HEPATIC AND EXTRAHEPATIC REGULATION OF CORTICOSTEROIDS

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INTRODUCTION

The purpose of this review is to present and discuss certain concepts of the production, peripheral metabolism, function and excretion of steroid hormones, with special emphasis on the corticosteroids. The four processes listed above are interrelated so that they influence one another.

De novo production of steroid hormones occurs by biosynthesis, a term we restrict to the formation of cyclopentanoperhydrophenanthrene derivatives (Fig. 1) (*i.e.*, derivatives of the four-ring system composing the nucleus of steroid compounds) by glands derived from the genital ridge—the adrenals, the ovaries, and the testes. No other organs have been shown to synthesize steroid hormones in demonstrable amounts starting with acetate.

Metabolism of steroids in non-endocrine tissues occurs by a series of oxidations and reductions which we designate as biotransformation. Since this peripheral metabolism in many instances leads to the production of biologically active compounds, we call it biotransformation to differentiate it from biosynthesis. Thus special cells of the endocrine organs may have unique biosynthetic capacities, whereas all cells may have in common some capacities to convert steroids to other steroids.

It should be noted that although peripheral metabolism precedes excretion, the metabolites formed exclusively by oxidation and reduction of the substituent

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groups on the steroid nucleus are excreted as such only in negligible amounts by the normal animal.

Biotransformation, then, influences the amount of biosynthetically produced compounds present in the periphery at any one time—and also profoundly affects functional capacities of steroids since function is closely related to the oxidative state of chemical groups on the steroid molecule. For example, cortisol (which has hydroxyl groups at C-11 and at C-17) is a much more powerful destroyer of lymphocytes than cortisone (which has a keto group at C-11 and an hydroxyl at C-17) which is, in turn, more active than corticosterone (C-11—OH and no hydroxyl at C-17) (100). It so happens that the conversion of cortisol to cortisone is a biotransformation which is reversible.

If a reversible reaction which helps to maintain a sufficient supply of a compound (or type of compound) needed for a particular effect becomes irreversible, then there will be a greater requirement for the more active compound than before. The cortisol-cortisone transformation in lymphocytes illustrates this point (48). Unlike normal lymphocytes, malignant lymphocytes can transform cortisone to many products which have no lymphocytokaryorrhectic effect at all (19). Therefore, lymphocyte destruction becomes more dependent on a constant supply of cortisol when the cells are malignant.

Endocrinopathies result if the altered reactions occur in the biosynthetic mechanism in the endocrine glands, while a variety of pathologic difficulties arises when the cells in the periphery begin to metabolize steroids in an abnormal way.

The authors wish to emphasize that this review will not attempt a detailed presentation of every step involved in formation and metabolism of steroids. Such information is given in several extensive and excellent review articles (34, 35, 68, 99). The present review deals mainly with peripheral (hepatic and extrahepatic) metabolism of adrenocortical hormones and the cells which carry out these metabolic reactions.

Figure 1 is included here in order that the reader may have a convenient reference for nomenclature of the compounds discussed in this review.

A. Biosynthesis of steroid hormones by glands derived from the genital ridge

Figure 2 represents what is assumed to be the manner by which steroid hormones are formed from acetate and cholesterol in the glands derived from the genital ridge. The particular tissues capable of performing each reaction are indicated in Figure 2, and an asterisk is used when peripheral tissues can also carry out the reaction. We have not attempted to indicate all the intermediates which have been isolated from *in vitro* incubation of precursors, but have included only those which are thought to be the actual products of endocrine tissue cells *in vivo*. *In vitro* studies have given much information on the manner by which steroids are formed in the endocrine organs. However, it should be realized that when a known or supposed precursor is incubated with an endocrine glandular tissue and a new product is isolated, this does not necessarily prove it to be a compound normally secreted by that gland.

