INHIBITORS OF ADRENAL STEROID BIOSYNTHESIS[1]

T. EUGENE TEMPLE AND GRANT W. LIDDLE
Department of Medicine, Vanderbilt University, Nashville, Tennessee

The entire history of pharmacologic inhibition of adrenal steroid biosynthesis spans a period of only two decades. Every hormonal steroid is synthesized through a long series of enzymatically governed steps (1); and, theoretically, any biosynthetic step that is enzymatically controlled might be inhibited by an appropriate pharmacologic agent. Thus, the synthesis of a biologically active steroid is vulnerable to pharmacologic inhibition at many points. The present review will summarize what is known about the several agents that have thus far been shown to inhibit one or another step in steroid synthesis, thereby altering, qualitatively and quantitatively, the hormonal output of the adrenal cortex.

Because the adrenal cortex is homeostatically regulated (2, 3), the inhibition of steroid biosynthesis leads to a "compensatory" increase in adrenal stimulation by one or both of the "tropic" substances, adrenocorticotropic hormone (ACTH) and angiotensin. Therefore, the pharmacologic inhibition of a particular step in steroid biosynthesis results not only in a decrease in the formation of steroids that normally appear after the inhibited step but also in a secondary increase in the formation of steroids that occur prior to the inhibited step. The pattern of steroid production is thus distorted.

By virtue of their specificity as inhibitors of certain steps in steroid biosynthesis, some of the agents to be considered have proved to be uniquely valuable as heuristic, diagnostic, or therapeutic tools.

PATHWAYS OF STEROID BIOSYNTHESIS (1, 2)

The most important steroids produced by the human adrenal cortex are the glucocorticoid, cortisol, and the mineralocorticoid, aldosterone. Adrenal estrogens are synthesized in only minor quantities, and adrenal androgens, though formed in large quantities, are physiologically of low potency. The major steps in the biosynthesis of these hormones are represented in a simplified scheme in Figure 1. The synthesis of aldosterone occurs in the zona

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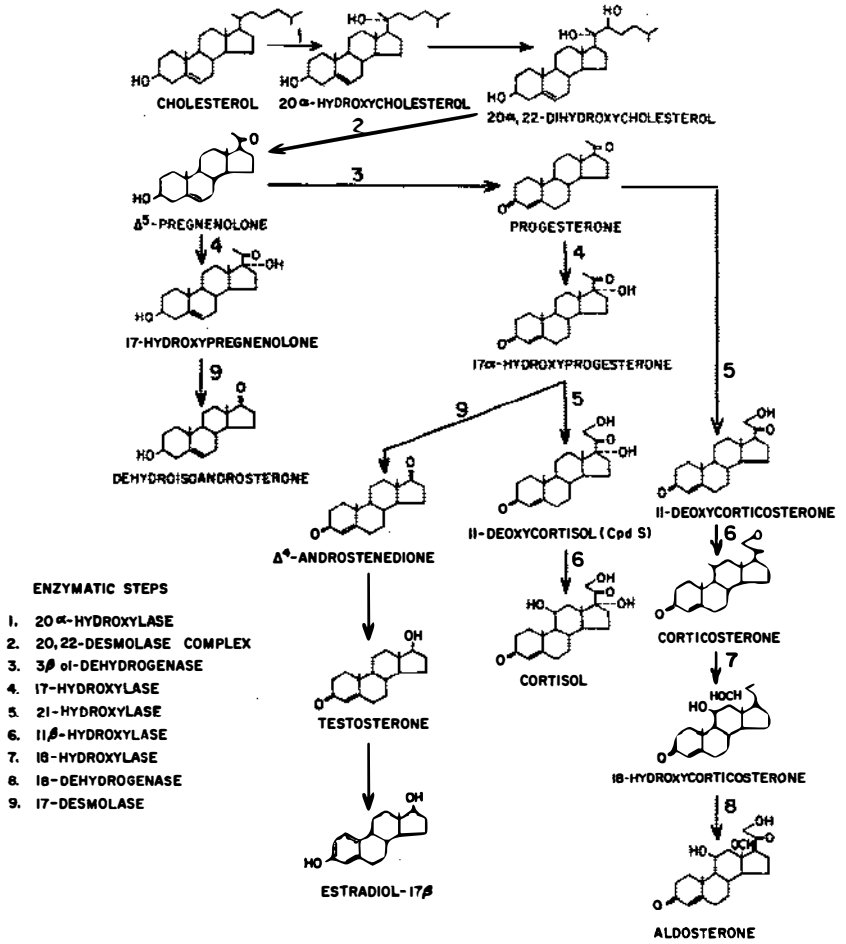

FIGURE 1. Biosynthesis of adrenal steroids.

glomerulosa. Cortisol, androgens, and estrogens are formed principally in the zona fasciculata.

Cholesterol, an intermediate in the biosynthesis of all hormonal steroids, is both synthesized by the adrenal and taken up from the circulation by the adrenal. Under normal circumstances the rate-limiting step in steroid biosynthesis is that at which cholesterol is converted to 20α-hydroxycholesterol. This is the step which is accelerated by ACTH and, presumably, is the step regulated by angiotensin as well. Once formed, 20α-hydroxycholesterol is thought to serve as a precursor for all hormonal steroids. It is first converted to 20α,22-dihydroxycholesterol. Then, by the action of a mitochon-

drial desmolase, the two adjacent hydroxylated carbon atoms, 20 and 22, undergo cleavage to form Δ^5-pregnene-3β-ol-20-one (pregnenolone) and isocaproic acid. By a series of enzymic reactions, pregnenolone is then converted to hormonal steroids. In this review these reactions will be considered only summarily.

