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# How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions*

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**ABSTRACT**

The secretion of glucocorticoids (GCs) is a classic endocrine response to stress. Despite that, it remains controversial as to what purpose GCs serve at such times. One view, stretching back to the time of Hans Selye, posits that GCs help mediate the ongoing or pending stress response, either via basal levels of GCs permitting other facets of the stress response to emerge efficaciously, and/or by stress levels of GCs actively stimulating the stress response. In contrast, a revisionist viewpoint posits that GCs suppress the stress response, preventing it from being pathologically overactivated. In this review, we consider recent findings regarding GC action and, based on them, generate criteria for determining whether a particular GC action permits, stimulates, or suppresses an ongoing stress-response or, as an additional category, is preparative for a subsequent stressor. We apply these GC actions to the realms of cardiovascular function, fluid volume and hemorrhage, immunity and inflammation, metabolism, neurobiology, and reproductive physiology. We find that GC actions fall into markedly different categories, depending on the physiological endpoint in question, with evidence for mediating effects in some cases, and suppressive or preparative in others. We then attempt to assimilate these heterogeneous GC actions into a physiological whole. (*Endocrine Reviews* **21:** 55–89, 2000)



## I. The Decline and Modern Revision of Glucocorticoid Physiology

OVER THE past half century, an extraordinary range of glucocorticoid (GC)[1] effects upon target tissues have been uncovered. When first studied in the 1930s by Hans Selye, one of the founders of the study of stress, GCs were a topic for the physiologist. Few contemporary endocrinol-

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[1] Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; AVP, arginine vasopressin; CBP, CREB-binding protein; COMT, catechol-*O*-methyltransferase; COX-2, cyclooxygenase 2; CREB, cAMP response element binding protein; CSF-1, colony stimulating factor 1; DTH, delayed-type hypersensitivity; EAE, experimental allergic encephalomyelitis; G-6-Pase, glucose-6-phosphatase; GC, glucocorticoid; GM-CSF, granulocyte monocyte colony stimulating factor; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GRIP, glucocorticoid receptor interaction protein; GTF, general transcription factor; HAT, histone acetyltransferase; HPA, hypothalamic-pituitary-adrenal; ICAM-1, intercellular adhesion molecule 1; IFN-γ, γ-interferon; IL-1, -2, interleukin-1, and -2; IRS-1, insulin receptor substrate-1; MAO, monoamine oxidase; MIP-1α, macrophage inflammatory protein-1α; MR, mineralocorticoid receptor; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; nGRE, negative glucocorticoid response element; NK cell, natural killer cell; NMDA, *N*-methyl-D-aspartate; PEPCK, phosphoenolpyruvate carboxykinase; PNMT, phenylalanine-*N*-methyltransferase; PPAR, peroxisome proliferator-activated receptor; RANTES, regulated on activation normal T cell expressed and secreted; SRC, steroid receptor coactivators; Stat, signal transducer and activator of transcription; TAF, TBP-associated factors; TBP, TATA box-binding protein; TGF-β, transforming growth factor β; Th1, Th2, T helper 1 and 2 cells; TIF, transcription intermediary factor; TNF-α, tumor necrosis factor-α; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

ogists view GC actions as part of a coherent physiological picture, or see the need to do so. Today the focus is on the molecular and cell biology of GC action, *e.g.*, GC receptors as ligand-activated transcription factors or GC-induced apoptosis in lymphocytes.

Two broad explanations have been offered for the decline of GC physiology as a discipline (1):

1. Disparate and new GC actions emerged (notably the antiinflammatory actions reported in 1949) (2), which did not fit into the existing paradigm of stress physiology, namely that GCs enhanced the response to stress. Many physiologists either dismissed these effects by declaring them to be pharmacological or ignored them (3, 4) (despite the antiinflammatory effects accounting for more research and publications on GCs after 1949 than all the traditional physiological effects together).

2. Selye, one of the most prolific champions of GC physiology, turned out to be profoundly wrong about critical features of the physiology that he espoused. His claim throughout the early 1940s that GC excess could cause arthritis, allergies, and collagen-related disorders was shattered by the discovery of GC's antiinflammatory actions. This debacle discouraged further attempts to make physiological sense of GC actions. Instead, attention moved to the dramatic new clinical applications of these hormones and, eventually, to their cellular and molecular actions.

A consequence of this withering of GC physiology was that it seemed irrelevant to ask the question that dominated earlier research—how do GCs help in surviving stressors? An earlier review (1) aimed to restore the integrative role of GC physiology by means of a new paradigm to encompass the disparate actions of GCs and remove the unconvincing physiological/pharmacological dichotomy. The authors proposed that GCs, rather than enhancing the stress response, through their suppressive actions limit its size and contribute to recovery from it. To quote (1):

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

Unknown to the authors of Ref. 1, a paper by Marius Tausk in 1951 (5) published in the periodical of a pharmaceutical firm, included the germ of this idea, which unfortunately did not enter the regular literature. Tausk illustrated his thought pithily by comparing stress to a fire and the role of GCs to that of preventing water damage rather than putting out the fire.

The permissive and suppressive effects of GCs have been suggested to complement each other, the former preparing or priming defense mechanisms for action and the latter, limiting the actions (6). The present review represents a synthesis of the classical view of Selye (that stress-induced secretion of GCs enhances and mediates the stress response), of Ingle (that basal GC levels are permissive of the stress response; 3), and of the emphasis on GCs as limiting the stress response and contributing to the recovery from it (1, 5, 6). The goals of the review are 4-fold: 1) to define the ways in which GCs influence the response to stress; 2) to propose criteria by which to discriminate between these roles in particular cases; 3) to apply those criteria to a broad spectrum of GC actions as organized by physiological systems, extending the analysis into areas not contemplated previously; 4) to attempt a synthesis of the physiological implications and evolution of these GC actions and establish why particular combinations of them make biological sense. As an important caveat, while we are reviewing a considerable body of facts (*i.e.*, a large percentage of the literature concerning the physiology of GC actions) that are generally accepted within the endocrine community, our interpretations and emphases represent a very personal perspective.

## II. Definition of Terms, and Criteria for Analyzing the Role of GCs in the Stress Response

### *A. The prototypical stress response*

We begin by outlining a prototypical acute vertebrate stressor, to review the basic parameters of the endocrine stress response, to define the classes of GC actions, and to determine criteria for classifying particular GC actions.

In this prototypical stressor, a herbivore, with no prior warning, is attacked by a predator. Injured, it manages to escape, but continues to be stalked and chased over the next hour, until the predator gives up. Note that this stressor includes physical injury, a demand for skeletomuscular activation, cognitive vigilance, as well as a perceived challenge to well-being that constitutes "psychological" stress. Note also that the lack of prior warning precludes any anticipatory stress.

We outline the broad features of the endocrine response to this stressor, concentrating on hormones whose responses are most consistent across stressors and whose actions are best understood (Fig. 1A). The first wave, occurring within seconds, involves: 1) enhanced secretion of catecholamines (epinephrine and norepinephrine) from the sympathetic nervous system; 2) hypothalamic release of CRH into the portal circulation and, perhaps 10 sec later, enhanced secretion of pituitary ACTH; 3) decreased hypothalamic release of GnRH and, shortly thereafter, decreased secretion of pituitary gonadotropins; and 4) pituitary secretion of PRL and (in primates) GH, and pancreatic secretion of glucagon. In the case of a hemorrhage, this first wave also includes massive secretion of arginine vasopressin (AVP) from the pituitary and renin from the kidney (in contrast to the moderate AVP response after other stressors); this response is bracketed, since loss of fluid volume (as in hemorrhage) will be analyzed as a separate facet of the stress response.

A second, slower wave involves the steroid hormones. Over the course of minutes, GC secretion is stimulated and gonadal steroid secretion declines.

A time course also exists with which the stress-induced endocrine changes in Fig. 1A are "heard" as target tissue effects (Fig. 1B). Commensurate with their rapid secretion, the hormones of the first wave exert most of their effects through rapid second messenger cascades within seconds to a few minutes. In contrast, because the bulk of steroid actions are genomic (for an exception, see Refs. 7 and 8), few GC actions are exerted until about an hour after the onset of the

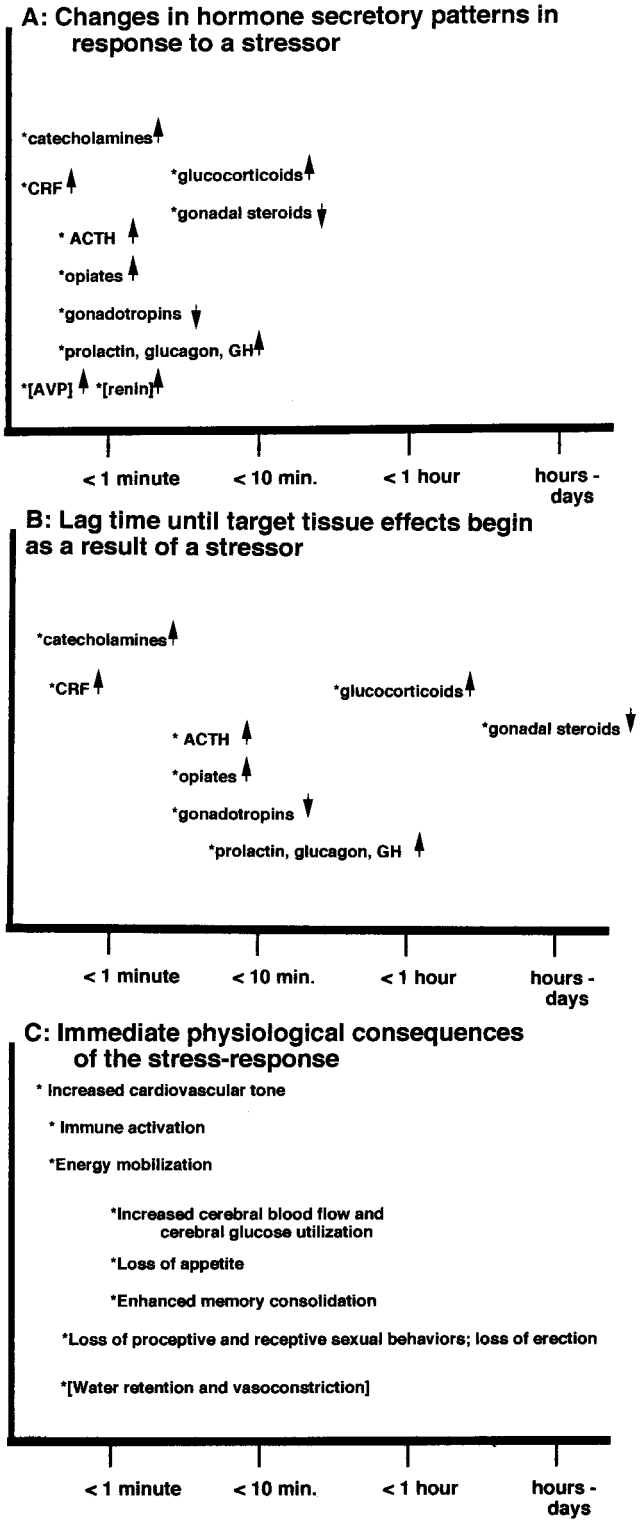


FIG. 1. Schematic overview of the typical endocrine stress response. A, The time course of changes in hormone-secretory patterns in response to a stressor. B, The lag time until target tissue begin as a result of a stressor. C, Immediate physiological consequences of the stress response. *Asterisks* approximate where on the time line the particular hormone is first having its effect (A and B) or when on the time line the physiological consequence is initiated (C). There is no formal y axis—hormones or consequences are simply spaced vertically to facilitate reading.

stressor, whereas the consequences of the decreasing reproductive steroid levels do not occur for several hours. The relatively slow effects of GCs become critical throughout this review.

These varied hormone effects bring about the major physiological changes of the stress response (Fig. 1C); the specifics of each set of changes will be considered in detail. On the scale of seconds to a few minutes, these include: 1) diversion of energy to exercising muscle (in the form of mobilization of stored energy, inhibition of subsequent energy storage, and gluconeogenesis); 2) enhanced substrate delivery to muscle via enhanced cardiovascular tone; 3) a stimulation of immune function; 4) inhibition of reproductive physiology and behavior (in the form of rapid declines in proceptive and receptive behavior in both sexes and loss of erections in males); 5) decreased feeding and appetite; 6) sharpened cognition and increased cerebral perfusion rates and local cerebral glucose utilization. In the specialized case of fluid loss due to hemorrhage, responses also include water retention through both renal and vascular mechanisms. Note that Fig. 1C only specifies the onset of these physiological responses; the duration of each will be a point of detailed analysis below. The critical point at this stage is to define the stress-induced physiological changes that precede stress-induced GC target tissue effects.

### *B. Definitions of the classes of GC actions*

We begin by analyzing what GCs do during stress with respect to this early wave of endocrine stress responses and physiological consequences. We distinguish between two classes of GC actions: modulating actions, which alter an organism's response to the stressor; and preparative actions, which alter the organism's response to a subsequent stressor or aid in adapting to a chronic stressor.

Among the modulating GC actions we distinguish the following:

1. *Permissive* actions are exerted by GCs present before the stressor and prime the defense mechanisms by which an organism responds to stress. Their consequences are first manifested during the initial stress response and occur whether or not there is a stress-induced increase in GC concentrations.

2. *Suppressive* GC actions are those attributable to the stress-induced rise in GC concentrations, and thus have an onset of from about an hour or more after the onset of the stressor. These relatively delayed GC actions rein in the stress-activated defense reactions and prevent them from overshooting.

3. *Stimulating* GC actions are also attributable to the stress-induced rise in GC concentrations, with an onset of from about 1 h or more after the onset of the stressor. These GC actions enhance the effects of the first wave of hormonal responses to stress and thus are the reverse of the suppressive actions. Because permissive and stimulatory actions both enhance the first wave of response to the stressor, we will refer to them collectively as helping to mediate the stress response.

Finally, *preparative* GC actions are defined as those that do not affect the immediate response to a stressor but modulate

the organism's response to a subsequent stressor. They can be mediating or suppressive.

These actions may best be illustrated with an analogy. In response to the stressor of an invading army, an immediate response would be to shoot at the enemy; this is akin to the actions of the first wave of stress-responsive hormones (catecholamines, CRH, etc.). Among actions that would modulate this response, permissive actions would be those already in place at time of the attack, such as setting up defenses. Stimulating actions enhance the response and are undertaken after the attack, *e.g.,* calling up active combatants from reserves. Suppressive actions, which constrain defense responses, might include calling off an attack to avoid self-destructive friendly fire (friendly fire being an example of defensive "overshoot", akin to autoimmunity). Preparative actions would be, for example, to institute rationing, an action designed not to repel the invader but to set up long-term measures for survival should the conflict continue, or enhance responsiveness to the next invasion (such as designing better systems for detection).

Permissive and suppressive actions have been recognized since the 1950s. Stimulating actions were once assumed to be responsible for the protection against stress afforded by stress-induced levels of GCs, but for which evidence has been weak (1). To our knowledge, the designation of some GC actions as preparative is new. As will be seen, it is rare that GC effects upon some physiological system consist of only one of these types of actions (*i.e.*, permissive, suppressive, stimulatory, or preparative). In principle, all could be exerted over the whole range of GC concentrations, with dose-response curves depending on the receptors through which they are produced. In actuality, permissive actions are typically associated with basal levels of GCs, and the other three types of actions with stress-induced levels, but as we will indicate, there are instances of permissive actions being induced by higher than basal levels of GCs, so long as they precede an actual stressor.

These actions are exerted generally through GC (GR, or Type II) receptors, although in some cases, the mineralocorticoid (MR, or Type I) receptor may be involved. Such actions exhibit monotonic dose-response curves, *i.e.*, response curves that continually either rise or fall with increasing GC concentrations in proportion to the number of GC-receptor complexes formed. The most convincing way to document that a GC effect is monotonic is to show that removal of GCs or their influence has a particular effect upon an endpoint, and that administration of physiological GC concentrations reverses the effect of removal. Monotonic dose-response curves are typical of classical GC effects used in bioassays for GC activity, such as liver glycogen deposition or thymus involution. Many GC actions are not monotonic, in that the steroid acts differently at low *vs.* high concentrations. This dichotomy can emerge, for example, from GCs permissively enhancing target tissue sensitivity to a cytokine, and simultaneously lowering the concentration of the cytokine, generating a bell-shaped or biphasic dose-response curve (6, 9). As will be seen, the biphasic nature is accentuated if the permissive actions are exerted through mineralocorticoid receptors (MRs) and the suppressive actions through glucocorticoid receptors (GRs), the former having more than 10 times greater affinity for natural GCs than the latter (cf. Ref. 10).