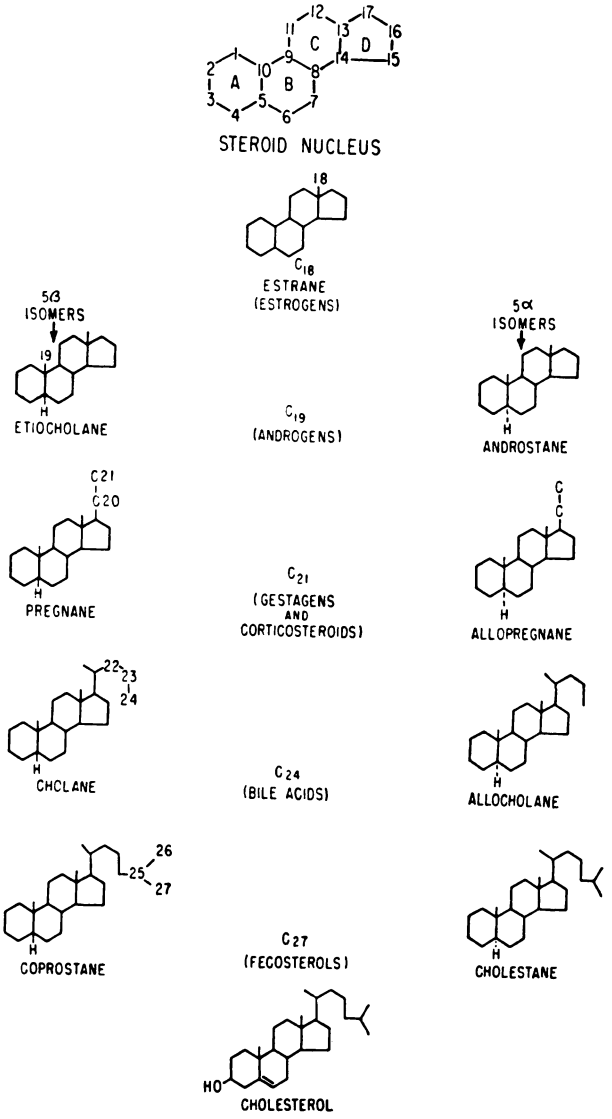


FIG. 1. Nomenclature of steroids

Chemically, the steroid nucleus is designated as cyclopentanoperhydrophenanthrene. In 1950 the name "gonane" was suggested (Ciba Foundation, London, May 30, 1950 [Chem. & Ind. SN1-11, June 23, 1951]).

The estrane configuration is represented here without isomerization at position 5, since the naturally occurring estrogens have an aromatic ring A (see Fig. 5). The aromatic ring A also prevents the presence of a methyl group at C-10. Other steroid compounds normally have the methyl group (the carbon of which is C-19) and when this group is lacking the lack is indicated by "19-nor," *e.g.*, 19-nor-testosterone.

A *beta* (β) preceding the name of a substituent group on the gonane nucleus means that the substituted group lies on the same side of the molecule as the C-18 and C-19 methyl carbons; an *alpha* (α) means that the group lies on the side of the molecule opposite to the C-18 and C-19 methyl carbons.