The classical sequence of events in converting pregnenolone to cortisol is as follows: (*a*) Pregnenolone is converted to Δ^4-pregnen-3,20-dione (progesterone). Two closely related enzyme systems are necessary for this conversion. The first is 3β-hydroxysteroid dehydrogenase which oxidizes the 3β hydroxyl group to a 3-oxo group. The second is a Δ^5-3-oxosteroid isomerase which catalyzes the migration of the double bond from the 5–6 position to the 4–5 position. (*b*) Progesterone is converted to Δ^4-pregnen-17α-ol-3,20-dione (17α-hydroxyprogesterone) by a 17α-hydroxylase system. (*c*) Through the action of a 21-hydroxylase system 17α-hydroxyprogesterone is converted to Δ^4-pregnen-17α,21-diol-3,20 dione (17α,21-dihydroxyprogesterone or 11-deoxycortisol or "substance S" of Reichstein). (*d*) Substance S is converted to Δ^4-pregnen-11β,17α,21-triol,3,20-dione (cortisol) by an 11β hydroxylase system.

The initial enzymic reactions for converting pregnenolone to aldosterone are the same as for cortisol except that 17α-hydroxylation is bypassed; so that the 17-deoxy compound, corticosterone, is formed rather than cortisol. Corticosterone undergoes 18-oxidation in two stages. First, corticosterone is converted to 18-hydroxycorticosterone; then, this intermediate undergoes 18-hydroxy dehydrogenation to form 18-aldocorticosterone (aldosterone). The fact that cortisol and aldosterone are produced by different zones of the adrenal cortex is accounted for by the fact that the cortisol-secreting zona fasciculata contains 17α-hydroxylase but not 18-hydroxysteroid dehydrogenase, while the aldosterone-secreting zona glomerulosa contains 18-hydroxysteroid dehydrogenase but not 17α-hydroxylase. These two zones appear to have differential sensitivity to adrenal stimulators, the cortisol-secreting zone being more sensitive to ACTH while the aldosterone-secreting zone is more sensitive to angiotensin. [Rat adrenals lack 17α-hydroxylase; therefore, in this species the major product of the zona fasciculata is corticosterone (17-deoxycortisol)].

In the enzymic conversion of pregnenolone to androgens and estrogens, the general reactions are the same as in the formation of cortisol up through the process of 17α-hydroxylation. Minor quantities of 17-α-hydroxyprogesterone are cleaved at the 17–20 carbon linkage, forming the androgen, Δ^4-androstene-3,17-dione, which can undergo reduction at the 17α position to form Δ^4-androstene-17β-ol-3-one (testosterone). The formation of estrogen can occur as a result of 19-oxidation, followed by 19-demethylation and consequent aromatization of ring A of the steroid nucleus to give estradiol.

Dehydroisoandrosterone (Δ^5-androsten-3β-ol-17-one) is a derivative of 17α-hydroxypregnenolone and thus shares only the early portion of the bio-

synthetic pathway for cortisol. It is a major precursor of urinary 17-ketosteroids.

It is recognized that the "classical" biosynthetic sequences, as outlined here, are not the only sequences that can occur within the adrenal cortex. However, the major points to be brought out in this review can be adequately explained without resorting to a more detailed exposition of normal physiology.

It is important to note that the chemical reaction through which a particular modification is brought about in the biosynthesis of one steroid is similar to (and perhaps identical to) the chemical reaction through which the analogous modification is brought about in the biosynthesis of another steroid. Therefore, a pharmacologic inhibitor of a given step in steroid biosynthesis often affects many steroids and not just one. For example, an inhibitor of 11β-hydroxylase (4, 5) blocks the formation of not just one but all 11β-hydroxysteroids including cortisol, corticosterone, and aldosterone.

General Physiologic Sequelae of Adrenal Inhibition

In biochemistry it is well known that a series of biosynthetic intermediates in the synthesis of a "product" might be unrecognized until some metabolic error impairs the enzymatic conversion of a specific precursor to a specific derivative (3, 4). Then one observes the accumulation of the precursor that serves as the substrate for the impaired reaction and diminished formation of the derivative. When adrenal inhibitors are studied *in vitro* the increase in the amount of precursor that is recovered might be equivalent to the decrease in the amount of derivative that is obtained. However, when inhibitors are studied *in vivo* one often observes an increase in the secretion of precursor which actually exceeds the observed decrease in secretion of derivative (4, 5). This stems from the fact that, in the normal animal, adrenal steroid biosynthesis is regulated by extra-adrenal mechanisms which are suppressed directly or indirectly by the adrenal biosynthetic end-products but not by their precursors. For example, in man the adrenal biosynthetic processes which culminate in cortisol secretion are driven by ACTH, and the secretion of ACTH is restrained by cortisol but not by cortisol precursors. Therefore, impairment of any step in the biosynthesis of cortisol results in an increase in ACTH secretion, and ACTH in turn leads to accelerated production of precursors appearing in the biosynthetic pathway prior to the inhibited step. Depending upon the degree of inhibition and the adequacy of the compensatory increase in ACTH secretion, cortisol secretion might rise toward, but should not exceed, normal values. Given sufficient time, ACTH also induces hyperplasia of the adrenal cortex (6, 8).

In the rat, corticosterone is the principal end-product of adrenal steroid biosynthesis, and in this species the secretion of ACTH is restrained by corticosterone. Consequently, inhibition of any step in the corticosterone biosynthetic pathway leads to increased secretion of ACTH (7), adrenal hy-

perplasia (7, 9), and increased synthesis of the precursors of corticosterone which occur prior to a particular inhibited step.