Duration and timing of hormone exposure can have major influences on responses. Excess GCs, while beneficial or harmless for a few days, can be fatal if prolonged. Just as there is diurnal and even minute-to-minute variation in GC levels, so there may be diurnal and minute-to-minute variation in responses to stress (11, 12). How soon GC effects are manifested after the hormones bind to their receptors may vary from a few minutes to days, and how long a hormone effect takes to decay after the hormones have been removed may vary from hours to days to weeks, depending on the life spans of the mRNAs and proteins that transmit the effects. Generally we have not examined the effects of long-term or chronic GC excess and deficiency, as in Cushing's and Addison's disease. In such conditions the primary physiological adaptations to altered GC levels that concern us here are often obscured by widespread and pathological secondary changes that are probably irrelevant to normal physiology and to evolution of the role of GCs in stress.

Finally, we will interpret with caution results obtained with synthetic GCs such as prednisolone or dexamethasone. These substances are extraordinarily useful clinically and experimentally, but may not be good substitutes for the natural GCs in physiological settings. They often do not bind to MRs, and may interact with GRs with different kinetics or affinities than the natural GCs (13).

### *C. Criteria for analyzing the role of GCs in the stress response*

Does a particular GC action modulate the stress response through permissive, suppressive, or stimulatory actions, or prepare the organism for the next stressor?

To analyze systematically these actions we will apply a set of criteria for discriminating among them, using several styles of evidence. The criteria concentrate on the critical implications of the idea that GCs keep the primary defenses from overshooting (1, 5, 6), and, in the aftermath of the stress response, reduce the actions of those primary defenses to bring about recovery. While additional criteria may be valid, and each current criterion has some flaws, we have found these to be useful in judging the nature of each of a variety of GC effects upon various organs and physiological systems.

*1. The criterion of conformity.* Does a particular GC action enhance or reduce the effects of the first wave of stress-responsive hormones in Fig. 1A (*e.g.*, catecholamines, CRH)? If the action reduces their effects, then by this criterion the GC action is suppressive. If the effect is enhancement, and due to basal levels of GCs present before that first wave, it would be viewed as permissive, while enhancement by the subsequent stress-induced levels of GCs would be viewed as stimulatory. Note that a GC action can be viewed as enhancing that first wave without having to have the identical effects—distributing guns and building aircraft carriers are both defensive reactions.

*2. The criterion of time course.* Suppressive or stimulating actions of stress-induced levels of GCs have an onset of minutes

to hours after the onset of the stressor. A particular action of stress-induced levels of GCs can be considered to be suppressive (or stimulating) only if it suppresses (or stimulates) the immediate stress response (*i.e.,* something that occurs in Fig. 1C before GCs begin to exert their effects). Thus, for example, if stress-induced levels of GCs stimulate appetite, this would qualify as a suppressive action only if appetite is suppressed during the first minutes of the stress response. In contrast, permissive effects require the presence of GCs before the stressor and result in enhancement of the initial stress response.

*3. The criteria of hormone subtraction and replacement.* What happens to the physiological stress response (Fig. 1C) if there is no stress-induced rise in GC activity? If some feature of Fig. 1C is attenuated, then this supports the classical view that GCs stimulate the stress response. In contrast, if an effect is enhanced (either in the form of "overshooting" with a higher peak, and/or a delayed recovery from the stress response), this supports the revisionist view that the stress-induced rise in GCs suppresses the stress response. Administering exogenous GCs post-stressor to replicate stress-induced secretion should restore the stress response to that obtained with normal endogenous GCs.

Similar outcomes should occur when GC actions are eliminated for days before a stressor, unless the stress response requires permissive GC actions. If permissive actions are required, however, then allowing previously established permissive actions to decay should attenuate or abolish the stress response, and neither stimulating nor suppressive actions would be manifested. To restore the normal stress response, exogenous GCs would have to be administered not only at stress-induced levels after the stressor as above, but at basal levels before the stressor.

The usual method for subtracting endogenous GCs is adrenalectomy. It subtracts other hormones as well and can require days of postoperative recovery. More specific subtraction is achieved with the GC antagonist RU486 (which also antagonizes progestins and sometimes displays agonist activity). It has been used *in vivo* and *in vitro* to reversibly block GC actions via GRs acutely or for extended periods. It does not block GC actions via MRs. To establish that changes in stress responses due to such manipulations result specifically from lack of GC activity, one must show that appropriate administration of exogenous GCs reverses the changes. (Note: we are not concerned here with effects of GC subtraction on endpoints in the absence of stress, which can nonetheless inform about tonic effects of GCs).

*4. The criterion of homeostasis.* Given the nature of the stressors experienced by most organisms and the adaptations needed to survive them by restoring homeostasis, does a particular GC action make more physiological sense as permitting, stimulating, or suppressing the stress response, or as preparing the organism for the next stressor?

Collectively, we feel that these criteria help identify GC actions that are either permissive, stimulatory, or suppressive. Somewhat by default, if an action fails to fit into any of these categories, we will consider whether this constitutes preparative action, a "bystander" effect, or if the action is simply not well understood (Table 1).

## III. GC Actions in the Context of These Criteria

Most organs and physiological systems are sensitive to GCs. We will concentrate on the half dozen best-studied branches of GC physiology, *i.e.,* cardiovascular tone, fluid volume and the response to hemorrhage, on immunity and inflammation, on metabolism, neural function and behavior, and on reproduction. In each section, we will review the effects of the first wave of stress-responsive hormones (from Fig. 1A, whose latencies until actions are shown in Fig. 1B) and their role in bringing about the relevant physiological changes (Fig. 1C). We will then review GC effects upon that particular system. With those data in hand, we will then apply the criteria to categorize GC actions in that realm.

TABLE 1. Application of the four criteria for determining whether a particular GC action is permissive, stimulatory, suppressive, or preparative

| A GC action is considered to be | Criterion | | | |
|---|---|---|---|---|
| | Conformity | Timecourse | Subtraction | Homeostasis |
| Permissive if: | Basal levels of GCs enhance the actions of the first wave of stress-responsive hormones | Basal levels of GCs enhance the earliest physiologic changes following the onset of a stressor | Lack of GCs for some time before the stressor attenuates a physiologic response to a stressor | Basal actions of GCs appear advantageous in mediating the response to a stressor |
| Stimulatory if: | Stress-induced GC levels enhance the actions of the first wave of stress-responsive hormones | Stress-induced GC levels enhance the earliest physiologic changes following the onset of a stressor | Elimination of stress-induced GC levels attenuates a physiologic response to a stressor | The actions of stress-induced levels of GCs appear advantageous in mediating the response to a stressor |
| Suppressive if: | Stress-induced GC levels inhibit the actions of the first wave of stress-responsive hormones | Stress-induced GC levels inhibit the earliest physiologic changes following the onset of a stressor | Elimination of stress-induced GC levels augments a physiologic response to a stressor | The actions of stress-induced levels of GCs appear advantageous in keeping the response to a stressor from overshooting |
| Preparative if: | Stress-induced GC levels interact with the first wave of stress-responsive hormones in a subsequent stressor | Stress-induced GC levels alter the earliest physiologic responses to a subsequent stressor | Elimination of stress-induced GC levels alters some feature of the physiologic response to a subsequent stressor | The actions of stress-induced levels of GCs appear advantageous in altering the quality of a subsequent stress-response |

*A. Cardiovascular effects*

In this section we consider GC actions upon blood pressure, heart rate, and cardiac output during stress. For a number of reasons, we separate this from the next section, which focuses on GCs effects on the related subject of fluid volume during hemorrhage. First, we will suggest that cardiovascular changes are a central feature of adaptation to most physical stressors, whereas fluid volume changes are critical to the specialized stressor of hemorrhage. Moreover, the mechanisms underlying GC actions in the two realms appear quite different. Finally, the conclusions regarding mediation or suppression of the stress response are opposite in these two arenas, and we do not wish to obscure these differences.

The cardiovascular stress response and the roles of hormones from the first wave (Fig. 1A) are both well understood. Since the days of Walter Cannon, who first described the fight or flight response at the beginning of this century, rapid activation of the cardiovascular system has been viewed as the *sine qua non* of surviving a physical stressor. Such activation involves elevated arterial pressure, heart rate, and cardiac output, accompanied by diversion of blood to muscle via constriction of mesenteric and renal vessels and dilation of vessels supplying skeletal muscle (14). Subtle and important qualifiers have been introduced in recent years. For example, a different picture emerges for stressors that demand quiet vigilance (such as an avoidance task, or an organism remaining immobile to evade detection by a predator). Such vigilance involves decreased heart rate and cardiac output, and increased vascular resistance in all target tissues (15).

Despite this elaboration, stressors that produce a physical output as a coping response consistently cause rapid cardiovascular activation. The mediation of such activation by catecholamines is part of the canon of autonomic physiology. More recent work also implicates CRH. In addition to CRH regulating ACTH release, the peptide occurs diffusely in the brain and serves as a neurotransmitter that mediates sympathetic arousal, providing an important link between the adrenocortical and autonomic branches of the stress response (16). As such, intracerebroventricular administration of CRH elevates plasma catecholamine concentrations, blood pressure, and heart rate (17–19). This represents a central action of CRH, in that it occurs in hypophysectomized animals (20, 21), and is physiologically relevant, as sympathetic activation is partially attenuated with CRH antagonists (22).

The effects of GCs upon the cardiovascular stress response are also well understood. They increase blood pressure and cardiac output, as demonstrated by the positive inotropic effect of GCs (23), the hypotension and feeble cardiac function of adrenalectomized individuals, or by the hypertension of Cushings patients or individuals treated with GCs. We review these actions in the context of the criteria.

*1. The criteria of conformity and of time course.* Insofar as catecholamines and neurotransmitter CRH cause cardiovascular activation, GC actions are not only similar to these, but are inextricably intertwined with them. These GC actions are permissive, in that most involve "permitting" catecholamines and other vasoconstrictors to exert their full actions (24, 25). Treatment of normal rats with RU486 decreases vascular reactivity to norepinephrine and angiotensin II (26). GCs exert their permissive effects upon catecholamine action in both vascular and cardiac tissue (27–34) [as well as in the lungs (35, 36)]. This is thought to arise in a number of ways. GCs induce phenylalanine-*N*-methyltransferase (PNMT), the rate-limiting enzyme in epinephrine synthesis (37, 38). Furthermore, GCs prolong catecholamine actions in neuromuscular junctions by inhibiting catecholamine reuptake and decreasing peripheral levels of catechol-*O*-methyltransferase and monoamine oxidase (39, 40). They also enhance cardiovascular sensitivity to catecholamines by increasing the binding capacity and affinity of $\beta$-adrenergic receptors in arterial smooth muscle cells (41, 42), receptor-G protein coupling, and catecholamine-induced cAMP synthesis (43–45). In other tissues, such as nasal mucosa, GCs increase adrenergic receptor mRNA levels (46). Finally, by inhibiting PG synthesis at basal levels, GCs block their vasodilatory effects (47, 48). While the physiological relevance of this last mechanism has been questioned (24), there is evidence for it being the main route by which GCs elevate blood pressure in Cushing's syndrome (25).

GCs can also inhibit a few features of sympathetic function (49). For example, GCs inhibit catecholamine release in response to some stressors (50, 51) and decrease cardiac norepinephrine turnover (52, 53). Nonetheless, in most cases GCs facilitate sympathetic interactions, and their overall physiological effects are to permissively augment cardiovascular activation during stress. Thus, by the criteria of conformity and of time course, GCs mediate the cardiovascular component of the stress response through their permissive actions.

*2. The criteria of subtraction and replacement.* These criteria support the view of GCs permitting the cardiovascular stress response. Both Addisonian and adrenalectomized individuals are characterized by basal hypotension (due in part to lack of aldosterone). Furthermore, as noted, RU486 decreases vascular reactivity to vasoconstrictors. In Addisonians, such hypotension can progress into an acute Addisonian crisis when the individual is challenged with a physiological stressor (infection, surgery, a burn). At such times, blood pressure is unresponsive to exogenous catecholamines. Thus, rather than removal of GCs causing a cardiovascular overshoot during stress, there is an undershoot.

This conclusion should be considered in the context of adrenalectomy being associated in some cases with elevated norepinephrine concentrations in response to a stressor (18, 50, 54–56), which has been interpreted by some authors as evidence for GCs constraining the cardiovascular stress response from overshooting (50). However, circulating concentrations of catecholamines, the endpoint in the studies just cited, are not equal to cardiovascular endpoints (blood pressure, heart rate, etc.). The varied GC effects upon catecholamine stability in the sympathetic synapse, upon the efficacy of catecholamines at their receptors, and upon postreceptor mechanisms apparently counteract the endpoint of circulating catecholamine concentrations (with the increased catecholamine concentrations after adrenalectomy perhaps being appropriately viewed as a partial compensation for the absence of these other GC effects). Thus, the total effect of GC

underexposure is an attenuated cardiovascular stress response.

*3. Criterion of homeostasis.* The logic of activating the cardiovascular system during most stressors is apparent and has figured in thinking about the physiology of the stress response since Cannon's *The Wisdom of the Body* (57). The complexity of regulatory factors uncovered since that time reinforces the conclusion that the mobilization of cardiovascular tone, as contributed to by GCs, represents a vital adaptation to stress.

All four criteria lead to the conclusion that GCs help mediate, rather than suppress, the cardiovascular stress response. These mediating effects involve permissive actions over the entire GC dose range (57). Whether these mediating actions include stimulatory ones as well (*i.e.,* effects that are amplified by stress-induced elevations of GC concentrations) remains untested.

*Conclusions:* Varied stressors trigger cardiovascular activation; this effect is primarily mediated by the sympathetic nervous system, with GCs, over their entire dose range, enhancing these effects. Removal of GCs impairs the cardiovascular stress response, rather than causing it to overshoot. These findings, plus the logic of enhancing cardiac output in coping with a stressful physical challenge, suggest that GCs help mediate permissively the cardiovascular stress response.

### *B. Fluid volume and hemorrhage*

As earlier, hemorrhage is a quite different and specialized stressor than is the sprint across a savanna. Because of this and, most importantly, the nature of GC actions, we have treated them separately.

Hemorrhage (as induced experimentally by controlled blood withdrawal) causes the robust stress response of Fig. 1A, along with enhanced secretion of AVP and renin, producing water retention and vasoconstriction. GCs indirectly inhibit the release of AVP (by restoring the actions of inotropic and vasoconstrictive hormones, resulting in reflexive inhibition of secretion), increase glomerular filtration rate, and increase the secretion and efficacy of atrial natriuretic polypeptide (58, 59), all of which enhance water excretion. These actions occur in response to both basal and stress-induced levels of GCs, and rate of excretion of a water load has been used to test patients for adrenal insufficiency (60). The implications of these actions during a hemorrhage has different implications than the cardiovascular responses to more general stressors. This view arises from a meticulous series of studies (61–63), in which the hemorrhage insult was a moderate one, involving withdrawal of 15 ml of blood/kg over a 5-min period from rats.

The authors first demonstrated that adrenalectomy robustly potentiated secretion of the vasoactive hormones (including hypersecretion of AVP and norepinephrine but, because of the adrenalectomy, obviously not epinephrine) (61). In other words, GCs normally constrain the size of the vasoactive response to hemorrhage. When such a hemorrhage in adrenalectomized rats was coupled with fasting, the hemorrhage invariably proved fatal (in contrast, intact rats, whether fed or fasted, always survived a similar hemorrhage) (62).

The authors thereupon dissected the complex chain of events underlying the death (62, 63). The critical step appeared to be the AVP overshoot, resulting in a vast vasoconstriction of the hepatic and coronary circulation. This produced ischemia in these organs and also led to a profound hypoglycemia (which arose because there was minimal hepatic gluconeogenesis in the absence of perfusion through the liver). The authors suggested the following features to this cascade:

1. The cause of death was probably the ischemia due to circulatory failure, rather than the hypoglycemia. As evidence, intravenous infusion with glucose did not prevent death (63).

2. This seemed to contradict the authors' finding that feeding prevented hemorrhage-induced death in adrenalectomized rats. However, feeding not only elevated circulating glucose concentrations, but also stimulated blood flow to the gut and liver (via gastrointestinal distention) (64), apparently enough to override the vasoconstriction induced by the AVP.

3. In the adrenalectomized rats, it was the overshoot of the AVP stress response, rather than of the norepinephrine or renin responses, which proved fatal. As evidence, a replacement regimen with GC concentrations in the low basal range, which normalized the norepinephrine and renin responses, but not the AVP response, did not prevent death (63). Protective effects were seen only when circulating GC levels were raised to the range seen during the circadian peak.

