injected together or later (133). From this and other observations, they concluded that corticosterone could be responsible for the antigenic competition seen in their experiments (133). They proposed, furthermore, that the delayed increase in corticosterone levels was due to release by activated lymphocytes of chemical mediators, *i.e.* lymphokines, that stimulate the adrenal via the hypothalamic-pituitary-adrenocortical axis (131, 132). In support of this idea, they found that intraperitoneal injections of solutions containing lymphokines raised blood corticosterone levels within less than an hour (134), and that this effect was accompanied by increased plasma ACTH levels and abolished by hypophysectomy or dexamethasone treatment (135).

According to Sorkin and colleagues, the physiological function of the delayed rise in glucocorticoid levels during the primary immune response may be to preserve the antigenic specificty of the response by preventing lymphocytes with little affinity for the antigen from proliferating in an unrestricted way that could lead to autoimmunity (132–135).

Craddock (137), apparently unaware of these studies, has made a very similar suggestion, emphasizing, however, the potential physiological importance of stress-induced levels of glucocorticoids in preventing development of autoimmunity to self-antigens exposed by disease or trauma.

Thus, we see that from an immunological standpoint it is reasonable to consider that in two different situations, that of a normal immune response to a foreign antigen and that of a potentially dangerous response to auto-antigens, elevated levels of glucocorticoids perform the physiologically vital function of preventing the immune system from over-reacting and generating autoimmunity. The immunological point of view, therefore, leads to the same position in regard to the function of glucocorticoids as the one we have reached from endocrinological considerations, namely, that the glucocorticoids suppress normal defense mechanisms to prevent them from causing damage.

Glucocorticoids and carbohydrate metabolism

Central to the study of glucocorticoids since the 1930s has been their role in regulation of carbohydrate metabolism. The hypoglycemia of adrenocortical insufficiency, the glucose intolerance, hyperinsulinemia and occasional hyperglycemia of Cushing's syndrome, are well-known phenomena, understood to reflect the anti-insulin activity of glucocorticoids.

This basic antagonism to insulin, we believe, underlies the metabolic role of the glucocorticoids in stress. As we have already mentioned, an early view held that glucocorticoids enhanced resistance to stress by providing more glucose to the stressed organism for muscle work, tissue repair, *etc.* By contrast, and in line with our general hypothesis, what we propose is that the function of the glucocorticoids is to prevent insulin from causing dangerous hypoglycemia.

A very similar point of view emerges from recent studies on the synergism between glucocorticoids, glucagon and catecholamines in countering the actions of insulin (138, 139). According to DeFronzo *et al.* (139), insulin may be viewed as the prime regulatory hormone of blood glucose levels; epinephrine, glucagon and glucocorticoids are counterregulatory hormones. Of the latter, epinephrine and glucagon act fast, whereas glucocorticoids act slowly. The main synergistic action of the glucocorticoids is to enhance and prolong for several hours the increase in blood glucose due to epinephrine or glucagon.

Other known effects of the glucocorticoids that would raise blood glucose are stimulation of hepatic gluconeogenesis, and inhibition of glucose uptake in several peripheral tissues (20). In addition, as we noted in the last section, there is evidence that glucocorticoids inhibit the secretion of insulin and stimulate secretion of glucagon (94, 95). Thus the glucocorticoids can be viewed as making use of a variety of mechanisms to protect the organism against overactivity of the prime regulator of blood glucose, insulin.

Glucocorticoids and fluid balance

Two familiar actions of glucocorticoids on fluid balance, the suppression of ADH secretion, and the increase

in glomerular filtration rate, in some way contribute to the ability of glucocorticoids to promote excretion of water (15, 22). The physiological function of these actions is completely unknown.

We suggest that the function is to reverse the fluid retention that is part of normal defense reactions to hemorrhage and other forms of fluid loss. Fluid retention is partly due to ADH, which is known to be secreted in response to stress. Thus, by promoting fluid excretion the glucocorticoids may prevent excessive fluid retention and possible water intoxication (15, 22).

Glucocorticoids and shock

We take up this topic with some hesitation since it is one of the most complex and controversial in all of glucocorticoid endocrinology. However, we believe that our hypothesis offers at least a useful point of view on the subject. Already in connection with the antiinflammatory actions we considered the question of the pharmacological doses of glucocorticoids necessary for treatment of inflammation and shock, and pointed out that they did not require us to assume that the underlying molecular mechanisms mediating glucocorticoid effects in these cases were different from those mediating physiological effects.