Inhibition of aldosterone biosynthesis (5, 10–17) is likely to result in increased sodium excretion, hypovolemia, increased production of renin and angiotensin, and increased biosynthesis of the precursors of aldosterone which occur prior to the inhibited step.

Adrenal Inhibitors and Their Sites of Action (Figure 2)

(*a*) Amino-glutethimide [Elipten (R); α-ethyl-p-aminophenyl-glutarimide] has been shown to inhibit the conversion of cholesterol to 20α-hydroxy-cholesterol (18). As a consequence of this action, cholesterol accumulates in the adrenal cortex, and there is diminished production of all hormonal steroids including cortisol and aldosterone. Cash et al. (19) have examined this inhibitory process utilizing a method (20) for measuring the "desmolase activity" of adrenal mitochondria, in which cholesterol with a labeled side chain is employed as substrate. The rate of generation of la-

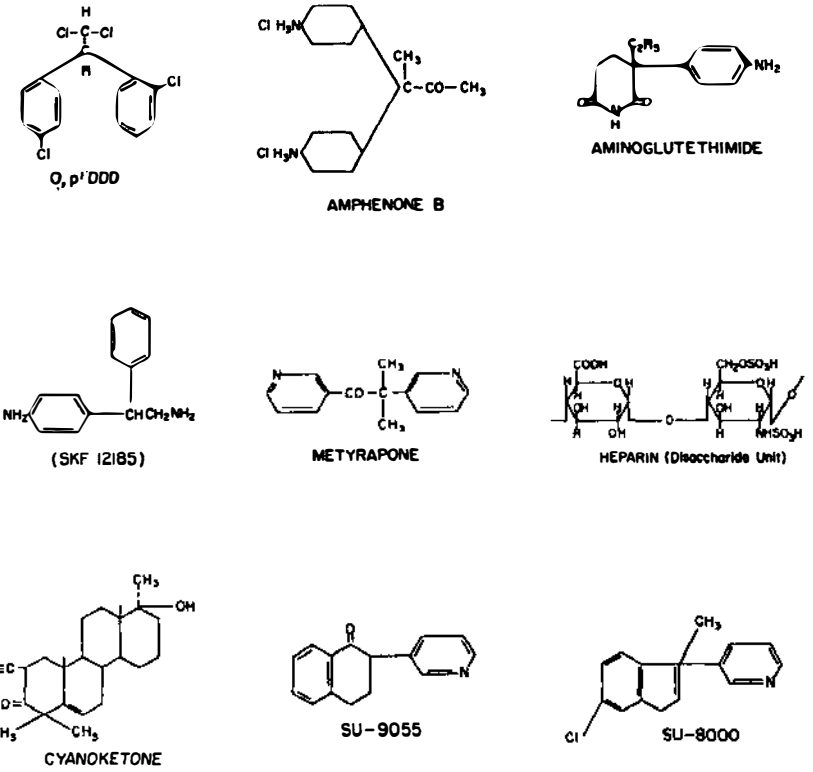

Figure 2. Structures of inhibitors of adrenal steroid biosynthesis.

beled isocaproic acid is used as an index of desmolase activity. Using this method, these investigators found that desmolase activity was greatly diminished in the presence of amino-glutethimide.

(*b*) SU-9055 [3- (1,2,3,4-tetrahydro-1-oxo-2-naphthyl)-pyridine] and SU-8000 [3- (6-chloro-3-methyl, 2-indenyl) -pyridine] have been shown to inhibit 17α-hydroxylation, thus blocking the synthesis of cortisol by the adrenal cortex (21) and androgens by the testis (22, 23). While diminishing the secretion of 17-oxygenated steroids, these agents lead to increased secretion of the 17-deoxysteroid, corticosterone.

These two pharmacologic agents also inhibit aldosterone biosynthesis. According to Raman et al. (24) SU-9055 inhibits the conversion of corticosterone to the aldosterone precursor, 18-hydroxycorticosterone, while SU-8000 inhibits the dehydrogenation process through which 18-hydroxycorticosterone is finally converted to aldosterone.

Bledsoe et al. (25) have shown that the administration of SU-9055 to human subjects results in a decrease in aldosterone secretion accompanied by an increase in corticosterone secretion. SU-8000 has not yet been used in man.

(*c*) Cyanoketone (2α-cyano-4,4,17α-trimethyl-androst-5-en-17β-ol-3-one) has been shown to inhibit 3β-hydroxysteroid dehydrogenase activity both in bacteria (Pseudomonas testosteroni) (26–29) and mammals (rat and bovine adrenals) (29–31). Acting possibly as a "stoichiometric inhibitor," cyanoketone blocks the conversion of pregnenolone to progesterone (32). It thus impairs the synthesis of the major corticosteroids, all of which have a Δ^4-3-ketone configuration, while permitting the secretion of Δ^5-3β-hydroxysteroids.

(*d*) Metyrapone [Metopirone (R); 2-methyl-1,2,-bis-(3-pyridyl)-1-propanone; SU-4885] and SKF 12185 [2-(p-aminophenyl)-2-phenylethylamine] have been shown to be relatively selective inhibitors of 11β-hydroxylase (4, 33–35). Although SKF 12185 is the more potent agent, it has been less thoroughly investigated because metyrapone had become generally accepted as an heuristic, diagnostic, and therapeutic tool before the SKF agent became known.