4. Hemorrhage in the fasted, adrenalectomized rats caused a decrease in vascular sensitivity to AVP (but not to norepinephrine or renin) (62). This can be viewed as a protective down-regulation in response to the vastly increased AVP signal, a compensation that was nevertheless insufficient to prevent death.

*Conclusion:* These data are commensurate with a picture of GCs suppressing, rather than mediating, the fluid volume response to a hemorrhage stressor. The stressor leads to a rapid burst of secretion of vasoconstrictive stress hormones, and of vasoconstriction itself, both of which are opposed by GCs. Thus, by the criteria of time course and conformity, GCs are suppressive. Moreover, adrenalectomy results in a (potentially fatal) overshoot of the secretion of AVP, satisfying the criterion of subtraction. From the point of view of homeostasis, the importance of the suppression by GCs of the response to hemorrhage is that it prevents the organism from being injured or killed by its own defense mechanisms.

These findings, when combined with those concerning GC effects upon cardiovascular physiology, generate a subtle but important insight. As reviewed, insufficient GCs can lead to enhanced catecholamine overflow during a stressor. However, such insufficiency also blunts sensitivity of cardiovascular tissues to the catecholamines. Similarly, lack of GCs leads to hypersecretion of the vasoconstrictive hormones after hemorrhage and to damped target tissue sensitivity to the critical AVP. In the former cardiovascular case, the loss of tissue sensitivity most likely reflects the numerous GC actions upon catecholamine half-life in the synapse and upon the efficacy of receptor and postreceptor mechanisms. In the

case of hemorrhage, the desensitization is speculated to arise more directly from the down-regulatory effects of the excessive AVP (since GCs themselves have been reported to increase AVP receptor number (65–67). In both systems, however, adrenalectomy leads to both an enhanced signal and a decreased sensitivity to that signal.

Despite this similarity, the outcomes are the opposite. In the case of general stressors that activate the cardiovascular system, the result of those two opposing consequences of adrenalectomy is a marked hypotension during stress (*i.e.*, the stress-response is attenuated). In the specialized case of a hemorrhage stressor, the result is ischemic vasoconstriction (*i.e.*, the stress-response overshoots). GCs often have opposite effects upon the strength of a particular signal and the target tissue sensitivity to the signal (6), and as described later, the combination of those two trends can produce a bell-shaped curve of dose responsiveness. The GC effects upon the signal and upon the sensitivity to that signal need not mirror each other perfectly, and depending upon which predominates, GC can enhance or damp the system. GC actions upon the general cardiovascular stress response, and upon the response to a hemorrhage stressor, appear to represent difference balancings of those two opposing trends.

### *C. Immunity and inflammation*

We now consider GC effects upon immunity and inflammation, an area of great confusion in making a physiological whole of GC actions. We begin by considering the immunological and inflammatory effects of the first wave of hormones secreted as stress response (Fig. 1A). This is an arena of considerable complexity, as such hormones have both stimulating and inhibitory effects (reviewed in Refs. 68 and 69). For example, CRH decreases T cell proliferation and natural killer (NK) cell cytotoxicity; this is a centrally acting event, as it can be reversed with intracerebroventricular infusion of CRH antibodies (70, 71). CRH (at extremely high doses that also cause hypotension) can also act as an antiinflammatory and antiedemic agent, reducing inflammatory exudate volume and cell concentration in models of injury to skin, mucosa, brain, or muscle (72–74). In contrast, CRH can also be an immune stimulant, enhancing B cell proliferation and the proliferative lymphocyte response to various mitogens and increasing interleukin 2 (IL-2) receptor number (75, 76).

We next consider the rapid physiological effects of stress upon immune function (Fig. 1C). Various infectious stressors cause rapid immune activation that precedes adrenocortical activation. These include exposure to endo- or exotoxins and inoculation with an infectious microorganism or antigen (Refs. 77–79; also, see Ref. 80). Surprisingly, the same can be triggered by noninfectious stressors. For example, psychological stressors, such as placement of rats in open-field settings or conditioned aversion stress, will trigger cytokine release and its associated fever response before there is a rise in GC concentrations (81–83). Thus, rapid activation of the immune system appears to be a response to a number of generalized stressors.

This link is made more interesting by the fact that this immune activation contributes to the subsequent GC release. First, the activated immune system can synthesize ACTH-like molecules (84). However, the bioactivity of those peptides is probably insufficient to be of much physiological relevance (85, 86).

Second, as postulated by Besedovsky and colleagues (87–90), various cytokines emanating from activated immune cells can stimulate the adrenocortical axis. For example, IL-1 can release CRH from the hypothalamus (91–93) and can directly release ACTH from the pituitary (94), although this is controversial (78). Since then, other cytokines, including IL-2, IL-6, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and interferon-$\gamma$ (IFN-$\gamma$), have been shown to stimulate the adrenocortical axis, although none with the potency of IL-1 (reviewed in Ref. 79).

We now consider the GC effects in this realm. The immunosuppressive and antiinflammatory actions of GCs have been recognized for decades (6, 68, 77, 95–101) and is the rationale for their clinical use to control autoimmune diseases and inflammation and to prevent organ rejection after transplantation.

The most general effect of GCs is to inhibit synthesis, release, and/or efficacy of cytokines and other mediators that promote immune and inflammatory reactions, both in cell culture systems and in whole organisms (reviewed in Refs. 21 and 102). These include IL-1, IL-2, IL-3, IL-4 [inhibited in human but stimulated in murine cells (103)], IL-5, IL-6, IL-12, granulocyte monocyte colony-stimulating factor (GM-CSF), IFN-$\gamma$, TNF-$\alpha$, chemokines like IL-8 (62, 81), RANTES (regulated on activation normal T cell expressed and secreted) (104), and macrophage inflammatory protein-1$\alpha$ (105), and inflammatory mediators and enzymes such as histamine, bradykinin, eicosanoids, nitric oxide (106–108), collagenase, elastase, and plasminogen activator. GCs reduce eicosanoid synthesis by inhibiting expression of the inducible form of cyclooxygenase, cyclooxygenase 2 (COX-2) (109–113). They inhibit 12-*O*-tetradecanoylphorbol-13-acetate and TNF-$\alpha$ induction of intercellular adhesion molecule 1 (ICAM-1) (114). GCs can inhibit antigen presentation and expression of major histocompatibility complex (MHC) class II proteins, reduce activation and proliferation of T and B cells (memory cells being much less sensitive than naïve cells), and shift responses from Th1 cells (which predominantly secrete IL-2 and IFN-$\gamma$) to Th2 cells (which secrete IL-10 among other antiinflammatory cytokines) (115, 116). They increase activity of transforming growth factor-$\beta$ (TGF-$\beta$), an antiproliferative cytokine that inhibits activation of T cells and macrophages (117, 118) and may induce expression of lipocortin-1 (119), which can regulate immune reactions (120).

Trafficking and function of peripheral cells are altered transiently by GCs, which rapidly lower circulating levels of lymphocytes (T more than B cells, and CD4 helper cells more than CD8 cytotoxic cells and NK cells), eosinophils, basophils, macrophages, and monocytes, but increase levels of neutrophils. This redistribution of cells is probably due largely to alterations in cell adhesion molecules (121, 122). Lymphocyte, monocyte, and granulocyte chemotaxis are suppressed, with reduced accumulation of phagocytic cells at inflammatory sites. GCs also atrophy the thymus and, to a lesser extent, other lymphoid tissues, triggering apoptotic

death in immature T and B cell precursors and mature T cells. The lymphocytolytic actions of GCs are central in treatment of lymphocytic leukemias and lymphomas. During prolonged exposure to GC therapy, they may contribute to immunosuppression. Physiologically, their role may be to facilitate both negative and positive selection of the T cell repertoire (95, 123, 124) and to remove potentially toxic activated cells (125).

Despite evidence of the suppressive actions of GC stretching back decades, enhancement of immune functions by GCs has been reported, and some recent results are striking. Jefferies (126, 127) argues for the importance of enhancing effects—which he ascribes to permissive actions—and laments their neglect in clinical practice. He cites instances where physiological doses of GCs improve the condition of patients or experimental animals, *e.g.*, by enhancing resistance to infection. Which immune functions are enhanced in such cases is unclear. As Jefferies notes, one that has been observed fairly consistently *in vitro* is the stimulation of immunoglobulin synthesis by cultured B cells (128–133). For such stimulation, GCs generally are required early in a culture, consistent with them being permissive. Some of these effects could be secondary to GC modulation of cytokine production or activity, such as the shift from T helper 1 and 2 cells (Th1 to Th2 cells) already mentioned, or the induction of cytokine receptors described below (134). However, inhibition of immunoglobulin production in culture has also been reported occasionally (135), and GCs inhibit some of the steps preceding B cell differentiation to antigen-secreting state (132) and suppress immunoglobulin production in whole organisms (97). Thus, the physiological role of these influences on B cell functions is difficult to evaluate.

While most reports indicate that GCs suppress T cell function, enhancement has been observed in humans and rats. Barber *et al.* (136) demonstrated suppression of TNF-$\alpha$ and IL-6 responses to endotoxin in humans by cortisol administered within 6 h of endotoxin. They gave cortisol (as hemisuccinate) in 6-h intravenous infusions that raised plasma cortisol levels to the micromolar range, corresponding to high stress-induced levels. By contrast, they also showed that if cortisol is given 12, 36, 72, or 144 h before endotoxin, TNF-$\alpha$ and IL-6 secretion are markedly enhanced, suggesting that permissive actions can be induced by high GC concentrations.

GCs can also enhance T cell responses in rats *in vivo* and *vitro* (102, 137–139). The response to the mitogen concanavalin A by peripheral T cells from rats adrenalectomized for 1 week was reduced 65% compared with cells from sham-operated rats. It was restored by administering low physiological plasma levels of corticosterone (~17 nM, maintained with subcutaneous pellets) and almost totally suppressed by high levels (~170 nM). Corticosterone *in vitro* at all concentrations suppressed the mitogenic response of cultured cells from either adrenalectomized or sham-operated rats, an effect blocked by RU486 at 500 nM. However, RU486 at 50 nM changed the suppression by 10 nM corticosterone to stimulation (137). Similar observations were obtained with splenic lymphocytes, stimulated with either concanavalin A (138, 139) or with the more specific stimulus of anti-T cell antigen receptor (139). In the experiments with anti-T cell antigen receptor, corticosterone had to be added within the first hour of stimulus to enhance; enhancement seemed to be due to increased expression of IL-2 receptors on T cells. In other experiments, even brief preexposure to corticosterone or aldosterone (with subsequent washing out of the steroid) enhanced the response to concanavalin A several days later (138). From these and other results, Wiegers *et al.* (139) propose that, as previously inferred from GC effects on hippocampal slices, corticosterone at low concentrations enhances T cell responses through MRs, and at high concentrations suppresses those responses through GRs (137–139).

GCs also play permissive and suppressive roles in the acute-phase response, a general systemic response to immune and inflammatory reactions triggered by injury and infection (140, 141). Cytokines and other mediators such as IL-1 and TNF-$\alpha$ are released into the circulation and stimulate hepatic synthesis of acute-phase proteins such as serum amyloid A, C-reactive protein, and complement components. GCs enhance the hepatic acute-phase response by increasing sensitivity to mediators, while suppressing the overall response by inhibiting mediator production (140).

A final example of GC-induced immune enhancement comes from an unexpected reinterpretation of classic data. Even relatively minor increases in GC concentrations can deplete circulating leukocytes. This has typically been interpreted as a decline in immune competence, as most evidence suggested that such leukocytes were being sequestered, inactive, in immune tissues. However, such depletion might instead involve diversion of circulating leukocytes to local areas of need (such as in inflamed skin) (101, 142–146). In an example of immune activation, delayed-type hypersensitivity (DTH), acute stress experienced immediately before the administration of an antigen to the skin significantly enhances a cell-mediated immune response directed against the antigen (147) [while, in contrast, chronic stress over a period of weeks suppresses the DTH response (148)]. Thus, rather than being immunosuppressive, this would represent, in the apt words of the authors, GC-induced migration of leukocytes to "battle stations."

We now consider these GC actions in the context of the criteria. The criterion of conformity—do GCs have effects on the immune system that are similar to, opposite to, or different from the more rapid stress-responsive hormones?—offers little information because, as noted, there is no consensus as to the effects of that first wave of hormones.

As discussed, the first wave of immune responses to various stressors is one of activation. Thus, the criterion of time course suggests that the inhibitory effects of GCs upon immunity and inflammation should be viewed as suppressive, whereas the more recently appreciated enhancing effects are permissive. For example, as noted, exposure of humans to cortisol for up to a week before a challenge with endotoxin enhances TNF-$\alpha$ and IL-6 levels, whereas cortisol at the time of or after endotoxin suppresses the cytokine response (136); in rats, preexposure to corticosterone *in vivo* or *in vitro* enhances mitogenesis (137–139). Furthermore, the fact that the enhancing effects of GCs in rats are seen with low levels of the hormone and can be mediated by the MR supports the permissive scenario of such enhancement occurring under

basal conditions in place at the onset of a stressor. In contrast, the requirement for higher concentrations of GCs and GR involvement for the emergence of the inhibitory effects supports the picture of suppressive actions occurring as GC concentrations rise into the stress-induced range.

The criteria of subtraction and replacement—is there an overshoot of immunity or inflammation in circumstances of diminished adrenocortical activity, and can the overshoot be counteracted with GCs?—strongly support the view that GCs suppress immunological and inflammatory stress responses. The earliest such report came in 1922, with the observation by Kepinov (discussed in Ref. 119) that adrenalectomy sensitizes guinea pigs to bronchial anaphylaxis. Adrenalectomy has also long been known to cause the thymus and other lymphoid organs to hypertrophy. Flower *et al.* (149), in a direct test of the hypothesis that endogenous GCs suppress inflammatory responses, found that adrenalectomy markedly enhanced the response to carrageenin. Moreover, the response of normal rats is enhanced by administration of RU486 (150). Bacterial endotoxin-induced sepsis in rats causes GC secretion secondary to the actions of cytokines upon the adrenocortical axis (151, 152), adrenalectomy significantly increases fever and mortality induced by the sepsis, and GCs reverse these effects (81, 153, 154). Doses of IL-1 or TNF-$\alpha$ that are readily survived by intact rats prove fatal in adrenalectomized animals (155); this effect also is reversed with GC supplementation. Circulating levels of TNF-$\alpha$, IL-6, and epinephrine stimulated by endotoxin in humans were diminished by cortisol administered within 6 h of endotoxin (136, 156). Adrenalectomized rats, and intact rats treated with RU486, developed substantially higher levels of plasma IL-6 than control rats after injection of endotoxin, an effect attenuated by administration of GCs (81, 157). In some circumstances, basal GC concentrations do not prevent immune or inflammatory overshoot; stress concentrations of GCs must be attained (81, 158). Miller *et al.* (159), however, found a linear correlation over the entire dose range between the extent of binding of GCs to splenic GRs and the extent of inhibition of mitogen-induced T cell proliferation, showing that GCs can suppress immunity over their entire concentration range.

A striking example of inflammatory overshoot is the Lewis rat, in which cytokines such as IL-1 fail to stimulate CRH synthesis or secretion so that an inflammatory stressor does not stimulate GC secretion. Lewis rats are exceptionally susceptible to experimental arthritis induced with streptococcal cell wall polysaccharide when compared with Fischer rats, and can be protected by treatment with GCs (160, 161). Similarly, Fischer rats, normally resistant to experimental arthritis, become susceptible when GC actions are blocked with RU486 (160, 161) or adrenalectomy (78, 162). Lewis rats are also very sensitive to carrageenin-induced inflammation (72) and to induction of experimental allergic encephalomyelitis (EAE), a model of multiple sclerosis (163). In normal rats the stressor of induction of EAE triggers substantial GC secretion, most probably via the stimulating actions of cytokines, and adrenalectomy significantly increases EAE-induced mortality; this increased mortality is prevented by administration of GCs that produce circulating concentrations in the stress range, but not in the basal range (158, 163, 164). Immune overshoot also occurs in obese strain chickens that spontaneously develop autoimmune thyroiditis (99, 165, 166). Their hypothalamic-pituitary-adrenal (HPA) axes are resistant to cytokine activation (79); furthermore, the biological potency of any secreted GCs is greatly decreased because of a doubling of circulating transcortin concentrations (167).

Clinical reports show parallels to these findings. Individuals with Addison's disease are prone to bronchial asthma, various allergies, and autoimmune adrenalitis (168–170). Moreover, unilateral adrenalectomy to remove an adrenocortical adenoma can cause a flare-up of autoimmune thyroid disease (171); whether the adrenalitis or thyroid disease in these two cases is more readily triggered in circumstances of stress is not known. Furthermore, individuals with inflammatory arthritis (*i.e.*, rheumatoid), but not those with degenerative arthritis (*i.e.*, osteoarthritic), have significantly impaired GC stress responses (69, 172).