A distinction that will be particularly important in the discussion that follows is the one we have already made between permissive or normalizing and regulatory or stress-induced levels of glucocorticoids. An organism completely lacking glucocorticoids acquires significant tolerance to mild stress when administered normalizing doses. To tolerate intense stress, however, the organism requires larger doses.

Much confusion has been engendered by comparing the response to stress of adrenalectomized animals with that of glucocorticoid-treated adrenalectomized animals, and assuming that the difference in responses represents the protective function of glucocorticoids in stressed normal animals. Adrenalectomized animals start at a physiological level far lower (in terms of resistance) than normal animals, which in turn require extra glucocorticoids to tolerate severe stress. Use of adrenalectomized animals for the comparison will therefore reveal either the normalizing effects or some combination of normalizing and regulatory effects, but not regulatory effects alone. These points are obvious to the clinician who is aware that a glucocorticoid-deficient patient requires maintenance doses of glucocorticoids for normal conditions and extra doses in stress. In relation to pressor responses to catecholamines the points are illustrated clearly by the experimental observations that, whereas glucocorticoid-deficient animals and humans have diminished pressor responses which can be normalised by administration of glucocorticoids (120), acute pretreatment of normal subjects with large doses of glucocorticoids has no effect on their pressor responses (140).

That large doses of glucocorticoids under experimental conditions do increase survival of normal animals following shock is not in doubt, however (128). The question is, how do they do it? As we have already pointed out, permissive enhancement of the pressor effects of catecholamines is a mechanism that has frequently been invoked in this connection (*c.f.* 120, 141). The experiments just described, however, show that such enhancement is not seen in normal subjects.

Our hypothesis suggests that high levels of glucocorticoids should, if anything, reverse the actions of the catecholamines. This idea is not new and there is evidence to support it. Weil and Whigham (128) pointed out many years ago that glucocorticoids are vasodilators in dogs and humans and suggested that by opposing the compensatory vasoconstriction caused by hemorrhage the glucocorticoids may improve tissue perfusion and reduce ischemic injury. In their extensive review, Ramey and Goldstein noted that the catecholamines appear to have two effects on the vascular bed: they cause both vasoconstriction and toxic damage. Whereas the glucocorticoids potentiate the first effect, they protect against the second (120).

We do not wish to give the impression that the idea that glucocorticoids protect against excessive or toxic effects of the catecholamines is universally accepted by workers on glucocorticoids and shock. What we do mean to convey, however, is that our hypothesis is consistent with significant observations as well as with important ideas in this field.

Although we have focussed on the catecholamines, which are among the most carefully studied vasoactive agents, many other substances or mediators are involved in shock and some of these are suppressed by glucocorticoids. The lethal actions of arachidonate have already been mentioned in this connection; they are blocked by glucocorticoids (70, 71). Myocardial depressant factor (MDF) is a cardioinhibitory factor that may play a significant role in the pathogenesis of shock; its formation and that of several similar factors is prevented by large doses of glucocorticoids (142).

Glucocorticoid-induced enzymes and other proteins

One of the seminal observations in the field of steroid hormone action was the discovery by Knox and colleagues that glucocorticoids rapidly induced hepatic tryptophan oxygenase and tyrosine transaminase (*cf.* 19). From this discovery came the proposal, since supported by a mass of results, that an essential step in steroid hormone action is stimulation of protein synthesis.

For all their significance to the development of ideas of steroid hormone action, the enzymes found to be

rapidly induced by glucocorticoids have contributed little to our understanding of glucocorticoid physiology. With few exceptions, early hopes that they would fulfill key roles in metabolic pathways stimulated by glucocorticoids have not materialized (16).

A physiological function suggested by our hypothesis for such enzymes, which are induced *via* the glucocorticoids during stress, is that of eliminating or detoxifying some of the mediators or metabolites released by stress. Precisely that function was envisaged many years ago by Curzon and Green (143–145) for tryptophan oxygenase in relation to serotonin. They proposed that the high levels of the enzyme induced by stress lowered the levels of serotonin in the brain, and showed that injection of cortisol into rats caused a slight but significant decrease in concentrations of brain serotonin following the increase in hepatic tryptophan oxygenase. Serotonin, which we already discussed among the mediators of inflammation, may have many effects peripherally as well as in the brain (146, 147).

Another glucocorticoid-induced enzyme is glutamine synthetase, which catalyses formation of glutamine from glutamate and ammonia (148). It is induced in many cells and tissues (16, 149, 150) including neural tissue, but to our knowledge no function has been suggested for its regulation by glucocorticoids. We believe that a possible physiological function for elevated levels of glutamine synthetase in neural tissues is to lower concentrations of glutamate and ammonia which are raised by stress (148). Glutamate is a known neurotransmitter substance; ammonia is a toxic metabolite to which the brain is particularly sensitive (148).