In vitro studies (4, 36–38) have demonstrated metyrapone to be a competitive inhibitor of the 11β-hydroxylase system that is responsible for converting 11-deoxycorticosterone to corticosterone and 11-deoxycortisol to cortisol. *In vivo* studies (4, 5, 11, 33–35, 39, 40) have shown metyrapone and SKF 12185 to diminish the secretion of the 11β-hydroxysteroids, aldosterone, cortisol, and corticosterone, while secretion of 11-deoxycorticosteroids is relatively unimpaired.

(*e*) Heparin and a heparinoid, ROI-8307, (N-formyl-chitosan polysulfuric acid) have been shown to inhibit the processes of 18-oxidation through which corticosterone is converted to aldosterone (12, 14). The action is relatively slow in onset and offset, requiring days to become fully apparent and weeks for complete reversal. The functional selectivity of heparin, affecting

as it does only the aldosterone biosynthetic pathway, has its anatomical counterpart in selective cytochemical changes that are apparent only in the zona glomerulosa (12, 17, 41, 42).

(*f*) O,p'DDD [2,2-bis(2-chlorophenyl-4-chlorophenyl)-1,1-dichloroethane] is a highly selective cytotoxic agent which induces degenerative changes in adrenal mitochondria culminating in gradual dissolution of the cells of the zona fasciculata and zona reticularis (43). The zona glomerulosa is largely spared (43–45). Degeneration of the inner zones of the adrenal cortex is accompanied by loss of the normal response of the adrenal to ACTH whether this be measured in terms of cortisol secretion (45–47) or tissue concentrations of glucose-6-phosphate dehydrogenase (48).

There are marked species differences in susceptibility to the adrenocorticolytic action of o,p'DDD. The dog is extremely sensitive, showing distinct morphologic (43–47, 49, 50) and functional changes (45–47, 50) within a few days. The rat (49, 51, 52) and guinea pig (48) are extremely resistive to the action of o,p'DDD, showing minor effects only after prolonged treatment with high doses. The nontumorous human adrenal shows intermediate susceptiblity to o,p'DDD (53–59). Several months of treatment in patients with adrenal hyperplasia results in gross impairment of cortisol secretory capacity while aldosterone secretory capacity remains intact (59).

(*g*) Amphenone B [3,3-bis(p-aminophenyl) 2-butanone dihydrochloride] (6, 60) was one of the earliest adrenal inhibitors to be studied, but its specific mode of action remains poorly understood. In several species it has been found to diminish the secretion of cortisol and aldosterone (61–66) and to result in the accumulation of cholesterol in the adrenal cortex (6, 61). The latter observation suggests that the drug might, like amino-glutethimide, inhibit 20α-hydroxylation. Other studies have suggested that amphenone might also inhibit 11β, 17α, and 21-hydroxylation (67). In recent years interest in this historic compound has waned because of the relative frequency with which its early clinical use was accompanied by untoward reactions (61, 62).

Heuristic Use of Adrenal Inhibitors

Four examples will illustrate the use of adrenal inhibitors as heuristic tools to aid in the elucidation of physiological problems.

The use of amino-glutethimide to determine whether ACTH affects the early part of the steroidogenic pathway.—It has been considered for some time that the principal action of ACTH is to stimulate the conversion of cholesterol to pregnenolone (2). The latter compound is converted by adrenal enzymes to hormonal steroids almost equally well in the presence or absence of ACTH (68). In order for ACTH to be fully effective in stimulating steroidogenesis over prolonged periods of time, it is essential that there be some mechanism for replenishing adrenal cholesterol. Two mechanisms could be postulated. Either ACTH could have a direct stimulatory effect on the accumulation of cholesterol by the adrenal, or the adrenal could have an

intrinsic homeostatic mechanism which would permit self-correction of any losses of cholesterol from the gland. Simple measurements of adrenal cholesterol in the absence and again in the presence of ACTH have failed to provide definitive information concerning this question. The fact that the concentration of cholesterol in the adrenal does not change significantly during treatment with ACTH is consistent with either of the mechanisms postulated above.

Dexter et al. (9) solved this problem with the help of aminoglutethimide. This drug was used to inhibit the conversion of cholesterol to steroids during treatment with ACTH. It was reasoned that, in the amino-glutethimide-blocked gland, ACTH should have no effect on adrenal cholesterol concentration if the mechanism for regulating adrenal accumulation of cholesterol were an intrinsic homeostatic function of the adrenal gland itself. On the other hand, if ACTH had a direct stimulatory effect on cholesterol accumulation, it should (in the amino-glutethimide-blocked gland) lead to a supernormal concentration of cholesterol.

The crucial experiments were performed in hypophysectomized rats maintained on amino-glutethimide in doses sufficient to minimize the conversion of cholesterol to steroids. The adrenals of rats that did not receive ACTH had adrenal cholesterol concentrations within the normal range, but the adrenals of rats that did receive ACTH had striking (100 per cent) increases in adrenal cholesterol concentrations.

It was inferred that ACTH has a direct stimulatory effect on adrenal cholesterol accumulation and that this action of ACTH enables the adrenal gland to maintain adequate stores of cholesterol to keep pace with the accelerated utilization of cholesterol which results from the action of ACTH in stimulating the rapid biosynthesis of steroids.

The use of amino-glutethimide to determine whether the adrenal cortex might play a role in the pathogenesis of essential hypertension.—Although "steroid hypertension" occurs frequently in patients with hypersecretion of cortisol, 11-deoxycorticosterone, or aldosterone, it has not been definitely established whether the adrenal cortex plays an important role in the pathogenesis of "essential hypertension."