The criterion of homeostasis—do GC effects in this realm during stress make sense?—has long presented a challenge, because of the classical inhibitory actions of GCs. As noted, one response of many GC physiologists has been to relegate them to pharmacology. Other attempts at incorporating them into physiology now appear quite unsatisfactory, such as the speculation that immunity is suppressed to spare energy during the prototypical physical stressor (173) or that GC-induced lymphocytolysis provides substrate for gluconeogenesis and tissue repair (174).

More recent work has helped clarify the homeostatic logic of the immunosuppressive effects of GCs, as well as their predominance at higher concentrations and only after the first wave of the stress response. Immunosuppression is logically viewed as suppressing the stress response to an infectious stressor to decrease the likelihood of autoimmune overshoot. Antigenic challenges to the immune system trigger polyclonal responses, raising the risk of autoimmunity where epitopes recognized by some of the clones overlap with those of normal body constituents. It has been suggested that under physiological conditions GCs are selective, "sculpting" the immune response so that superfluous or autoimmune-prone components are selectively inhibited (175). This is due to the preferential targeting by GCs of lymphocytes that are less active or that produce antibodies with lower affinities for the antigen (176, 177). Consistent with this role of GCs, after an infectious stressor, GC concentrations peak when the antiantigen response peaks (80, 178), which may be days later. A similar synchrony of ACTH, corticosterone, and IL-6 responses follows an inflammatory stressor (179). Another argument for the homeostatic value of GC suppression is that many cytokines induced by stressors can be toxic in excess, independent of their stimulation of immune and inflammatory reactions, and thus their levels need to be controlled (180, 181).

Thus, the criterion of homeostasis suggests that the enhancing effects of GCs be viewed as permissive, while the delayed inhibiting effects are suppressive.

Why were enhancing, permissive effects of GCs so rarely observed in earlier studies? Surprisingly, the results of Barber *et al.* (136) were obtained with large doses of GCs administered to subjects with normal GCs. Classical permissive effects, such as those on gluconeogenesis or cardiovascular

functions, have generally been elicited with basal levels of GCs in subjects with subnormal or no GCs. This illustrates the earlier point that permissive effects probably have dose-response relations similar to other GC effects, but whose effects at high doses of GCs are usually obliterated by suppressive effects. In the experiments of Barber *et al.*, permissive and suppressive effects were separated by timing of GC administration. Wiegers *et al.* (139), in trying to account for the differences between their results and those of others with rats, mention that the density of cells in culture may be a critical variable for the responses of T cells. They also suggest that in the other studies, high and prolonged GC exposure may have suppressed enhancing effects. One of their tools for uncovering permissive effects was the GR antagonist RU486, which does not block MRs. Thus, if permissive effects on T cell functions are generally mediated by MRs, a reinterpretation may be necessary of experiments in which administration of RU486 exacerbates immune or inflammatory responses. Exacerbation has usually been interpreted as being caused simply by blocking of suppressive GC actions, but could also be due partly to RU486 uncovering permissive enhancement by GCs through MRs. Synthetic GC agonists like dexamethasone, which are often used for immunosuppression both experimentally and clinically, would be unlikely to reveal permissive effects through MRs since they are effective immunosuppressants at much lower concentrations than corticosterone or cortisol and would activate MRs much less than the natural GCs. Finally, another reason for the dearth of earlier reports of permissive effects of GCs on T cell functions may be that not all T cell-mediated responses require permissive enhancement.

Enhancement by GCs via up-regulation of hormone, cytokine, and growth factor receptors has been proposed to underlie permissive activation of several physiological systems (134, 139, 182, 183). Among such receptors are those for IL-2 (139), IL-6, IFN-γ, GM-CSF, and CSF-1 (6, 100). For example, GC up-regulation of GM-CSF can explain GC synergism with GM-CSF to increase MHC class II expression (184). Such effects could also account for the generally beneficial influences of GCs in culture media (182). GC inhibition of production of mediators that act through many of these receptors is initially paradoxical. However, a simple mathematical model shows that combined stimulating and inhibitory effects, even with identical dose-response curves, generate a bell-shaped dose-response curve according to which GCs activate homeostatic mechanisms permissively at basal levels reached during normal diurnal variation and suppress them at stress-induced levels (Fig. 2) (6, 9). The bell-shaped curve generated via GC receptors extends GC influences over a wide concentration range, which is even further extended at low concentrations if permissive GC actions are mediated via MRs, as just described for T cell mitogenesis (139). Although there is no time axis in the figure, permissive actions should be thought of as preceding, and suppressive actions as following, a stressor.

*Conclusions:* With infectious stressors, immune activation precedes (and contributes to) the eventual increase in GC concentrations at which suppressive effects occur. Furthermore, GC deficiency is associated with pathological overshoot of inflammatory and immune responses; GC secretion induced by stress protects against this overshoot, sculpting and restraining the immune response. Even complete absence of GC activity does not diminish inflammatory and immune responses, as would be expected if permissive GC actions were required to enhance or "prime" those responses. Thus, most GC actions on immune and inflammatory reactions are suppressive, even under conditions of exposure to basal GC concentrations, while evidence is mounting that permissive actions also play important roles. Thus, it appears that GCs present in advance can permissively help mediate the immune activation demonstrable during the first moments of response to a variety of stressors, whereas stress-induced GCs later act to rein in that same activation.

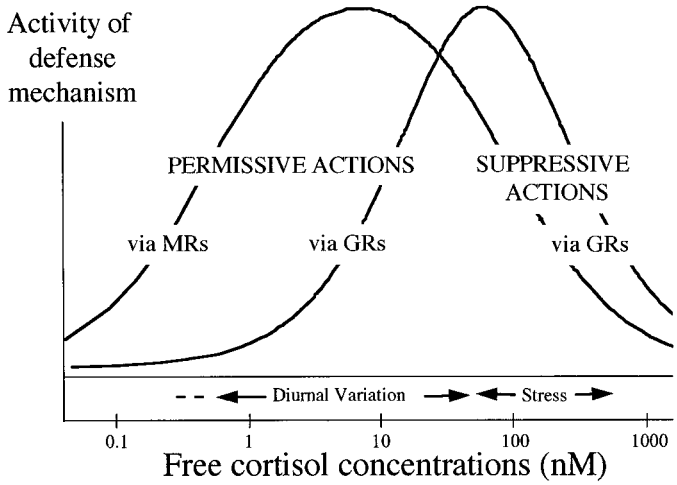


FIG. 2. Regulation by GCs of defense mechanisms through permissive and suppressive GC actions. The two *bell-shaped curves* are derived from a mathematical model of a GC-regulated defense mechanism composed of a mediator, its receptor, and the mediator-receptor complex that generate activity (6). Cortisol is assumed to permissively induce mediator receptors via either GC receptors (GRs) or mineralocorticoid receptors (MRs), and to suppress mediator levels via GRs. Thus, with increasing cortisol concentrations, activity first rises over the basal cortisol range as mediator receptors increase but then decreases as mediator levels are suppressed by cortisol in the stress-induced range. Cortisol actions are calculated using a $K_d$ of cortisol for GRs of 30 nM, and of cortisol for MRs of 0.5 nM, assuming the actions are proportional to the concentration of cortisol-receptor complexes. Approximate values are given for ranges of basal diurnal and stress-induced free cortisol concentrations in humans.

### *D. Metabolism*

The early phases and endocrine mediators of the metabolic stress response have been understood for decades (Fig. 1, A and C). Blood glucose levels are elevated rapidly, in part by mobilization from existing stores, and by inhibition of further storage through a rapid insulin resistance (185); thus, energy is diverted from storage sites to exercise muscle. These changes are brought about by catecholamines, glucagon, and GH.

The preeminent effect of GCs upon metabolism is their ability to increase circulating glucose concentrations. This is accomplished through a number of mechanisms. One, discussed later, is via stimulation of appetite by low levels of GCs (186). In addition, when GCs are present for hours before the stressor, there is 1) the stimulation of glycogen-

olysis and gluconeogenesis by glucagon and catecholamines that constitute the immediate stress response; 2) stimulation of hepatic gluconeogenesis and glycogen deposition; and 3) inhibition of peripheral glucose transport and utilization (reviewed in Refs. 187–193). In addition, GCs mobilize lipids through lipolysis in fat cells, and amino acids through inhibition of protein synthesis and stimulation of proteolysis in various muscle types.

The criteria yield a clear interpretation of these GC actions. By the criterion of conformity, GCs help to mediate permissively the metabolic stress response, synergizing with catecholamines, GH, and glucagon to stimulate lipolysis and to elevate circulating glucose concentrations by stimulating glycogenolysis and gluconeogenesis (cf. Refs. 189, 192, and 193). Epinephrine and glucagon act quickly, whereas GCs act slowly to enhance and prolong for several hours the increase in blood glucose due to epinephrine or glucagon (189).

A similar conclusion is reached by applying the criteria of time course and subtraction: during a physical stressor, Addisonian and adrenalectomized individuals are impaired in mobilizing the necessary energy substrates, a defect corrected with maintenance doses of GCs. As early an investigator as Selye (194) showed that this impaired capacity to mobilize substrates becomes fatal during stress when the organism is already food deprived. Furthermore, from the standpoint of homeostasis, it makes abundant sense for the metabolic stress response to be one of mobilization of substrate stores and their diversion to the subset of tissues that need them.

With regard to the slower stimulation of gluconeogenesis and inhibition of peripheral glucose utilization by stress-induced GCs, they clearly supplement the permissive actions and may be responsible for extending and prolonging the stress response. They can therefore be categorized as stimulatory. Stimulation of liver glycogen deposition, however, which similarly takes a few hours, can have little influence on the stress response, but by restoring glycogen levels prepares for the next one. It thus is best classified as preparative.

*Conclusions:* All four criteria suggest that during a prototypical stressor, GCs help mediate the metabolic response through both permissive and stimulating actions and also have preparative actions. These actions appear to arise from a mixture of monotonic and biphasic effects over the GC dose range. For example, GC inhibition of glucose uptake is monotonic (195). Fat depletion is stimulated by GCs over their entire dose range (188). In contrast, the muscle-wasting effects of GCs appear to occur only in the stress range (196). These mediating GC actions should be viewed as both permissive and stimulatory. The preparative GC stimulation of hepatic glycogen deposition gives a classic monotonic dose-response curve.

These interpretations of the roles of GCs in metabolic stress responses differ from those in Ref. 1, where GC actions were viewed as "counterregulatory" to those of insulin, and therefore suppressive (202). This shift in interpretation can be understood by distinguishing between the effects of GCs upon metabolism, and those of GC-induced insulin secretion. During the normal daily fluctuations of fasting and feeding, of repose and activity, each with their associated metabolic demands, and after injury or during disease states, the metabolic actions of GCs are intertwined with those of insulin and certain other hormones. In these interactions a central physiological variable is the level of blood glucose, which must be kept from falling below some threshold for normal brain function and may have to be raised acutely to satisfy a sudden need for energy. GC actions generally oppose but sometimes synergize with those of insulin. For example, GCs and insulin have opposite actions on blood glucose levels, as well as on appetite, gluconeogenesis, glucose transport, protein synthesis, muscle wastage, lipolysis, lipogenesis, and fat deposition in adipose tissue (197); they synergize in stimulating hepatic glycogen deposition and lipogenesis (188, 198, 199). Elevated GCs raise insulin concentrations; whether this is due to direct GC stimulation of secretion or is secondary to the metabolic actions of GCs is unclear (188, 200). Sustained GC secretion causes sustained insulin secretion after a delay of a few hours. Chronically elevated GCs, as in Cushing's syndrome, cause pronounced muscle wastage, fat accumulation and redistribution, and are diabetogenic. Thus, in analyzing the actions of GCs, the concurrent effects of insulin must be taken into account. True GC effects are most readily demonstrated in the absence of insulin secretion (*e.g.,* in streptozotocin diabetic rats), in which GC's lipolytic, proteolytic, and gluconeogenic effects are dramatic (188, 198, 199, 201).

Catecholamines, glucagon, GH, and GCs are known as "counterregulatory" hormones, reflecting their ability to counteract the hypoglycemic activity of insulin by raising blood glucose levels (203–204). This term is often used to describe how the secretion of these hormones, stimulated by the postprandial elevation of insulin levels (188, 205) or by insulin administration in the diabetic patient, protects against hypoglycemia. However, in a mammal sprinting across the savanna, it is the secretion of the "counterregulatory" hormones that comes first, mobilizing energy substrates. Only with the abatement of the stressor do insulin's opposing actions emerge, reversing the metabolic actions of these other hormones.

Insulin administration to a laboratory animal or normal human has long been used to stimulate an endocrine stress response or simulate the rise in insulin levels that follow a meal. This reflects not only the convenience of the method, but the importance that the understanding and management of diabetes has in clinical endocrinology. Within that framework, GCs are "suppressive" as they prevent insulin-induced hypoglycemia from overshooting (1). However, an insulin surge and a sprint across the savanna are different stressors. The latter, we believe, is the more logical setting to understand the evolution and physiological relevance of GC secretion during stress, although the former, which utilizes the same hormonal actions and metabolic pathways, also carries survival value.

If stress physiology had a tradition of drawing upon ethologists rather than diabetologists, insulin would perhaps be termed a "counterregulatory" hormone. However, under basal, nonstressed circumstances, GCs, catecholamines, GH, and glucagon interact with insulin in complex ways that justify the view that each class of hormones counterregulates the other at some point.

*E. Neurobiological effects*

The neurobiological actions of GCs were only briefly touched on in Ref. 1. Since then, numerous studies have reported electrophysiological and neurochemical effects of GCs (cf. Ref. 206). Unfortunately, most of these findings are too reductive to be interpreted physiologically. For example, consider that GCs modulate the effects of a neurotransmitter upon turnover of a second messenger in a particular brain region (207), or that GCs modulate the levels of mRNAs for a particular subtype of the *N*-methyl-D-aspartate receptor (208). It is unlikely that information exists as to the time course and dose responsiveness of effects such as these, the effect of the rapid stress-responsive hormones on these endpoints, and the preparative value of any such actions.

For this reason, we have chosen three topics among the neurobiological and behavioral effects of GCs. They are interpretable in the context of adapting to stress, and there is information as to the effects of the early wave of stress-responsive hormones on these endpoints, plus dose-response information regarding GC actions.

*1. Cerebral glucose transport and utilization.* Stress increases local cerebral glucose utilization within seconds (209), an effect mediated by sympathetic activation. It is probably not due to catecholamines directly acting upon glucose transport mechanisms in neurons or glia, since catecholamines do not readily pass the blood-brain barrier. Instead, sympathetic arousal stimulates cardiovascular tone and increases cerebral blood flow.

GCs are well known for inhibiting glucose transport in various peripheral tissues (210). This phenomenon appears to extend to the brain. *In vivo*, GCs inhibit local cerebral glucose utilization throughout the brain (211–214) and inhibit glucose transport in neurons, glia, and possibly endothelial cells *in vitro* (215, 216). The effect requires stress levels of GCs (a minimum of 100 nM) and is GR-mediated. The mechanisms underlying the inhibition are understood. Over the course of minutes to hours, GCs cause the translocation of glucose transporters from the cell surface to inactive intracellular storage sites (217–219). In addition, over the course of hours to days, GCs also decrease the level of mRNA for the glucose transporter (220).

These findings yield a consistent categorization when the criteria are applied. Insofar as GCs do the opposite of catecholamines, by the criterion of conformity GC actions are suppressive. GCs are also suppressive by the criterion of time course in that they reverse the stimulation of glucose utilization occurring in the early seconds of the stress response. Adrenalectomy increases glucose utilization throughout the brain (211), suggesting a suppressive action by the subtraction criterion.

*2. Appetite and feeding.* Stress suppresses feeding in less than 1 h, even in food-deprived animals (221). This effect is probably mediated by CRH; the peptide is a potent anorexic agent, and CRH antagonists block the anorexic effects of stress (222). These CRH actions reflect a neurotransmitter role, as the effect occurs in hypophysectomized animals, or after intracerebroventricular injection of CRH (223).

In contrast, GCs stimulate appetite over days in rats. Adrenalectomy decreases feeding and food-seeking behavior (224), which is reversed by GC administration. Appetite normally peaks at the time of the circadian cycle when GC concentrations peak, and this peak can be shifted with GC treatment (188). These GC actions appear to center in the paraventricular nucleus of the hypothalamus, where crystalline implants of GCs also stimulate feeding (225, 226).