Hepatic cytochrome P-450, a hemoprotein enzyme, is known to be induced by glucocorticoids (151–153). This induction may account for old observations that stress or glucocorticoid treatment decrease the duration of the response of animals to certain drugs, apparently because the drugs are metabolized more rapidly (154). It has been proposed that a general function of these enzymes is to protect the organism from the toxicity of foreign chemicals in the environment (153). Our hypothesis suggests no particular function for glucocorticoid induction of the enzymes since it is not clear that they have endogenous substrates. Recent work, however, indicates that they may be important in the metabolism of arachidonic acid (155).

Metallothionein is another protein that is induced by glucocorticoids in many cells (156). It has received much recent attention from molecular biologists interested in the mechanism of regulation of its synthesis (*cf.* 157). We mention it here only as a particularly intriguing example of the many glucocorticoid-induced proteins that could conceivably have detoxifying functions of the kind we have proposed. Its outstanding property is that of binding metals such as zinc, cadmium, and copper with extraordinarily high affinity, and it has frequently been suggested to play a role in detoxification or metabolism of metals, particularly zinc (156, 158).

Finally, we should mention that the idea that the adrenal glands are involved in mechanisms of detoxification and thereby might aid in stress is an old one (3, 4). The original versions of this idea, however, are very different from the one we have proposed.

Conclusions

In this last section we have been engaged mainly in interpreting glucocorticoid endocrinology from a fresh point of view. Our objective has been to show that several of the most important physiological and pharmacological actions of the glucocorticoids find natural physiological explanations within the framework of our general hypothesis that the function of these hormones in stress is to suppress normal defense reactions. We have deliberately left out many topics, conscious of the danger of trying to fit every fact to the Procrustean bed of our hypothesis. At the same time we are not aware of significant observations that would make the hypothesis untenable.

Even if considered only as a reinterpretation of established facts, the point of view we advocate has evident advantages. The most important is undoubtedly that it reveals an underlying unity in many apparently unrelated actions of the glucocorticoids and largely eliminates the arbitrary divisions of the past between physiological and pharmacological effects. An aspect of unity that we have not emphasized in this action is the regularity with which glucocorticoid actions appear to be expressed through control of intercellular mediators. Rapidly advancing research in this area promises to reveal many new interrelationships. Some, perhaps, will be as unexpected and redolent of clues to our evolutionary past as those already made apparent by glucocorticoid regulation of neuropeptides that can act on the immune system and of lymphokines that can act on the brain.

Summary

Almost any kind of threat to homeostasis or stress will cause plasma glucocorticoid levels to rise. The increased levels have traditionally been ascribed the physiological function of enhancing the organism's resistance to stress, a role well recognized in glucocorticoid therapy. How the known physiological and pharmacological effects of glucocorticoids might accomplish this function, however, remains a mystery.

A generalization that is beginning to emerge is that many of these effects may be secondary to modulation by glucocorticoids of the actions of numerous intercel-

lular mediators, including established hormones, prostaglandins and other arachidonic acid metabolites, certain secreted neutral proteinases, lymphokines, and a variety of bioactive peptides. These mediators participate in physiological mechanisms—endocrine, renal, immune, neural, *etc.*—that mount a first line of defense against such challenges to homeostasis as hemorrhage, metabolic disturbances, infection, anxiety, and others.

Contrary to the traditional view that glucocorticoids enhance these defense mechanisms, however, it has become increasingly clear that glucocorticoids at moderate to high levels generally suppress them. This paradox, which first emerged when glucocorticoids were discovered to be antiinflammatory agents, remains a major obstacle to a unified picture of glucocorticoid function.

We propose that stress-induced increases in glucocorticoid levels protect not against the source of stress itself but rather against the body's normal reactions to stress, preventing those reactions from overshooting and themselves threatening homeostasis. This hypothesis, the seeds of which are to be found in many discussions of particular glucocorticoid effects, immediately accounts for the paradox noted above. Furthermore, it provides glucocorticoid physiology with a unified conceptual framework that can accommodate such apparently unrelated physiological and pharmacological effects as those on carbohydrate metabolism, inflammatory processes, shock, and water balance. It also leads us to suggest that some of the enzymes rapidly induced by glucocorticoids, such as glutamine synthetase, detoxify mediators released during stress-induced activation of primary defense mechanisms. These mediators would themselves lead to tissue damage if left unchecked.

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