One clue suggesting that the autonomous secretion of some mineralocorticoid might be a factor in some cases of essential hypertension stems from the observation that plasma renin activity is profoundly suppressed in about 20 to 30 per cent of patients with this disorder (69–75). The combination of hypertension and suppressed plasma renin activity is characteristic of primary aldosteronism, a disorder in which the autonomous secretion of aldosterone by an adrenal adenoma leads to sodium retention, elevation of the blood pressure, suppression of renin production, and diminished secretion of aldosterone by nontumorous portions of the adrenal (76). However, the suggestion that patients with essential hypertension and suppressed plasma renin activity might actually have primary aldosteronism has been rejected by most clinical investigators because the aldosterone secretion rates of

these patients are within normal limits (70, 72–75). This has not eliminated the more general possibility that hypersecretion of some (perhaps unidentified) mineralocorticoid might contribute to the hypertension and suppression of plasma renin activity in this group of patients.

The question has been explored by Woods et al. (77) using amino-glutethimide as an experimental tool. Advantage was taken of the knowledge that 20α-hydroxylation of cholesterol is a necessary step in the biosynthesis of all steroidal hormones and that this step is inhibited by amino-glutethimide (18). Correction of hypertension by amino-glutethimide could, then, serve as suggestive evidence that one or more steroidal hormones were important factors leading to hypertension. A double blind method was employed in order to eliminate subjective factors in the evaluation of drug effects. Control groups included normotensive subjects as well as patients with essential hypertension and normal plasma renin activity. Only the group with essential hypertension and suppressed plasma renin activity was found to respond to amino-glutethimide with a significant decrease in blood pressure. It was concluded that many patients with so-called essential hypertension and suppressed plasma renin activity might be suffering from an excess of one or more hormonal steroids. What steroids might be involved remains to be determined.

The use of metyrapone in determining whether cortisol can inhibit the pituitary-adrenal response to severe stress.—Prior to the time when ACTH assays could be performed satisfactorily on the blood of normal subjects, the question arose as to whether or not the pituitary-adrenal response to severe stress was regulated by the circulating level of corticosteroids. It was suggested that the secretion of ACTH by the pituitary was always subject to "negative feedback" control and that during severe stress the negative feedback regulator was merely set at a higher level than under nonstressful conditions (78). According to this theory, only enough ACTH would be secreted to raise corticosteroids to a level appropriate for that degree of stress. If that amount of corticosteroid were injected prior to stress, ACTH secretion would be correspondingly diminished.

This hypothesis was challenged by Liddle et al. (79) who found that large doses of the synthetic cortisol analogue, dexamethasone, did not inhibit the cortisol secretory response to the stress of laparotomy. However, cortisol rather than dexamethasone is the natural negative feedback regulator of ACTH secretion in man. Therefore, it was considered pertinent to extend the study to determine whether or not large infusions of cortisol would suppress the pituitary-adrenal response to laparotomy. The problem then became one of attempting to discern whether or not endogenous corticosteroids were being released in the presence of a large excess of exogenous cortisol.

The question was resolved (80) by the use of metyrapone to inhibit adrenal 11β-hydroxylase, thus forcing the adrenal (if it were to respond to stress) to secrete 11-deoxycortisol rather than cortisol. By solvent partition

and chromatography these two steroids could be separated from each other. Thus plasma 11-deoxycortisol could be measured as an index of endogenous adrenal activity while plasma cortisol could be monitored as an index of the amount of exogenous corticosteroid available in the circulation to serve as a suppressor of ACTH secretion. Cortisol and metyrapone were administered by continuous intravenous infusion to patients undergoing laparotomy. Prior to laparotomy only cortisol could be found in the plasma, but during laparotomy there was a sharp increase in 11-deoxycortisol, indicating that the pituitary-adrenal system had responded to stress despite the fact that plasma cortisol concentrations were four to ten times as high as those usually observed during laparotomy. It was, therefore, concluded that the secretion of ACTH by the pituitary during a major stress is not precisely regulated by the circulating level of corticosteroids. This conclusion was subsequently confirmed by direct measurements of ACTH in the plasma of patients who received cortisol infusions during laparotomy (80, 81).

The use of metyrapone to determine whether or not sodium depletion stimulates the early portion of the aldosterone biosynthetic pathway.—As mentioned earlier, aldosterone is synthesized by the zona glomerulosa through a series of steps that include cholesterol, pregnenolone, progesterone, 11-deoxycorticosterone, corticosterone, and 18-hydroxycorticosterone (1). Although sodium depletion has long been known to be a selective stimulator of aldosterone secretion (having no significant effect on cortisol secretion), it remained for Bledsoe et al. (82) to determine whether sodium depletion acted early in the biosynthetic pathway to stimulate the formation of aldosterone precursors or merely late in the pathway to facilitate the conversion of corticosterone to aldosterone. (The two possibilities are not mutually exclusive.) These studies were performed in human subjects undergoing multiple simultaneous corticosteroid secretion rate determinations utilizing isotope dilution techniques. In one key experiment the early part of the aldosterone biosynthetic pathway was "isolated" physiologically in the following manner. The subjects were maintained on constant treatment with dexamethasone to abolish ACTH-induced steroidogenic activity. Initially a high sodium diet was used, so that the aldosterone biosynthetic system was only basally active. Metyrapone was given continuously so that if the early part of the aldosterone biosynthetic pathway should become active this would be evident through the secretion of 11-deoxycorticosterone. While all other factors remained constant, a low sodium diet was then substituted for the high sodium diet. In all studies the low sodium diet stimulated an increase in 11-deoxycorticosterone secretion. There was no concomitant increase in 11-deoxycortisol secretion; therefore, it could be assumed that sodium depletion had selectively stimulated the aldosterone biosynthetic pathway and had not activated other adrenal biosynthetic pathways. It was inferred that one way in which sodium depletion stimulates aldosterone secretion is by stimulating the aldosterone biosynthetic mechanism prior to the formation of 11-deoxycorticosterone.