GCs stimulate appetite monotonically over the entire dose range in various species, including humans. There are two complications in reaching this conclusion. First, while basal concentrations of GCs stimulate appetite (188), stress concentrations decrease appetite, a finding that changes the interpretation of this section (227, 228). This inhibition was subsequently shown to be due to the high concentrations of GCs stimulating a burst of insulin secretion. The inhibitory effects of insulin upon appetite (229) more than offset the stimulating GC effects; in the absence of GC-induced insulin secretion (in streptozotocin diabetic rats), GCs stimulate appetite over the entire dose range (188).

Second, aldosterone, or GCs at concentrations that only occupy the MR, stimulate consumption of both carbohydrates and fats, whereas GR-specific agonists stimulate only carbohydrate consumption (226). However, despite low and high concentrations of GCs stimulating appetite in different ways, GCs nonetheless stimulate feeding in a monotonic manner over their entire dose range.

Thus, by the criteria of conformity and of time course, GC actions suppress these facets of the stress response. The criterion of subtraction leads to this categorization as well as the adrenalectomy data just noted.

In considering the criterion of homeostasis, we can perceive no way in which the relatively slow stimulation of appetite (by GCs) could help during a stressor such as a sprint across a savanna. In contrast, the earlier responses, which are then inhibited by GCs, are readily viewed in that manner. Feeding, a costly process that provides energy relatively slowly, is obviously expendable during a stressful crisis. Thus, this criterion suggests that GC actions suppress and aid the recovery from the anorectic facet of the stress response. In addition, to the extent that GCs stimulate appetite to the point that metabolic stores are ultimately greater than before the onset of the stressor [a pattern often seen (188)], there are preparative features to this GC effect, equipping the organism for the metabolic costs of a subsequent stressor.

Thus, by stimulating appetite and feeding, GC effects are mostly suppressive, with some preparative features as well. The fact that GCs have these effects over their entire dose range, plus the seeming involvement of both MRs and GRs, suggest that basal and stress levels of GCs tend to suppress this facet of the stress response. Moreover, insofar as feeding is preparatory for the energy expenditure of the next sprint across a savanna, there are preparative elements to these GC actions as well.

*3. Memory formation.* Acute stressors enhance memory formation, a phenomenon familiar to many in the form of vividly remembering where they were when some tragic historical news was announced (230). As a more controlled demonstration of this phenomenon, volunteers were read

one of two stories, equivalent in length and complexity and virtually identical in their beginning and end, but differing dramatically in the emotionally stressful content of the middle of the story (the first story being fairly neutral in content, and the second describing a disturbing accident). Memory of the emotionally laden component, but not of the neutral components of the second story, was enhanced relative to the first (231).

Catecholamine secretion appears to mediate this phenomenon, as it can be blocked with β-adrenergic receptor antagonists (231, 232). The sympathetically mediated increase in cerebral perfusion rate and glucose delivery to the brain during the early phases of the stress response probably plays a role in the memory enhancement. As evidence, peripheral or ventricular infusion of glucose in the ranges achieved during stress enhance memory formation (233–235), commensurate with the metabolic costs of neuronal plasticity during learning. Other mechanisms for the catecholamine involvement in this phenomenon have been advanced (230).

The effects of GCs upon memory are complex and are centered in the hippocampus, a brain region central to learning and memory (236) that possesses high levels of MR and GR. Basal levels of GCs enhance forms of synaptic plasticity thought to be underpinnings of learning (237–240). These effects are mediated by MRs (240–242). Moreover, basal levels of GCs, acting via MRs, enhance hippocampal excitability in general (243–246). This is probably accomplished by shortening and shallowing the hyperpolarized refractory period of hippocampal neurons after an action potential (243). As would be expected from these findings, adrenalectomy disrupts memory processes in animals, and occupancy of MRs with GCs restores function (247), while MR antagonists disrupt cognition (248, 249).

In contrast, stress levels of GCs, working via the GR, have opposite effects. Over the course of hours, GCs disrupt those same forms of synaptic plasticity and blunt general hippocampal excitability (by prolonged hyperpolarizations) (237–239, 243–246). These effects can be shown in hippocampal slices *in vitro*, suggesting direct intrinsic effects on these neurons. Insofar as stress levels of GCs inhibit glucose transport and utilization (see above), this should be an extrinsic, metabolic mechanism for disrupting memory formation as well [given the importance of glucose availability to memory (235)]. Prolonged exposure to stress levels of GCs atrophy hippocampal neuronal processes and, ultimately, cause neuron loss as well (although the relevance of these very gradual effects to the prototypical scenario of the sprint across the savanna is minimal). These GC effects have been relatively well documented in rodents and primates [emerging over the course of weeks and months, respectively (250)], and hints have emerged for a similar phenomenon in the human [emerging over the course of years (251)]. As would be expected, sustained exposure to elevated GC concentrations disrupts memory. This has been long recognized in patients prescribed high-dose corticosteroids for sustained periods and has been demonstrated as well in cross-sectional and longitudinal neuropsychological studies of such patients (252). Moreover, administration of GR agonists to healthy volunteers disrupts memory within a few days (253, 254).

The application of the criteria produces clear conclusions. The criterion of conformity suggests that basal levels of GCs are permissive, in that they enhance memory as do catecholamines. In contrast, by that criterion stress levels of GCs are suppressive. A similar dichotomy emerges when applying the criterion of time course. The same conclusion is reached in considering the careful adrenalectomy studies in which there was a distinction between replacement with low levels of GCs or with MR-specific agonists (in which adrenalectomy-induced memory problems are reversed), and replacement with high GC levels or GR-specific agonists [in which memory problems are worsened (247)].

The criterion of homeostasis is readily applied to some components of GC actions. It seems apparent that sharpening memory consolidation and retrieval is a valuable response to a stressor, in that it aids the recall of behaviors that worked previously, as well as the consolidation of memories meant to avoid this stressor in the future. In that regard, the enhancement of memory processes during the early stages of responding to a stressor can be viewed as logical and salutary. The value, if any, of disrupting memory with more sustained stressors, is unclear to us.

Thus, the criteria suggest that basal GC levels at the onset of a stressor permissively help mediate the cognitive stress response, whereas the subsequent stress-induced rise in GC concentrations suppresses the cognitive response.

*Conclusions:* The neurobiological and behavioral effects of GCs during stress discussed above can all be categorized as having suppressive elements. In the case of glucose utilization and transport, stress-induced GC concentrations suppress the earlier stress response, while both basal and stress-induced concentrations suppress appetite. The review of this literature also suggests that GCs might have some preparative actions in the realm of appetite.

This represents the conclusions only for these three neurobiological examples that were chosen because they represent the best examples of GC/nervous interactions that are understood on a reductive neuroendocrine level while also being interpretable within the larger context of coping with a stressor. Other aspects of GC neuroendocrinology might be categorized differently. For example, GCs can have rapid effects (over the course of seconds to minutes) on behavior in birds and reptiles (7, 8, 255, 225–42). These actions (which are probably mediated by membrane-bound receptors) include inhibition of sexual behavior, and stimulation of escape behavior, and have been interpreted as helping to mediate behavioral features of the stress response (255, 256). While those GC effects are limited to nonmammalian species, they suggest that our conclusions in this section should not be viewed as global statements about GC/nervous system interactions.

### *F. Reproductive physiology*

The 1984 review (1) did not consider the effects of GCs upon reproduction. Nevertheless, the wealth, consistency, and physiological and pathophysiological relevance of the data in this area lead us to include the topic now.

The onset of a stressor initiates inhibition of reproductive physiology and behavior. This involves a decline in portal

GnRH concentrations and pituitary release of gonadotropins within minutes (Fig. 1A). Moreover, there is rapid loss of erections in response to an acute stressor in males and a decline in sexual proceptivity and receptivity in both sexes.

The first wave of hormonal mediators of the stress response are central to this reproductive suppression. CRH inhibits reproductive physiology and behavior (257, 258), and administration of CRH antagonists partially reverses stress-induced suppression of LH release (259). The effect on the pituitary is secondary to inhibition of GnRH release, since intracerebroventricular rather than peripheral administration of CRH or its antagonists is effective (259–261), CRH does not directly blunt pituitary responsiveness to GnRH (262), and CRH can directly inhibit hypothalamic release of GnRH *in vitro* (263). Opiate release during stress is also reproductively suppressive and, like CRH, involves inhibition of hypothalamic GnRH release (264–273). The opiate inhibition of GnRH appears to be the proximal mechanism by which CRH exerts its antireproductive actions (262, 274). Finally, the sympathetic nervous system has antireproductive properties. For example, sympathetic activation blocks the parasympathetically mediated initiation of erections (275). Within the humoral realm, adrenomedullectomy or administration of sympathetic $\beta$-blockers attenuates the suppression of LH and FSH by stress (276).

The effects of GCs in this realm are well understood. GCs potently disrupt reproductive physiology through a number of mechanisms. They decrease hypothalamic GnRH release (277, 278) and basal or GnRH-stimulated release of LH from the pituitary (Refs. 279–287; this effect predominately occurs in females). In addition, GCs reduce gonadal responsiveness to LH and concentrations of LH receptors (Refs. 286 and 288–292; this effect predominately occurs in males). These patterns occur in both *in vivo* and *in vitro* systems and in rodents, humans, and other primates.

These studies have mostly used concentrations of GCs in the stress range. It is less clear whether basal GC concentrations have similar effects. Some studies suggest not. In one of those, treatment regimens of 20 or l00 $\mu$g/kg/day dexamethasone for 5 days did not lower basal LH concentrations in male rats, whereas 500 $\mu$g/kg/day did so dramatically (279). The lower, inefficacious dexamethasone doses produce GR occupancy roughly in the range seen for basal concentrations of corticosterone, whereas the higher doses are roughly comparable to a stress signal (293). Moreover, low doses of dexamethasone administered over a series of days failed to lower LH concentrations in castrated women (294). Similarly, some papers indicate that adrenalectomy of unstressed animals does not elevate testosterone concentrations (295). These results suggest that basal concentrations of GCs are insufficient to disrupt reproductive physiology. In contrast, other studies suggest that basal GC levels do exert a tonic inhibitory effect. For example, basal GC levels inhibit rat Leydig cell steroidogenic capacity (296). Corticosterone levels in these cells are regulated through inactivation by the oxidative activity of 11$\beta$-hydroxysteroid dehydrogenase 2 (11$\beta$-HSD2), which is itself induced by corticosterone, forming a local negative-feedback loop (296–298). Furthermore it has been reported that adrenalectomy of unstressed animals is indeed associated with elevated concentrations of testosterone (296, 294, 299). Knox *et al.* (300) found that antagonism of the GR (with RU486) in unstressed female rats increased LH concentrations (as an interpretive problem in this study, the effects of the RU486 could have been by antagonism of progesterone receptors). Thus, it remains unclear whether basal levels of GCs disrupt reproductive physiology.

Application of the various criteria yield a consistent conclusion. By the criterion of conformity, GCs appear to mediate the reproductive stress response, insofar as they have the same broad antireproductive effects as do catecholamines, opiates, and CRH during stress. Similarly, the criterion of time course argues against GCs suppressing the stress response; with a handful of exceptions among only subgroups of animals (301, 302), there is no evidence for enhanced reproductive physiology during the first minutes of stress.

The criterion of GC subtraction leads to the same conclusion. Were the antireproductive effects to be suppressive, reining in the stress response, then adrenalectomized rats, Addisonian humans, and obese strain chickens should all show some manner of reproductive overshoot during stress (*e.g.,* elevated concentrations of gonadal steroids, superovulation, hyperplastic sperm production, or premature puberty). To our knowledge, no such patterns have been reported.

The criterion of homeostasis suggests that the antireproductive effects of GCs during stress are mediating. Reproduction is a highly costly anabolic state, particularly in a female, and should logically be deferred during a stressor. This logic dominates classic models in natural selection theory and ecology regarding the stressful effects of overcrowding, habitat degradation, and social subordinance (303, 304). [Of note, this logic does not apply to a few species that are semelparous (*i.e.*, in which breeding occurs only once in the lifetime). In such cases, it is not evolutionarily logical for stress to suppress that sole opportunity for reproduction. Those species appear to have evolved mechanisms by which the gonadal axis is resistant to the suppressive effects of stress. For example, semelparous marsupials and salmonids (such as the Pacific salmon) secrete vast amounts of GCs at the time of the single bout of breeding; while such GCs have numerous deleterious effects throughout the body, reproductive behavior and physiology are unperturbed (305); it has been hypothesized that tissues of the gonadal axis downregulate corticosteroid receptor number at the time of breeding (306). As another route seen in some semelparous bird species, climatic stressors that would normally cause robust GC secretion fail to do so during the sole mating season (255, 256, 307)].

*Conclusions:* Stress (and perhaps basal) GC levels inhibit reproduction in most species. These effects are intercalated with those of the hormones of the first wave of the stress response and have effects similar to those seen during the first moments of the stress response. These antireproductive effects can be rationalized as a logical contributor to the stress response, insofar as they triage an expensive physiological process until a more auspicious time. Finally, in the absence of GCs, there is not evidence of a "pro-reproductive" overshoot during stress. Collectively, these findings consistently suggest that GC actions are not suppressive.

In preceding sections of this review, strong evidence against a suppressive role was accompanied by complementing support for a mediating role (either permissive and/or stimulatory). It is less clear whether GC actions in this realm are truly mediating. As noted, very shortly after the onset of a stressor, broadly integrated antireproductive effects occur, in the form of loss of sexual receptivity and proceptivity in both sexes, and loss of erections in males. It is not clear, however, whether GCs play any role in these phenomena beyond the sparse data noted in birds and reptiles, which suggest rapid, membrane receptor-mediated suppressive effects of GCs on reproduction. In contrast, in mammals, the antireproductive effects of GCs are manifested more slowly, beginning with a contribution to the decline in circulating sex steroid concentrations (over the course of hours), to far more integrative endpoints (such as disruption of ovulatory cycles) that take days or weeks to emerge. It is difficult to view such GC actions as helping to mediate responses to a stressor as outlined at the beginning of this review. Thus, we tentatively conclude that the GC effects on reproductive physiology during stress should be thought of as having preparative elements as well.

## IV. An Integration

Table 2 presents a summary of the various categories of GCs actions, as derived through the application of the four criteria. Some categorizations are straightforward. Others are not. For example, in the realms of immunity and memory, GCs exert both permissive and suppressive effects; in the cases of appetite, immunity, and fluid volume, the suppressive GC actions are demonstrable at basal as well as stress levels; metabolic GC actions in relation to stress may be permissive, stimulating, and preparative (and in relation to feeding, suppressive); finally, the actions of GCs upon reproductive physiology and behavior are probably best thought of as preparatory for the next stressor (given the cautions we voiced about categorizing an effect as "preparative").

TABLE 2. Summary of categorizations of glucocorticoid actions

| GCs as helping to mediate the ongoing or pending stress-response | | GCs as helping to rein in the stress-response | |
|---|---|---|---|
| Permissive | Stimulatory | Preparative | Suppressive |
| 1. Cardiovascular effects | | | |
| Yes | | | |
| 2. Effects on fluid volume | | | |
| | | | Yes[a] |
| 3. Immunological effects | | | |
| Yes | | | Yes[a] |
| 4. Effects on metabolism | | | |
| Yes | Yes | Yes | |
| 5. Effects on glucose transport and utilization in the brain | | | |
| | | | Yes |
| 6. Effects on appetite | | | |
| | | Yes | Yes[a] |
| 7. Cognitive effects | | | |
| Yes | | | Yes |
| 8. Effects on reproductive behavior and physiology | | | |
| Yes | Yes | Yes | |

[a] Indicates suppression by both basal and stress-induced GC levels.

### A. The logic of the heterogeneity of categories of GC actions

This lengthy analysis was prompted by the earlier revisionist synthesis of Munck and colleagues (1) and differs from it considerably. Of the six physiological systems considered in this review, two were not considered in that prior synthesis (neurobiological and reproductive issues), and of the remaining four areas analyzed in both, markedly different conclusions are reached about two of them (metabolism and cardiovascular function). Considering this new synthesis allows one to conclude that the classic emphasis of Selye (194) on the stimulatory actions of GCs, the later focus by Ingle (3) on the permissive actions, and the more recent revisionist emphasis on the suppressive effects (1) all have validity. Permissive and suppressive actions clearly predominate among those we have identified. We find only one clear-cut instance of stimulatory actions, and two of preparative actions, but as discussed later, preparative actions may have a much wider scope than we have considered so far. Is there a way to make a coherent whole out of the disparate ways in which GCs influence stress responses? By this, we mean, is there any underlying logic as to *why* certain GC actions are permissive, suppressive, stimulatory, or preparative? We offer a few tentative speculations.