Diagnostic Use of Adrenal Inhibitors

(*a*) Amino-glutethimide. In describing the site of action of amino-glutethimide on adrenal steroid synthesis, we indicated that this agent inhibits the secretion of all known corticosteroids, including aldosterone (10). In studies of patients with primary aldosteronism it has been found that amino-glutethimide is efficacious in reducing the aldosterone secretion rate and in correcting the classical consequences of aldosterone hypersecretion, hypokalemia, and hypertension. Theoretically, a therapeutic trial of amino-glutethimide might provide useful information as to whether or not a patient is suffering from an excess of aldosterone-like steroids (77) and whether or not such a patient should be expected to respond favorably to the surgical removal of adrenal tissue.

(*b*) Metyrapone. Since 1958 the most widely used adrenal inhibitor has been metyrapone, and its most common use has been in the diagnostic evaluation of "pituitary-adrenal reserve" (4, 83–88).

Metyrapone inhibits the final step in cortisol biosynthesis, that of 11β-hydroxylation (4, 33, 36). Therefore, the administration of metyrapone results in a decrease in cortisol secretion, a "compensatory" increase in ACTH secretion, and a resultant increase in the secretion of 11-deoxycortisol. This relatively inert steroid and its major excretory product, tetrahydro-11-deoxycortisol, are easily measurable by such routine methods as the Porter-Silber (89) reaction for quantifying 17, 21-dihydroxy-20-ketosteroids (17-OHCS) and the Norymberski (90) reaction for quantifying 17-ketogenic steroids (17-KGS). Normal individuals respond to a standard test dose of metyrapone (10 mg/Kg body weight every 4 hours) with a two- to four-fold increase in urinary 17-OHCS or 17-KGS. Failure to respond in such a fashion can occur if exogenous cortisol-like steroids are administered so that there is no cortisol deficiency to stimulate a "compensatory" increase in ACTH secretion. More importantly, however, the pituitary gland that has lost its normal capacity to respond to cortisol deficiency may fail to respond to a metyrapone-induced decrease in cortisol secretion with a sufficient compensatory increase in ACTH secretion to cause a normal rise in 17-OHCS or 17-KGS. Patients with clinically obvious ACTH deficiencies invariably fail to respond normally to metyrapone. In addition, however, many patients with subtle disorders of the hypothalamus or pituitary fail to show normal responses to metyrapone even though they do secrete enough ACTH to avoid clinical adrenal insufficiency under nonstressful circumstances and even though their adrenal glands secrete near-normal quantities of cortisol when stimulated by exogenous ACTH. Such patients are said to have "limited pituitary-adrenal reserve." Among the clinical disorders that have been found to be associated with "limited pituitary reserve" are pituitary dwarfism, chromophobe adenomas of the pituitary, eosinophilic adenomas of the pituitary, craniopharyngiomas, metastatic carcinoma secondarily involving the pituitary, hypothalamic-pituitary

sarcoidosis, cachexia, post-partum pituitary infarction, and post-traumatic diabetes insipidus. Irradiation of the pituitary can lead to "limited pituitary reserve" without frank hypopituitarism, as can prolonged treatment with cortisol-like steroids (83–85).

An interesting example of drug interaction which has a bearing on the metyrapone test has recently been described by Meikle et al. (91). These investigators found that prior treatment with diphenylhydantoin induced hepatic enzymes that convert metyrapone into inactive metabolites. Thus, in a patient receiving diphenylhydantoin, a standard dose of metyrapone would not result in the usual concentration of the test agent in the circulation; and the pituitary-adrenal response would consequently be subnormal. By giving a larger dose of metyrapone, however, it was possible to raise blood concentrations of metyrapone to effective levels and thus induce normal pituitary-adrenal responses even in patients receiving diphenylhydantoin.

Of considerable practical value is the use of the metyrapone test in distinguishing between patients with hypercortisolism (Cushing's syndrome) due to a primary excess of ACTH secreted by the pituitary and that due to a primary excess of cortisol secreted by an adrenocortical tumor (83–85). In the former condition the response to metyrapone is qualitatively normal, and there is a large increase in 17-OHCS or 17-KGS; in the latter condition there is no rise in 17-OHCS or 17-KGS in response to metyrapone.

Therapeutic Use of Adrenal Inhibitors

(*a*) Amino-glutethimide. Because it interrupts corticosteroid formation at an undifferentiated step in the biosynthetic pathway (7, 8), amino-glutethimide can be used in the treatment of either hypercortisolism or hyperaldosteronism (10, 19).

Dramatic correction of cortisol hypersecretion has been observed in patients with Cushing's syndrome caused by autonomous adrenal neoplasms (10, 92, 93). In patients with Cushing's syndrome caused by an excess of pituitary ACTH, however, amino-glutethimide has been of only modest therapeutic value (10, 19, 94). The basic difficulty is that such patients respond to adrenal inhibition with further increases in ACTH, which in turn raise cortisol levels toward the pretreatment range. On the other hand, some patients who had previously undergone therapeutic irradiation of their pituitaries, with resultant curtailment of ACTH reserve, have shown satisfactory therapeutic responses to amino-glutethimide. In patients with autonomous production of ACTH by nonpituitary tumors, amino-glutethimide can be used to diminish the adrenal secretory response to this "ectopic" ACTH (95).

Several patients with aldosterone-secreting tumors have responded to amino-glutethimide with immediate normalization of their aldosterone secretion rates and gradual correction of their potassium depletion and hypertension (10).

(*b*) Metyrapone, by inhibiting 11β-hydroxylation, interferes with the se-

cretion of both cortisol and aldosterone (4, 5). Like amino-glutethimide, it is most dramatically effective when used in the treatment of patients with autonomously functioning adrenal neoplasms. Cushing's syndrome caused by adrenal tumor responds quickly to metyrapone (83, 96). Successful management of patients with the ectopic ACTH syndrome has also been reported (97, 98). In the latter condition, hypercortisolism is secondary to the secretion of ACTH by nonpituitary neoplasms. Although metyrapone does nothing to affect the primary neoplastic disorder, it is useful in limiting the secretion of cortisol and thus correcting the major metabolic consequences of excess ACTH secretion.

Metyrapone inhibits aldosterone secretion both in primary and secondary aldosteronism (5, 99). If this were the sole effect of metyrapone on adrenal function, one would expect it to promote sodium excretion and potassium conservation. However, in inhibiting the formation of corticosterone, metyrapone brings about the secretion of significant quantities of the precursor, 11-deoxycorticosterone, which is itself a mineralocorticoid. The net effect of metyrapone on electrolyte excretion varies from patient to patient and can be understood in terms of the relative inhibition of aldosterone secretion and "stimulation" of 11-deoxycorticosterone secretion (5).

(*c*) Heparin and heparinoids have been shown to decrease aldosterone secretion in both primary and secondary aldosteronism (12–15). In so doing, these agents promote sodium excretion, lower the blood pressure, and correct edema.

(*d*) O,p'DDD is uniquely valuable in treating metastatic adrenal cortical carcinoma (100–106). Although it has not been known to effect a permanent cure, it is, nevertheless, the only agent that has been shown to have clearcut adrenocorticolytic action. Bergenstal et al. (101) reported that of 18 patients treated with o,p'DDD, seven responded with objective regression of tumor masses and decreased steroid production. An additional seven responded with decreased steroid production only, and four failed to show convincing improvement of any sort. A later summary of clinical experience with o,p'DDD in the treatment of adrenal carcinoma (102) indicated a favorable effect of the drug on the neoplastic disease in 34 per cent and decreased steroid levels in 72 per cent of the patients treated.

O,p'DDD has selective destructive effects on the zona fasciculata and zona reticularis (43–45). For this reason it is peculiarly suitable for use in treating Cushing's syndrome due to adrenal hyperplasia (53–59); it decreases cortisol production while sparing aldosterone secretion (59). As an alternative to bilateral adrenalectomy in the treatment of this variety of Cushing's syndrome, o,p'DDD offers the advantage that it does not leave the patient dependent upon exogenous mineralocorticoids.

Limitations of Adrenal Inhibitors as Therapeutic Agents

In achieving full therapeutic responses to various adrenal inhibitors it is often necessary to accept the risk of undesirable side effects. Since the side

effects may not necessarily be related to the adrenal inhibitory actions of the drugs, it might be possible to develop other agents with superior therapeutic ratios. In some instances it appears that a two- to ten-fold improvement in the therapeutic ratio would provide us with highly useful adrenal inhibitory agents.

(*a*) Amino-glutethimide can be used safely over long periods of time in doses up to 1 gram per day. A common side effect appearing after about 1 week of treatment is a macular skin eruption which clears spontaneously within a few days even if the drug continues to be administered. Doses in excess of 1 gram daily are usually accompanied by symptoms of drowsiness and ataxia which seriously limit the use of amino-glutethimide as a therapeutic agent (10). In addition, reversible nontoxic goiters have been reported in a few cases (107).

(*b*) SU-9055 in daily doses of 4.8 grams frequently causes abdominal distress and diarrhea (25). Therefore, larger doses have not been employed. Smaller doses are not very effective in inhibiting adrenocortical function.

(*c*) Cyanoketone has been shown in fetal female rats to cause genital abnormalities of a type that would suggest increased androgen production (108). If true, this effect would need consideration prior to clinical use of this agent, especially before administration to women or children.

(*d*) Metyrapone has been employed in doses up to 4.5 grams per day without serious toxicity. Transient vertigo occurs if the drug is absorbed too rapidly, but this can be avoided by having the patient take the medication with food or milk. Since metyrapone results in increased secretion of 11-deoxycorticosterone and adrenal androgens (4, 5, 39), it might be anticipated that its long-term employment in large doses would result in hypertension and mild virilization. These theoretical limitations have not diminished the utility of metyrapone in the treatment of selected cases of Cushing's syndrome in which the beneficial effects of curtailed cortisol secretion outweigh the undesirable effects of increased 11-deoxycorticosterone secretion (96–99).

(*e*) Heparin and heparinoids are of limited usefulness in treating hyperaldosteronism because of their anticoagulant properties and because they must be administered parenterally. In addition, prolonged administration of heparin to patients with cardiovascular disorders has been associated with osteoporosis (109).

(*f*) O,p'DDD is limited as a therapeutic agent by its tendency to cause nausea, vomiting, drowsiness, and ataxia when given in doses of more than 6 grams per day (59, 101, 102). Furthermore, it is rather slow-acting as an adrenocorticolytic agent.

(*g*) Amphenone B has been abandoned as a clinical agent because of the many toxic effects encountered during its early trials as an adrenal inhibitor. The toxic effects included drowsiness, cutaneous eruptions, methemoglobinemia, impaired liver function, and gastrointestinal symptoms (61, 62).