We are struck by a dichotomy between generalized *vs.* specialized stressors. GCs help mediate some of the most generalized responses to a broad array of physical stressors. Regardless of the physical stressor, it is useful to prime an organism to mobilize energy for immediate utilization and increase substrate delivery to exercising muscle by enhancing cardiovascular tone, or to defer costly anabolism. Thus, GCs help to mediate the "backbone" of the generic stress response. In contrast, GCs appear to suppress responses to some rather specific and unique stressors—joint injury, hemorrhage, infection. As such, during a "generalized" stressor (*e.g.*, the sprint across the savanna), the organism might derive the immediate benefits of the anticipatory permissive effects of GCs on cardiovascular function and metabolism, while the more specialized and delayed "suppressive" features (suppressing immunity, inflammation, and water retention) are neutral in their effects. In contrast, during a specialized stressor—a joint injury or hemorrhage, for example—the organism may derive the benefits of the "mediating" GC actions while GCs are still preventing excessive inflammation or vasoconstriction.

### B. An appreciation of permissive GC actions

To state a tautology, a stressor triggers the secretion of GCs into the stress-induced concentration range. This prompts an understandable focus on the relevance of such elevated concentrations for coping with a stressor, and myriad laboratory studies have examined the magnitude and consequences of GC secretion many minutes or hours into the stress of immobilization, exposure to an aversive learning or shock paradigm, forced swimming, sustained hypoglycemia, and so on. Often unappreciated is that some of the most threatening of stressors in more naturalistic settings last for only seconds. For example, the median chase times of zebras and wildebeest by hyenas are 46 and 43 sec, respectively (308); similar

numbers apply to lions (309). This is much faster than the latency for GCs to first exert the genomically mediated actions that constitute the bulk of their effects (Fig. 1B). This forces a critical clarification: a significant proportion of the duration of many stressors, and the entire duration of some stressors, occurs long before stress-induced GCs have exerted any significant effects.

This reaffirms the importance of Ingle's pioneering emphasis on permissive GC actions (3). These were defined as instances where basal GC concentrations permit or normalize responses to stress and various agents, including other hormones, by priming in advance some of the body's homeostatic defense mechanisms. Permissive GC actions may be virtually identical to the maintenance actions exerted basally by GCs. Basal GC levels peak at the beginning of the activity phase of the daily cycle, as though preparing the organism for action (6). Their essential role may become evident only in the face of a stressor. As a measure of the value of permissive GC actions, an animal with no GCs, when exposed to a stressor (Fig. 3A) is less likely to survive than an animal with basal levels throughout (Fig. 3B). Less explored is whether other parameters of permissive actions also protect. For example, are basal GC levels before a stressor and stress-induced levels after (Fig. 3C) more protective than no GCs prior but with stress-induced levels after a stressor (Fig. 3D)? Would the pattern in Fig. 3C be more protective than that in Fig. 3E? The extent of the importance of permissive actions awaits further study.

Of clinical relevance, the "permissive" scenario emphasizes how much survival of a stressor revolves around relatively low circulating GC concentrations. This suggests that less exogenous GCs are needed to maintain patients with adrenal insufficiency than often assumed, as shown in some recent studies (310–312).

A secondary consequence of this reemphasis on permissive actions is that suppressive GC actions that occur only in the stress range (*i.e.*, the effects on glucose utilization in the brain and on cognition) can be central to recovery from the rapid stressor. Thus, while many naturalistic stressors are indeed sustained (as for either actor in a sustained hunt, most competitive interactions between members of social species, or adverse ecological conditions), the existence of many very rapid stressors forces a rethinking as to the meanings of basal and stress-induced GC levels.

### *C. The relevance of preparative actions in an ethological context*

The previous section suggests that for many naturalistic stressors, the effects of basal GC concentrations are more important than are the effects of stress-induced levels. Thus, the question remains as to the purpose of the stress-induced rise in GCs when the stressor is completed before the biological effects of that GC rise occur.

We have introduced here the categorization of some GC actions as being "preparative," adapting the organism for responding to the next stressor rather than the present one. In the case of such rapid stressors, perhaps GC actions should be thought of as preparative. In other words, if basal, permissive actions of GCs play a more significant role in coping with an ongoing stressor than previously appreciated, then elevations of GC concentrations might be as much about preparing for the next stressor as recovering from the current one.

Support for this idea would come from the demonstration that there are frequent instances in which animals in naturalistic settings elevate GC concentrations in anticipation of a challenge, rather than merely in response to one. This requires that stressors must frequently be predictable; this is often the case for naturalistic stressors. To show this, we begin by noting two examples in which GCs are secreted in preparation, rather than in response to a stressor:

1. Numerous studies have explored a phenomenon that can be schematized as follows: two food-deprived rats have

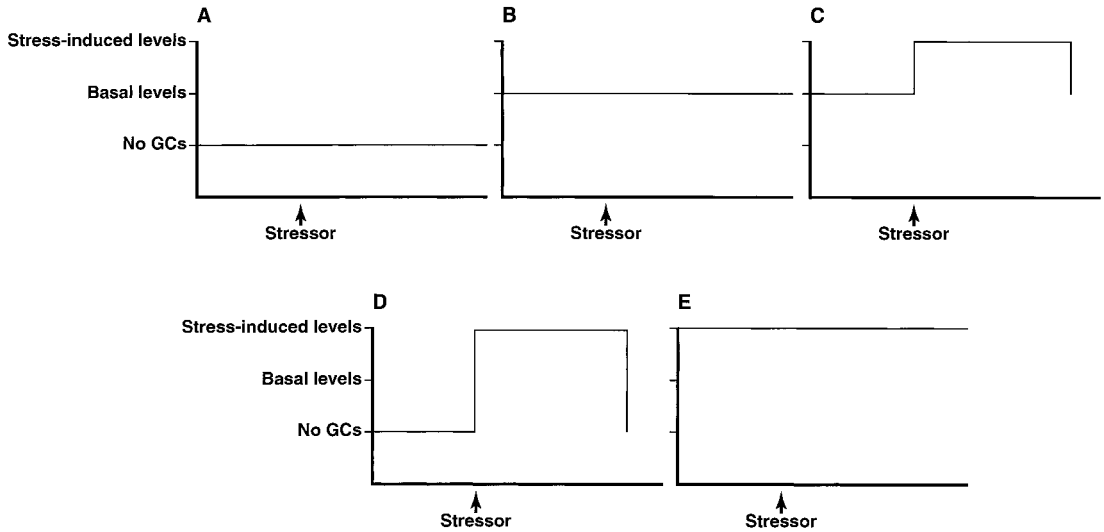


FIG. 3. Schematic diagrams indicating possible GC profiles in response to experimental manipulations. In panel A, there is a complete absence of GCs throughout. Panel B shows maintainence of basal levels throughout. Panel C shows the generation of a stress response such that a stressor causes a rise from basal levels to those typical of a stressor. Panel D shows a stress-induced rise this time from an initial absence of GCs. Panel E shows constant stress-typical levels of GCs.

been trained to lever press on a reinforcement schedule that delivers one food pellet per 100 bar presses. Typically, such rats will have moderately elevated ACTH and GC concentrations at the beginning of the session. Each rat bar presses 100 times, each exerting the same physical effort—*i.e.*, both rats are experiencing an environment that is homeostatically stressful to the same extent. At the end of the 100 lever presses, only the first rat receives a food pellet. The first rat promptly suppresses ACTH secretion, whereas the unfed second animal initiates a substantial stress response (in response to a psychological state referred to by many investigators as "frustration"). To emphasize again, the rats have expended identical amounts of energy in reaching that point; where they differ is that the latter rat must now expend more energy in the hope of being fed. As such, the GC secretion is not in response to the physical stressor already undergone, but reflects the frustration of not being fed plus the preparation for the impending stressor (313).

2. A second example emphasizes the same point in a manner far less technical: a human will initiate a substantial adrenocortical stress response before a parachute jump, or if approached by a menacing mob (discussed in Refs. 313–315)—even though in each case, no substantial physical energy demands have yet been placed.

These two cases, gleaned from a vast literature concerning conditioned and anticipatory stress responses, demonstrate that GC secretion can be in preparation for a stressor. Humans, with their cognitive sophistication, are abundantly capable of such conditioned secretion and can even have such secretion in anticipation of a homeostatic challenge that proves illusory (often termed anxiety). To a surprising extent, however, stressors are often predictable for nonhuman species.

Within social species, many stressors are predictable. In tournament species (in which male reproductive access to females is based on outcomes of male-male competition and aggression) in which there is seasonal mating, territories must be established and defended at predictable times of the year (316). In nonseasonal tournament species, formation of transient consortships with fertile females involves the predictable stressor of excluding other males from mating (requiring decreased feeding and resting, increased activity and vigilance, and overt fighting) (317). Furthermore, in social species, dominance-related aggression is often the predictable culmination of hours or days of escalating threats and displays.

There is also a high degree of predictability of physical stressors related to food acquisition in predatory species. Most carnivores feed on intermittent meals that are dense in nutrients. Thus, hunger is a fairly reliable signal that the physical stressor of hunting will soon ensue, and hunger-induced GC secretion can prepare for the hunt. Another reliable trigger of preparatory GC secretion should be a failed hunt (a common occurrence in most predator species). Among lions, hyenas, wild dogs, and cheetahs, a failed hunt typically results in another attempt within a few hours (308, 318–320). Thus, for the same physical effort in the chase, an unsuccessful hunt should stimulate GC secretion more robustly than a successful hunt. This is akin to the laboratory example just discussed in which the GC responses are opposite, depending on whether appetitive behaviors are rewarded with a consumatory event.

This idea regarding food acquisition as a predictable physical stressor is less applicable to herbivores, who eat almost constantly [wildebeest graze 15 h a day, while lions feed an average of once every 4 days (319)]. Thus, under stable conditions, herbivores are never particularly hungry (nor particularly sated). Moreover, per unit time, the act of food acquisition by a herbivore is less physically stressful than by a carnivore—one does not have to chase tubers.

Are there major physical stressors that are predictable for prey species? The most physically stressful of activities for them must include evading a predator. Predatory attacks are often unpredictable, particularly in forest-dwelling ecosystems. However, in more open terrains (aquatic environments, grasslands, desert, and tundra), there are a number of circumstances in which an individual is predictably at a greater risk of being subject to a predation attempt:

1. During parturition, when the female is conspicuous and immobile (320).

2. Individuals at the perimeter of a social group. This can occur in harem species (where a single resident male breeds with large numbers of females) in which the harem male spends much of his time patrolling the perimeter to exclude other males. Among such species (such as gazelles), these individuals are disproportionately subject to predation (321–323). Individuals also wind up on the perimeter in species that form protective clusters against predators (such as wildebeest, who form such clusters nightly). The strategizing of individuals to wind up safely in the center of such clusters is termed "the geometry of selfish herd" (324); individuals on the perimeter are most likely to be predated (321). At an extreme, solitary individuals are predictably at the highest risk (323).

3. Conspicuously sick or injured individuals. An injured joint is readily apparent. In addition, chronic infections usually induce conspicuous physical and behavioral changes in organisms, centering around the pyrogenic, somnogenic, and cachectic consequences of chronic immune activation (325). A hallmark of predatory strategy in open environments is to cue on sick or injured animals (cited in Ref. 308). Likewise, for social species, illness or physical injury signal competitive conspecifics that this is an auspicious time to challenge the impaired individual with an aggressive dominance interaction (cf. Ref. 317). Moreover, conspicuous illness or injury decreases the likelihood of being chosen as a mate (326).

Of note, these are circumstances in which animals are likely to increase GC secretion. Parturition, injury, and illness are all potent stimuli of secretion (and, as described, immune release of cytokines during illness is a proximal mechanism for stimulating the GC secretion). While GC concentrations in harem *vs.* nonharem males are not known, the role of a harem male is typically transient and unstable, subject to frequent harassment by other males (316), and an unstable position in a dominance system is a potent stimulus of GC secretion (327).

Finally, seasonal and climatic changes are a reliable cue of stressors to come in temperate-zone species, in which there are abundant signals of a coming winter, and among equa-

torial species, in which there are cues of an impending dry season. This is particularly true for species (*e.g.*, numerous birds) in which seasonal cues also signal impending and metabolically costly migration (328, 329). There are also considerable cues for an acute weather challenge (*i.e.*, a storm). (256). At present, however, it is not clear whether GCs increase in anticipation of these events.

Thus, there are cues in many species whose onset indicates an increased risk of an imminent major physical stressor. Moreover, some of these cues are associated with elevated GC secretion. We are particularly struck by the fact that, arguably, two of the strongest instances in which GCs help to suppress the stress response—inhibition of immunity and inflammation—also decrease the conspicuous signs that may target a sick or injured individual for predation or dominance challenge.

Therefore, there are more circumstances in which GC actions can be viewed as preparatory than often appreciated, reinforcing the importance of the "preparative" effects of elevated GC concentrations.

## V. Molecular Mechanisms Underlying Actions of GCs in Stress

As explained in the next section, coping with stress involves most known actions of GCs. Understanding how the molecular mechanisms underlying these actions are coordinated to produce integrated physiological responses will require much broader and deeper knowledge of those mechanisms than currently exists. Here we will selectively survey the copious but fragmentary information available and try to discern unifying threads common to permissive, suppressive, or preparative actions. Not surprisingly, the most thoroughly investigated areas of GC actions are those that are most closely tied to therapeutic applications, particularly to the suppressive antiinflammatory and immunosuppressive actions. Permissive actions, for which GCs are used clinically only in the relatively rare instances of adrenocortical insufficiency, have by comparison been neglected.

Like other hormones of the steroid-thyroid-retinoid family, GCs initiate primary molecular interactions in their target cells through binding to their nuclear receptors. The known GC receptors, GRs and MRs, function as ligand-activated transcription factors to regulate transcription of target genes. We will assume that all GC actions start in this way. This assumption may eventually have to be modified. For example, some genomic steroid hormone actions may be mediated by as yet physiologically uncharacterized nuclear receptors (330). Mounting evidence also indicates that steroid hormones can exert rapid nongenomic effects, possibly via membrane or other nonclassical receptors (331). For GCs the physiological significance of these effects remains uncertain and will not be considered here. We will also not consider ligand-independent activation of nuclear receptors through other signal transduction pathways. Such mechanisms have been found with most of the nuclear receptors, but so far not with GRs or MRs (332). Finally, we will not deal with the recently discovered $\beta$-isoform of the GR, which lacks hormone-binding capacity. It has been proposed to modulate activities of the GR, but its physiological significance remains controversial (333–336).

### *A. Permissive and suppressive actions: MRs or GRs?*

A division of labor between GRs and MRs as mediators, respectively, of suppressive and permissive GC actions, might be expected from the fact that suppressive actions are characteristically produced by high, stress-induced GC levels, sufficient to modulate binding to GRs over a wide range, whereas permissive actions are anticipatory, generally occurring while GCs are at basal levels that suffice to nearly saturate the high-affinity MRs while occupying only a small fraction of GRs. There is indeed such a trend, although no absolute separation. For example, massive evidence from dose-response relationships, agonist and antagonist studies, and other sources indicates that most immunosuppressive and antiinflammatory GC actions are mediated through GRs, but there is at least one report of potential immunosuppressive effects being mediated by MRs (337). Although, as just mentioned, GRs and MRs have not been found to be activated through other signal transduction paths in ligand-independent fashion, of considerable interest in connection with the roles of GRs and MRs in stress are some recent observations showing that other signals may be important in modulating the relative transcriptional activities of liganded GRs and MRs (338, 339).

One of the clearest examples of permissive actions [or "proactive" actions as they have also been called (340)] being mediated by MRs comes from the studies with rat hippocampal slices referred to previously (341), in which neuronal excitability is enhanced by treatment with aldosterone or corticosterone in the 1 nM concentration range. In this system suppressive (or "reactive") actions, which require higher GC concentrations, are through GRs. In fact, a fairly wide range of observations on GC actions in the hippocampus, both *in vitro* and *in vivo*, are consistent with MRs mediating permissive or maintenance roles and GRs mediating suppressive roles (340). Similarly, permissive actions of GCs on some T cell immune responses (137, 139) and on transcription of the CRH gene in stressed rats (341) appear to be mediated by MRs, and suppressive actions by GRs. There are also examples, however, of permissive actions being mediated by GRs. For example, induction of IL-6 receptors, which probably underlie some permissive effects on inflammatory and immune responses, appear from dose-response curves to be via GRs (342). Induction of $\alpha$1B-adrenergic receptors (41) and $\beta$2-adrenergic receptors (42, 343) in DDT1 MF-2 smooth muscle cells, and of angiotensin II type 1 receptors in vascular smooth muscle cells (344), which are related to the important permissive effects of GCs on the cardiovascular system, also seems to be via GRs. Comparable conclusions apply to the permissive sensitization of adipose cells to lipolysis by $\beta$-adrenergic agonists, as exemplified by GC induction of $\beta$-adrenergic receptors in 3T3-F442A adipose cells (345).