Pathological Counterparts of Adrenal Inhibition

A number of inborn errors of metabolism simulate the actions of the adrenal inhibitors that have been described in previous sections.

Prader et al. described a form of congenital adrenal hyperplasia in which the affected patients died in infancy with a clinical illness resembling adrenocortical insufficiency (110). The adrenal glands were grossly hyperplastic and laden with cholesterol. Male infants with this disorder had inadequate masculine genital differentiation, presumably due to a deficiency of androgen production by the fetal testis. It seems likely that such patients have a deficiency of 20α-hydroxylase, the enzyme which converts cholesterol to 20α-hydroxycholesterol, preparatory to its transformation into hormonal steroids. The metabolic consequences of such an enzymic deficiency are qualitatively similar to those observed following the administration of aminoglutethimide (*7*, 8, 10).

Eberlein & Bongiovanni (111, 112) elucidated the nature of the disorder known as "hypertensive virilizing congenital adrenal hyperplasia" by demonstrating that such patients produce inordinate quantities of 11-deoxycortisol and 11-deoxycorticosterone and relatively small quantities of cortisol and corticosterone. They also produce large quantities of 17-ketosteroids, principally of the 11-deoxy series. These authors inferred that their patients suffered from an inborn deficiency of the 11β-hydroxylase system. The metabolic consequences of congenital 11β-hydroxylase "deficiency" are analogous to the effects of metyrapone (4, 5, 33, 39) and SKF 12185 (34, 35).

Bongiovanni (113, 114) has described a disorder in children which is attributed to a deficiency of 3β-hydroxysteroid dehydrogenase, the complex enzyme system that converts Δ^5-3β-hydroxysteroids to Δ^4-3-oxosteroids. There is underproduction of steroids of the Δ^4-3-oxo series (of which cortisol, aldosterone, and testosterone are members) and overproduction of steroids of the Δ^5-3β-ol series, of which dehydroisoandrosterone is a prominent member. The enzymic disorder apparently affects steroid production not only in the adrenal but in the fetal testis as well, since males with this condition have inadequate differentiation of their external genitalia. Females with this disorder are born with some degree of masculinization of their external genitalia, presumably caused by excessive quantities of the weakly androgenic steroid, dehydroisoandrosterone. The chemical and developmental consequences of this inherited defect in 3β-hydroxysteroid dehydrogenase can be simulated by treatment with cyanoketone (108).

Biglieri et al. (115), Goldsmith et al. (116), and Mallin (117) have described patients who have an inborn deficiency of 17-hydroxylase affecting both the adrenals and the gonads. All of the patients who have been described thus far have had subnormal cortisol and 17-ketosteroid values and supernormal levels of 17-deoxycorticosteroids such as corticosterone and 11-deoxycorticosterone. Clinically these patients have had sexual infantilism

(estrogens and androgens have 17-oxygen functions), high gonadotropins, high ACTH, and hypertension. In many ways the metabolic abnormalities associated with this clinical disorder are analogous to those that are produced by the 17-hydroxylase inhibitors, SU-9055 and SU-8000 (21–23).

Ulick et al. (118) have described a deficiency of aldosterone secretion. Underproduction of aldosterone was accompanied by overproduction of 18-hydroxycorticosterone; therefore, it was inferred that the specific defect was an inborn deficiency of 18-hydroxysteroid dehydrogenase activity. The clinical manifestations of this disorder stemmed largely from the incapability of the patients to conserve sodium. The chemical features of this disorder are mimicked by the drug SU-8000 (24). Other cases that might possibly belong in the same category have been described by Degenhart et al. (119) and Rappaport et al. (120).

Migeon et al. (121) have recently described a series of children who apparently have congenital absence of the zona fasciculata and zona reticularis with persistence of zona glomerulosa. They have deficiencies of cortisol secretion but not of aldosterone. Migeon et al. have postulated that these children have a congenital derangement of the adrenal cellular mechanism for reacting with ACTH. An alternative possibility is that they have congenital aplasia of the cells which would normally respond to ACTH. The superficial similarity between this congenital disorder and the selective adrenal atrophy induced by o,p'DDD is obvious (43-45, 59).

The most common inborn error of adrenocortical metabolism is a deficiency of 21-hydroxylase activity (122). In its classical form this condition is characterized by cortisol and aldosterone deficiencies and overproduction of 21-deoxycorticosteroids and 17-ketosteroids. 17α-Hydroxy-progesterone (the cortisol precursor which serves as the principal substrate for 21-hydroxylase) is excessively secreted and is metabolized to the diagnostically important excretory product, pregnanetriol. Although amphenone B has been said to interfere with 21-hydroxylase activity (67), it is not specific for this enzyme system; there has yet to be described a pharmacologic inhibitor of adrenal function which mimics this particular inborn error with fidelity.

Concluding Comments

There have been numerous exciting developments in adrenocortical pharmacology during the past two decades; yet much remains to be learned about the intimate biochemical mechanisms through which various agents inhibit the biosynthesis of corticosteroids. In the process of elucidating these mechanisms it may be possible to gain some insight into the basic nature of the inborn errors of metabolism which at least superficially resemble the pharmacologic inhibition of adrenal function.

It seems probable that the clinical application of adrenal inhibition will be greatly extended in the years ahead. Limited efforts to utilize adrenal inhibitors as heuristic and diagnostic tools have thus far been remarkably

fruitful, and the use of adrenal inhibitors as therapeutic tools seems even more promising. As long as there are people who might benefit by reduction of their levels of mineralocorticoids, glucocorticoids, androgens, or estrogens there will be at least a theoretical need for safe, effective, selective inhibitors of steroid biosynthesis.

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