Many factors influence the sensitivity of cells and tissues to GCs (340, 346). How much hormonal activity is transmitted to a cell by GCs through GRs and MRs depends in the first instance on how many hormone-liganded receptors are

formed in the cell, which in turn is determined by how many receptors there are, and by the concentration of free intracellular GCs to which the receptors are exposed. Regarding how many receptors there are, far more is known about GRs than MRs. Almost all cells have GRs; their number is highly variable from cell to cell and is subject to down-regulation by GCs (347). Down-regulation of GR expression, and GR activity and half-life, are in turn modulated by GR phosphorylation (348), which is itself up-regulated by GCs (349). Along with other influences, such dynamic controls on sensitivity to GCs can modulate an organism's response to stress, particularly when stress is prolonged (340, 346, 350).

### *B. Role of 11β-HSDs*

Regarding the concentration of free intracellular GCs to which receptors are exposed, far more is known about MRs than GRs. The enzyme 11β-HSD has already been mentioned in this connection. It comes in two isoforms, type 1 (11β-HSD1) and type 2 (11β-HSD2), with distinct and important roles. 11β-HSD2 almost irreversibly inactivates cortisol and corticosterone, oxidizing their 11β-hydroxy group to 11-keto to form, respectively, cortisone and 11-dehydrocorticosterone, which bind only weakly to MRs and GRs. 11β-HSD1 catalyzes both the oxidizing (inactivating) and reducing (activating) reactions and so can activate the 11-keto steroids. MRs in mineralocorticoid target cells are "protected" from the natural GCs by 11β-HSD2, which is present in the target cells and inactivates cortisol and corticosterone. Cells with MRs that mediate GC actions, such as those in the hippocampus, have little if any 11β-HSD2, although they may have 11β-HSD1 (340). As discussed earlier, 11β-HSD2 also protects GRs: in Leydig cells GCs through GRs inhibit testosterone production (296, 297), an effect that contributes to the preparative antireproductive GC actions. Leydig cells also have 11β-HSD1, and during development net 11β-HSD activity switches from reductive to oxidative (297). Similarly, in the uterus both 11β-HSD activities are present. They vary during the menstrual cycle, with 11β-HSD2 apparently protecting from excessive inhibitory effects of GCs (351) that may also exert preparative antireproductive GC actions in stress.

A metabolic role of 11β-HSD1, which is primarily hepatic, has been demonstrated with 11β-HSD1 knockout mice (352). The homozygous 11β-HSD1$^{-/-}$ mice, despite compensatory adrenal hyperplasia and increased GC secretion, during starvation had diminished activation of the key hepatic gluconeogenic enzymes glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK) when compared with normal mice. They also showed diminished hyperglycemia in response to stress and obesity. These observations indicate that 11β-HSD1 in the liver is important locally in eliciting a significant metabolic response via GRs to stress-induced GCs and might similarly modulate other responses to stress-induced GCs such as those in the hippocampus (340).

Even a synthetic GC like dexamethasone, which is not metabolized by the 11β-HSDs, does not necessarily have free access everywhere to GRs and MRs. In the brain it is pumped out by the multidrug resistance 1a (mdr1a) P-glycoprotein, which is expressed in the apical membranes of endothelial cells at the blood-brain barrier. Compared with the natural GCs, which are not affected by mdr1a, dexamethasone thus has limited access to brain receptors (340).

### *C. General mechanisms of transcriptional activation and repression by GCs*

What happens after GCs bind to receptors in cells? The initial steps are well known, although not well understood. There is far more information on GRs than MRs, but because of their similarities, what is known about the general behavior of GRs will probably hold for MRs. In their specific behaviors, however, they may differ (353). Unliganded GRs, which are predominantly cytoplasmic but probably cycle between the cytoplasm and nucleus, form large heterocomplexes with heat shock protein 90 (hsp90) and other heat shock proteins (354). On hormone binding the hormone-receptor complex rapidly undergoes "activation" or "transformation" (355), in the course of which the heterocomplex dissociates to an activated hormone-receptor complex monomer (354) that becomes more highly phosphorylated and binds to structures in the nucleus (356).

The activated hormone-receptor complex then finds its way to a target gene. At one time it was thought that both transactivation and transrepression required binding of the activated receptor, as a homodimer, to short palindromic sequences of nucleotides in the target gene promoter region called GREs or glucocorticoid response elements (MRs, progesterone receptors, and androgen receptors also bind to GREs) (357). That view is still generally accepted for transactivation, as well as for repression mediated through binding to negative GREs (nGREs), where the receptor displaces or interferes with positively acting factors at adjacent sites (357, 358).

Several GC actions are known to be transmitted through nGREs. One relevant to stress is the negative feedback suppression by GCs of the pituitary POMC gene. GCs inhibit POMC gene expression through an nGRE in the promoter region. In contrast to other nGREs, to which GRs bind as dimers, in this nGRE three GRs bind cooperatively, two as dimers and one as a monomer (359). Whereas it is unclear how GR binding to the nGRE represses transcription of the POMC gene, GC suppression of PRL gene expression via an nGRE is due to GR interference with binding on adjacent sites of two transcription factors that activate the gene (360).

There are many cases where activated GRs do not need to bind to GREs or nGREs, or even to DNA, to control transcription. Here the basic mechanism is what is often referred to as transcriptional "cross-talk" via factor tethering (358, 361). GRs, probably as monomers (362), bind directly to a transcription factor that activates transcription through its DNA binding site. The GRs sometimes synergize but usually interfere with the factor. Among the best known of these factors are the activator protein-1 (AP-1) proteins, cJun and cFos. With cJun-cFos heterodimers occupying the AP-1 site, GRs repress, but with cJun-cJun homodimers GRs synergize (358, 361, 363). Other such factors, many of which have been shown in cell-free systems to bind directly to GRs via protein-protein interactions, are cAMP response element binding

protein (CREB), and nuclear factor-κB (NF-κB), and Oct-1. For example, GCs suppress GnRH (an action related to the preparative effects on reproductive physiology) via GRs tethered to Oct-1, which is directly bound to the GnRH gene (364). Functional interactions between GRs and these factors are often reciprocal, GRs repressing activity of the factor and the factor repressing activity of GRs ("cross-talk" is an expression that is also used to describe other interactions at the cellular and molecular level).

A point of interest in this context is that whereas ligand-activated GRs and MRs appear to exert similar transcriptional activities through simple GREs, at a so-called "composite GRE" containing both a simple GRE and an AP-1 site in the proliferin gene (plfG), they behave very differently: GRs repress AP-1-stimulated transcription but MRs are inactive. The difference has been traced to a segment of the N-terminal domain of the GR that is required for repression (353, 365). Another potential source of diversity in the physiological roles of GRs and MRs is that they can modify each other's actions by forming heterodimers on GREs (366).

In the course of controlling gene expression, activated GR complexes probably interact not only with DNA and/or with DNA-bound transcription factors such as NF-κB, but with general transcription factors (GTFs) that compose the RNA polymerase II (Pol II) transcription complex, and with the transcription intermediary factors (TIFs) or coactivators that link the basic transcriptional machinery to nuclear receptors or other DNA-binding proteins (361, 367–369). Targets of nuclear receptors among GTFs may include the TATA box binding protein (TBP) and TBP-associated factors ($TAF_{II}$s). Among coactivators are the CREB-binding protein (CBP), its homolog p300, the steroid receptor coactivators (SRCs), and the GR interacting protein (GRIP). Coactivators, via a short leucine-rich motif (370), associate among themselves (*e.g.*, SRCs with CBP/p300), with nuclear hormone receptors, and with other transcription factors, thereby integrating hormonal responses and cross-talk between signaling paths. Such associations can give rise to cross-talk by "squelching", *i.e.*, competition between nuclear receptors and other transcription factors for a common coactivator present in limiting amounts (361, 369).

Reversal of the role of GRs appears in the relation of GCs to signal transducer and activator of transcription 5 (Stat5), where the GR is the coactivator. Stat5 is a signal transducer and transcriptional activator that mediates induction by cytokines, hormones, and growth factors of the JAK/STAT pathway. GCs enhance Stat5-dependent transcription via GRs, which bind to Stat5 and act as transcriptional coactivators (371).

Remodeling the structure of chromatin with which a regulated gene is associated is probably an essential step in regulation of transcription by GRs and other nuclear receptors (368). The chromosomal environment in which a gene is located may even determine hormone specificity (372). Involved in remodeling are such factors as the SWI/SNF complex (368, 373) and histone acetylases and deacetylases. Histone acetylation, long thought to participate in this remodeling process, recently has attracted renewed attention with the discovery that many elements of the transcriptional machinery possess histone acetyltransferase (HAT) activity. HAT activity has been reported for CBP/p300 and SRC-1, among other factors. Histone deacetylase (HDAC) activity has also been reported (369, 374). Histone acetylation is thought to play a role in transcriptional activation by weakening the association of histones with DNA, making the gene more accessible. Deacetylation does the opposite. Studies on chromatin remodeling have been carried out with only a few GC-induced genes (368). One is the hepatic tyrosine aminotransferase (TAT) gene (375, 376), which is rapidly activated by GCs and glucagon. A GC-responsive enhancer lies 2.5 kb upstream of the transcription initiation site, where there are several GREs, two of which cooperate in enhancing GC stimulation of gene expression. In this region GCs induce DNase I hypersensitive sites (377), reflecting disruption of chromatin structure (375, 376). Induction of hypersensitivity begins 10 min after addition of GCs, accompanied by stimulation of transcription, and is rapidly reversed on washing out hormone or on addition of RU486.

Few among the multitude of GC actions recognized at the physiological level are even moderately well understood at the level of gene regulation just described; for most actions there is simply no information. Furthermore, many observed actions of GCs on transcription of a gene may not be primary responses (responses due to direct interaction of the hormone-receptor complex with that gene), but secondary responses initiated, for example, by a GC-induced transcription factor controlling other genes in the same cell. Secondary responses are relatively slow in onset and can be blocked by inhibitors of protein synthesis. Among numerous examples, a recent one is GC activation of transcription of the rat arginase gene, where the primary GC-induced product is a CCAAT/enhancer binding protein (C/EBPβ), which secondarily activates the arginase gene (378).

The molecular mechanisms we have outlined occur in nature in many variations and combinations, but rarely in the pure forms that have been defined mainly in highly simplified artificial systems such as transfected cells and cell-free systems. Difficulties with understanding GC actions in whole organisms at the molecular level are accentuated by the fact that even apparently simple physiological GC functions often require interactions among many GC-regulated target cells and genes, as well as interactions with other hormones and mediators. Some of these interactions may take place among the steroid hormones themselves. Thus, GRs and MRs not only can form heterodimers when bound to DNA, but can modify each other's actions when coexpressed in a neuroblastoma cell line, raising the possibility that they engage in cross-talk while associated with GREs (366). Similar evidence exists for cross-talk between GRs and androgen receptors (379, 380).

Consideration of possible relations between molecular and physiological actions of GCs in stress leads to an obvious hypothesis: namely, that permissive GC actions, typically associated with stimulatory effects such as increased levels of mediator receptors, are induced mainly by gene transactivation; whereas suppressive GC actions, typically associated with inhibitory effects such as those on cytokine expression, are induced mainly by gene transrepression. Although the evidence is still sparse, the hy-

pothesis may be testable with results of GR gene targeting, as we will discuss later. Pharmacological testing may also be feasible, using synthetic GCs such as recent ones that exert strong AP-1 transrepression but little or no transactivation (381).

We now review molecular mechanisms of GC actions on immune and inflammatory processes, and on metabolism. These are the areas that have been studied most extensively among those we have dealt with in relation to GCs and stress. Research in most other areas has reached the molecular level in only a few scattered instances.

### *D. GC actions on immunity and inflammation*

We will focus on two of the most general GC actions underlying suppressive and permissive actions on immunity and inflammation: inhibition of cytokine activity and induction of cytokine receptors (9, 102). Much more information is available on the first of these, the inhibitory actions. GCs inhibit IL-1 by suppressing IL-1 transcription, translation, and secretion, by destabilizing its mRNA (382–385) and by inducing a so-called decoy receptor that binds and sequesters IL-1 without transmitting activity (57, 386). They block transcription of IL-2 (387–389), IL-3 (390), and IL-8 (391) and destabilize the mRNAs of TNF-$\alpha$ (392) and GM-CSF (393–395). Destabilization of mRNA is mediated by AU sequences in the 3′-untranslated region. GC induction of the receptors or receptor subunits IL-2R$\alpha$, IL-4R$\alpha$, IL-6R$\alpha$, IFN-$\gamma$R, GM-CSFR$\alpha$, CSF-1R, and TNF-R, is known to be accompanied by increased levels of their mRNAs (102).

GC repression of cytokine gene transcription has been associated so far with two general molecular mechanisms: GR inhibition of AP-1 and GR inhibition of NF-$\kappa$B. Both these topics have been reviewed recently (140, 361, 396). The GR connection with AP-1 was the subject of several 1990 reports on GC inhibition of basal and phorbol ester-activated transcription of the gene for collagenase (397–399), a major GC-suppressed mediator of inflammation. Those studies showed that GC inhibition depends on mutual interference between GRs and AP-1 by protein-protein interactions, probably through binding of GRs with cJun in the transcription complex rather than squelching, and independent of binding of GRs to GREs. These and related observations gave rise to the model described earlier for GR repression by cross-talk via tethering to other transcription factors. This mechanism has since been found to apply to inhibition by GCs of other important immune and inflammatory responses. In particular, it appears to account for GC repression of the IL-2 gene, in which AP-1 synergizes with the nuclear factor of activated T cells (NFAT) and both factors cooperate to mediate GC inhibition of transcription (96, 389). Similarly, GC repression of the IFN-$\gamma$ gene involves interaction of GRs with AP-1·CREB-activating transcription factor (ATF) complexes (400).

NF-$\kappa$B is a transcriptional activator protein that mediates key immune and inflammatory reactions, responding to signals from cytokines such as TNF-$\alpha$, IL-1$\beta$, and IL-17, as well as from antigens. Among proteins regulated by NF-$\kappa$B are the cytokines TNF-$\alpha$, IL-1$\beta$, IL-2, IL-6, M-CSF (monocyte colony stimulating factor), GM-CSF, the chemokine IL-8, and other chemokines, nitric oxide (NO) synthase, COX-2, ICAM-1, the IL-2R$\alpha$ receptor subunit, the T cell receptor $\beta$ subunit, and the serum amyloid A protein (140, 396). NF-$\kappa$B is a cytoplasmic protein found in most cells. It is a member of the Rel/NF-$\kappa$B family that has several variants. In its inactive form it is bound to the inhibitor protein I$\kappa$B, which also has several variants. Activation of NF-$\kappa$B is initiated when I$\kappa$B is phosphorylated, released from NF-$\kappa$B, ubiquitinated and degraded. NF-$\kappa$B then enters the nucleus and binds to NF-$\kappa$B sites in target genes. The activated NF-$\kappa$B is a heterodimer composed of two proteins, p65 (also known as relA) and p50 (361, 396, 401).

Transcription of I$\kappa$B is stimulated by NF-$\kappa$B and by GCs. GC-mediated induction of I$\kappa$B may account for immunosuppression via inhibition of NF-$\kappa$B in monocytes and lymphocytes (402, 403). In other types of cells, GC inhibition appears to be through binding of the activated GR to the p65 subunit of NF-$\kappa$B, a cross-talk mechanism that depends on the presence of neither I$\kappa$B nor GREs (361, 396, 401, 404–408). Through one or another of these mechanisms GCs have so far been shown to reduce expression of genes for IL-8 (409), ICAM-1 (408, 410), COX-2 (408), and IL-6 (407). The protein-protein interaction between hormone-activated GRs and NF-$\kappa$B led to reciprocal transrepression between the factors. They involve the p65 subunit of NF-$\kappa$B and require all domains of the GR (401).

Another possible mechanism for antiinflammatory and immunosuppressive GC effects stems from observations that the mutual inhibition exerted by GRs and NF-$\kappa$B depends on CBP and SRC-1 and is relieved by overexpressing these factors. Cross-talk between GRs and the p65 component of NF-$\kappa$B is proposed to be due in part to squelching through nuclear competition between GRs and NF-$\kappa$B for limited amounts of CBP and SRC-1 (411).

The induction of apoptosis is a GC action on immune cells that is probably very important although its physiological significance remains obscure (412, 413). Apoptosis, which occurs in many cell types other than lymphocytes, has received enormous attention in recent years. Little is known about how GCs kill cells. It has been postulated that GCs induce "death genes," implying a transactivating function of GRs. However, mutant GRs incapable of transactivation can still mediate GC apoptosis of human leukemic cells, suggesting that GCs interfere with the expression of "survival genes" (414). Other results relevant to this matter are described below.

### *E. Metabolic GC actions*

As discussed earlier, a central action in the metabolic response to stress and hypoglycemia via increased blood glucose levels is GC stimulation of hepatic gluconeogenesis. This action has permissive and stress-associated components that synergize with, or counter, effects of other hormones such as glucagon, catecholamines, GH, and insulin. It is due both to a GC-induced increase in the capacity of the liver for gluconeogenesis, and to GC-stimulated provision of substrates from peripheral tissues (415). The increase in the capacity of the liver is mediated by increased activities of several enzymes, primarily the two rate-limiting enzymes:

PEPCK, which catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, and G-6-Pase, which converts glucose-6-phosphate to glucose (416). Of these, the molecular mechanisms of regulation of PEPCK gene expression have been studied most intensively.

PEPCK activity is controlled principally through synthesis of the enzyme, which GCs induce both by activating transcription and by stabilizing PEPCK mRNA (416). Reflecting the multiplicity of hormones that control gluconeogenesis, expression of the PEPCK gene is activated not only by GCs but by glucagon (via its intracellular second messenger cAMP) and is repressed in dominant fashion by insulin (417). The gene has a GC response unit (GRU) that spans 110 bp, with two GREs and two accessory factor-binding sites. GC regulation requires all these sites. The GRU includes insulin- and retinoid-responsive elements (416, 418). GC induction of PEPCK depends, in large part, on the presence of C/EBP (CAAT/enhancer-binding protein), as judged from experiments with C/EBPb$^{-/-}$ mice, and may involve interaction of GRs with C/EBP through binding to CBP (417). Permissive GC enhancement of hepatic gluconeogenesis by glucagon and catecholamines is thought to depend on increased responsiveness to cAMP (415), but the molecular mechanisms underlying this phenomenon are not understood. Increased substrates for gluconeogenesis are primarily amino acids released from muscle and other peripheral tissues, and glycerol released from adipose tissue sensitized permissively by GCs to lipolysis by GH and catecholamines (419, 420). Molecular mechanisms of these so-called catabolic effects have received little attention.

GCs also regulate blood glucose levels by decreasing glucose uptake and utilization in several peripheral tissues. Primary molecular mechanisms of this action are not known, but in adipose tissue and fibroblasts the immediate cause is translocation of glucose transporters from the plasma membrane to intracellular sites (218, 219). There is some evidence that GC-induced proteins mediate these actions (421–423). GCs decrease levels of IRS-1 (insulin receptor substrate-1) in adipocytes (424), which may account partly for the antiinsulin activity of GCs on glucose uptake, but does not explain the inhibition of glucose transport by GCs in the absence of insulin. GC inhibition of glucose uptake in muscle may be caused indirectly by plasma fatty acids released through lipolysis (210, 425). Here again, GCs decrease levels of IRS-1 (426).

Another facet of metabolic responses to stress initiated by GCs is stimulation of liver glycogen synthesis. This putative preparative GC action is due to new synthesis of hepatic glycogen synthase, to activation by dephosphorylation of the inactive form of glycogen synthase, and to inactivation by dephosphorylation of phosphorylase a (427–430). Some of these changes may be due secondarily to GC-induced proteins (430).

Permissive GC effects on energy mobilization in stress, in particular on the lipolysis by epinephrine that raises plasma FFA, have been suggested to be due partly to induction of peroxisome proliferator-activated receptor (PPAR). PPAR is involved in control of several metabolic pathways. GCs raise PPAR levels by activating transcription of the PPAR gene via GRs, a primary action that is not blocked by cycloheximide (431). Whether a comparable mechanism accounts for permissive GC actions on hepatic gluconeogenesis stimulated by glucagon and catecholamines is not known.

### *F. Studies with transgenic mice*

Gene targeting experiments aimed at disrupting or modifying molecular mechanisms of GC actions through GRs and MRs may ultimately prove the most illuminating for understanding the roles of GCs in stress. So far results have been reported with so-called GR gene "knockdown" mice bearing a transgene for GR antisense RNA (432–434), with gene "knockout" mice in which the GR gene has been disrupted (340, 370), and with gene "knockin" mice in which a gene for a mutated GR that does not dimerize has been inserted in place of the normal GR gene (435). Relevant studies have also been conducted with mice in which the CRH gene is disrupted (436). Recently reported observations with MR knockout mice (437) deal only with mineralocorticoid deficiencies, so they will not be discussed here.

We begin with the studies on CRH-deficient knockout mice (436). Heterozygous and homozygous offspring of heterozygous parents are viable and phenotypically normal. Offspring of homozygous parents, however, despite normal appearance at birth, all died within 12 h. They suffered from severe lung abnormalities, including low surfactant mRNA. If the homozygous mothers were given corticosterone from 12 days of gestation to 14 days after birth, the offspring were normal, revealing an essential GC requirement for normal perinatal lung development. Stress raised corticosterone levels significantly in the CRH-deficient mice, but levels in females were about one fourth those in normal mice. Those in males were much lower than in females, about as low as values in normal mice at the nadir of diurnal variation, raising the possibility that such low levels are sufficient to exert essential permissive functions, especially in mice that may have accommodated to low levels throughout development. The implication is that for survival, stress-induced GC levels are not necessary.

Mice bearing a GR antisense gene, whether heterozygous or homozygous, have as their most striking phenotypic characteristic a great increase in fat deposition and reach up to twice the weight of normal mice (432). They eat 15% less than normals, suggesting that defective GC function affects energy balance by increasing energy efficiency (434). Expression of GR mRNA is low. The mice show evidence of a disrupted HPA axis, with high ACTH and low corticosterone levels. No sexual dimorphism is observed in GR development, in contrast to normal mice. Insensitivity of the immune system to GCs was evidenced by the inability of the high corticosterone levels to reduce thymus weight and the failure of dexamethasone to influence *in vitro* thymocyte and splenocyte proliferation. There is a shift of T cells toward the CD4+ CD8$^{-}$ phenotype, coupled with hyperresponsiveness of T cells to concanavalin A stimulation. The findings point to a major role of the GR in control of immune responses (433).

Disruption of the GR gene is fatal for most homozygous (GR$^{-/-}$) mice, which die within a few hours of birth from respiratory failure (370). These studies, therefore, like those with CRH-deficient mice, reveal a requirement for GCs in normal perinatal lung development. GR$^{-/-}$ mice had en-

larged and disorganized adrenal cortices, impaired development of chromaffin cells, and absence of PNMT in the atrophied adrenal medulla. They also had defective HPA feedback (evidenced by high levels of corticosterone and ACTH in both $GR^{+/-}$ and $GR^{-/-}$ mice), and impaired activation of the genes for the hepatic gluconeogenic enzymes G-6-Pase and PEPCK, as well as for TAT and serine dehydrogenase (370). Some surviving $GR^{-/-}$ mice were tested for brain functions (reviewed in Ref. 340). Their electrophysiological responses to 5HT and the cholinergic analog carbachol were defective, like those of adrenalectomized mice, indicating that GRs are necessary for development of MR-induced suppression of neurotransmitter responses in hippocampal CA1 neurons. In behavioral studies the mice were impaired in processing spatial information, again suggesting dysfunction of MRs, and also were deficient in long-term memory of spatial information.

Highly suggestive results have been reported with transgenic mice in which the normal GR is replaced by a GR carrying a mutation in the DNA-binding domain that impairs dimerization and hence, it is believed, binding to GREs (435). That defect therefore prevents the mutant GR from mediating GC actions via GRE-dependent transactivation but leaves intact transrepression functions that can be mediated by GR monomers, such as cross-talk with AP-1 and NF-κB and (435). These mice are termed $GR^{dim}$. Despite absence of disruption of transactivating GR functions, homozygous ($GR^{dim/dim}$) mutant offspring are viable and show no lung abnormalities. As expected, GRs in immortalized embryonic fibroblasts from $GR^{dim/dim}$ mice activated only minimally an MMTV-CAT (mouse mammary tumor virus-chloramphenicol acetyltransferase) reporter gene in response to dexamethasone, a standard system for assaying transactivation by GRs via dimerization to GREs. In $GR^{dim/dim}$ mice treated with dexamethasone there was no induction of liver PEPCK, TAT, and serine dehydrogenase, confirming that those mice lack transcriptional control depending on GR binding to GREs. Repressive functions of the mutant GRs are preserved. AP-1-mediated GC repression of the phorbol ester-activated collagenase-3 gene in immortalized $GR^{dim/dim}$ fibroblasts was nearly as efficient as in $GR^{+/+}$ cells. Although in $GR^{dim/dim}$ mice CRH expression was normal, POMC mRNA in the anterior pituitary was strongly elevated, as was ACTH. This result is consistent with repression of the POMC gene via GR dimerization on nGREs, as discussed earlier. Similarly, in the neurointermediate lobe PRL mRNA expression, also regulated through nGREs, was elevated. Despite elevated ACTH in the anterior pituitary, serum ACTH levels were normal, suggesting that GCs regulate ACTH secretion by a mechanism independent of GRE binding. The adrenal medulla in $GR^{dim/dim}$ mice was normal, as was PNMT expression, contrasting with the $GR^{-/-}$ mice described above.

The mutant mice also provide strong evidence that GC-mediated apoptosis of thymocytes requires GR dimerization and binding to GREs. Flow cytometric analysis of $GR^{dim/dim}$ thymocytes after 24 h of treatment with dexamethasone showed no sign of death, whereas most $GR^{+/+}$ and $GR^{+/dim}$ cells died. The results are in striking contrast to those with human leukemic lymphocytes described earlier and support the possibility that GC-induced "death genes" mediate GC apoptosis of thymocytes and normal T cells. $GR^{dim/dim}$ mice had no abnormalities in CD4/CD8 thymocyte profiles. They were deficient in erythropoiesis, for which GCs are known to be important (438), suggesting that this is another function that requires GR dimerization, GRE binding, and transactivation.

In considering what all these experiments with transgenic mice tell us about the role of GCs in stress, a few points are worth keeping in mind. It has long been known that GCs are not essential for viability, growth, and reproduction of laboratory mice and rats. Adrenalectomized rats and mice do very well without any GCs at all, if their lack of aldosterone is compensated with extra salt. So it is not surprising that the transgenic mice can survive without GC functions once they get through the perinatal period during which, as demonstrated conclusively here, GCs are absolutely required for lung development.

As we have documented in earlier sections, animals with impaired GC functions do not tolerate stress as well as their normal counterparts. Mechanisms for surviving stress, however, are far more essential for animals in the wild than for mice living sheltered laboratory lives and inbred over many generations. A general question that arises is to what extent such laboratory animals still retain mechanisms, including GC functions, that have evolved in the wild for dealing with stresses of predator-prey relationships imposed by the need to forage or hunt for food, and to survive and multiply in often unforgiving environments. In some respects, laboratory mice may be better models than wild mice for modern humans, since most of us also live sheltered, sedentary lives.

It seems unlikely that a wild mouse, deprived of major GC functions and released back into the wild, would survive for long. How well the transgenic mice, especially the $GR^{dim/dim}$ mice, can survive stress is unclear. If our hypothesis that permissive effects of GCs are predominantly mediated by transactivation is correct, then these mice should be severely impaired in permissive functions of GCs and be particularly sensitive to forms of stress that call on such functions.

## VI. Conclusions

Emerging from this survey of GC actions in stress is a picture of extraordinary diversity, whether viewed in terms of the target cells, the metabolic pathways, or the physiological functions that GCs regulate. How those diverse actions are coordinated to protect the organism from specific challenges to homeostasis has been the theme of our analysis. Now we turn to some of the broader implications of our findings.

Although we have not tried to be comprehensive, within our limited goal of discussing GCs only in relation to stress, we have encountered most of the textbook GC actions. (Among the significant exceptions are GC functions in development and parturition, and in bone and ion metabolism). Included implicitly in our survey are even major clinical applications of GCs, since the suppressive actions underlie GC use in treatment of inflammatory and immune disorders, and the permissive actions probably underlie GC use in

treatment of adrenal insufficiency (1, 6). Thus, not only are GCs essential for surviving stress, but most GC actions appear to have a role in stress, whether or not they have alternative roles. For example, as discussed, GC effects on carbohydrate metabolism are important both in the prototypical stress of a chase, and in day-to-day regulation of food disposal and blood glucose levels.

This review reaffirms the importance of the permissive actions of GCs. The evolution of the role in stress of permissive actions, rooted in basal GC levels, may well have been separate from that of suppressive (and stimulatory) actions, which are consequences of stress-induced levels of GCs. Key to the suppressive actions must have been the linkage between stress and the ensuing surge in GCs, and key, in turn, to that linkage must have been central nervous system (or comparably central) control of GC secretion. Such control mechanisms appear in all vertebrates including fish, in which cortisol plays the two roles that in mammals are exercised independently by mineralocorticoids and GCs, and may also exist in much more primitive species (see Refs. 439–441). Out of earlier roles of GCs in regulating osmotic and ion balance via such organs as gills during transfer from salt to fresh water and back—which might be regarded as a stressor, and is accompanied in some species of fish by elevation of GC levels (439, 440)—other tissues and functions may have become attuned to periodic surges in GC levels. Eventually, after GCs were relieved of their osmoregulatory role by aldosterone [with the aid of 11$\beta$-HSD and the renal-based renin-angiotensin system, and of separate GRs and MRs (442)], GCs could be harnessed to protect against a wider range of stressors and aid in recovery from the various stress responses.

A second major emphasis of this review has been the potential importance of what we have termed preparative functions of GCs. This view has relied heavily upon an ethological perspective, on the assumption that an understanding of stressors and stress responses in natural settings provides an important complement to the traditional study of stress physiology in the laboratory (cf. Ref. 327). We suspect that an ethological perspective will be useful for appreciating the evolution and larger physiological context of other facets of endocrinology as well. As a caveat though, it is always critical to appreciate an ethological setting within the framework of an organism, rather than the perception of the human studying that organism. A circumstance that might, to a human observer, appear to represent a stressful challenge to homeostasis might merely represent a normative life history stage for an animal with adequate metabolic reserves. For example, king penguins which, as a normal part of nesting behavior during the peak of the Antarctic winter, fast for weeks on end without a rise in GC levels (443).

This review also emphasizes the differences between the physiological role of GCs in surviving natural stressors and the pathological effects of prolonged GC elevation. GC physiology should be thought of as the salutary responses (be they mediating or suppressive) to noxious stimuli, whereas GC pathology occurs when the natural recovery phase to a noxious stimulus is prevented from occurring.

Finally, both this and our earlier review (1) noted the tendency of GC endocrinologists in recent decades to view the multitude of GC actions as reflecting a patchwork quilt of often unconnected pharmacological actions. We hope that the present review will stimulate further research within a framework of GC actions constituting a coherent, albeit complex and heterogeneous, physiological whole.

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## International Consortium of Familial Pheochromocytoma

We are pleased to announce the creation of a new consortium to search for the susceptibility gene for familial pheochromocytoma.

It is our aim to accrue the highest possible number of kindreds affected by pheochromocytoma, with either intra or extra-adrenal tumor location. Diagnosis of pheochromocytoma in more than two individuals consanguineously related is required for accrual. Candidate families will be considered as those in which multiple endocrine neoplasia type 2A (MEN2A) and 2B (MEN2B) as well as von-Hippel Lindau syndrome have been ruled out at least on a clinical basis. If at all possible, exclusion of these syndromes on a molecular basis is ideal, either by excluding linkage or by negative mutation screening within the RET protooncogene, in the cases of MEN 2A and MEN 2B, and the VHL tumor suppressor gene, in the case of von-Hippel Lindau disease. A genome-wide scan approach will be undertaken to map the susceptibility gene. Genomic DNA obtained from peripheral blood from candidate patients and their affected and unaffected first-degree relatives (at a minimum, patients and both parents), alongside a copy of the family pedigree and a summarized description of the studied cases, including tests performed to exclude MEN2 and VHL diagnosis, are required.

This Consortium is a joint effort by the Dana-Farber Cancer Institute (Dept. of Adult Oncology/Cancer Biology) and the Children's Hospital (Depts. of Neurooncology and Dept. of Genetics), both institutions affiliated with Harvard Medical School.

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