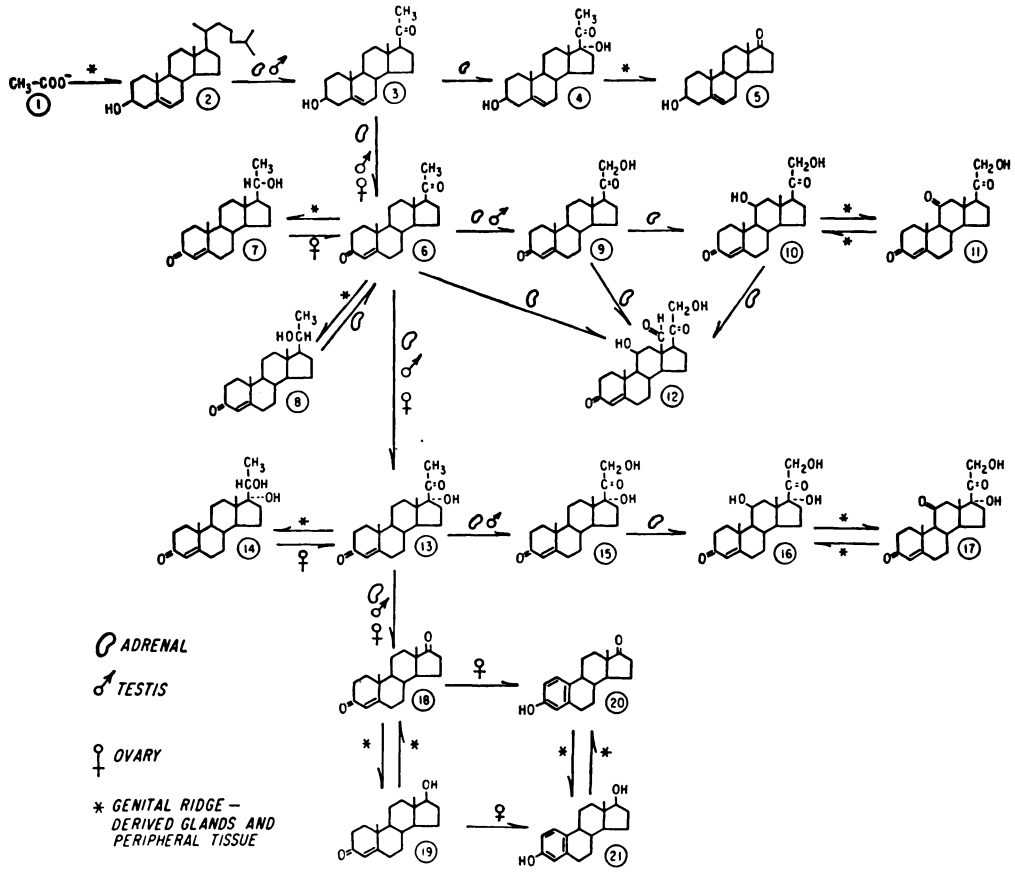


FIG. 2. Biosynthesis of the key steroid hormones by glands derived from the genital ridge
(1) Acetate, (2) cholesterol, (3) 5-pregnenolone, (4) 17α-hydroxy-5-pregnenolone, (5) dehydroepiandrosterone, (6) progesterone, (7) 4-pregnene-20α-ol-3-one, (8) 4-pregnene-20β-ol-3-one, (9) desoxycorticosterone, (10) corticosterone, (11) 11-dehydrocorticosterone, (12) aldosterone, (13) 17α-hydroxyprogesterone, (14) 4-pregnene-17α,20α-diol-3-one, (15) 11-desoxycortisol, (16) cortisol, (17) cortisone, (18) 4-androstene-3-17-dione, (19) testosterone, (20) estrone, (21) estradiol.

We have mentioned with reference to Figure 2 that certain types of cells in the periphery have in common with endocrine gland cells the ability to carry out particular reactions. In this context, it would be a mistake to assume that a reaction is peculiar to an endocrine tissue, without first testing the capacity of other tissues to accomplish the same process. An example of this is 6β-hydroxylation, which occurs in the adrenal cortex and might therefore be considered unique to biosynthetic mechanisms. However, 6β-hydroxylation occurs in many other tissues in addition to the adrenal gland, for example, the liver (1), placenta (15), and ovaries (2, 65 106a). Another example of a reaction which goes on in non-endocrine as well as in endocrine tissue is C-20 reduction (*e.g.*, $\begin{array}{c}|\\ C{=}O \rightarrow H{-}\\ |\end{array}$

C—OH), as when progesterone (no. 6 in Fig. 2) is converted to 4-pregnene-20α-ol-3-one (no. 7 in Fig. 2). This transformation is known to occur in the ovary (106a), in the placenta (99), and in a large number of other tissues (18, 86, 107, 113, 124). Even the tissue-cultured fibroblast is capable of C-20 reduction (8, 10, 17, 43, 90, 109).

1. Biosynthesis of corticosteroids. The key to the formation of corticosteroids is found in the hydroxylation of progesterone in positions 11, 17 and 21 (Figs. 2 and 3). These particular reactions require TPNH (reduced triphosphopyridine nucleotide) (94) and molecular oxygen (99, 106, 110). Of the steroid-producing endocrine organs, only the adrenal is capable of 11-hydroxylation. The last is worthy of note since the principal corticoid produced by mammalian species is either cortisol or corticosterone, which are both 11-hydroxylated.

The formation of desoxycorticosterone (DOC) (no. 9 in Fig. 2) from progesterone (hydroxylation at C-21) has now been found to occur not only in the adrenal gland, but also in the testis (99). It has been demonstrated that DOC cannot be hydroxylated at carbon 17 (68). Therefore DOC cannot be converted

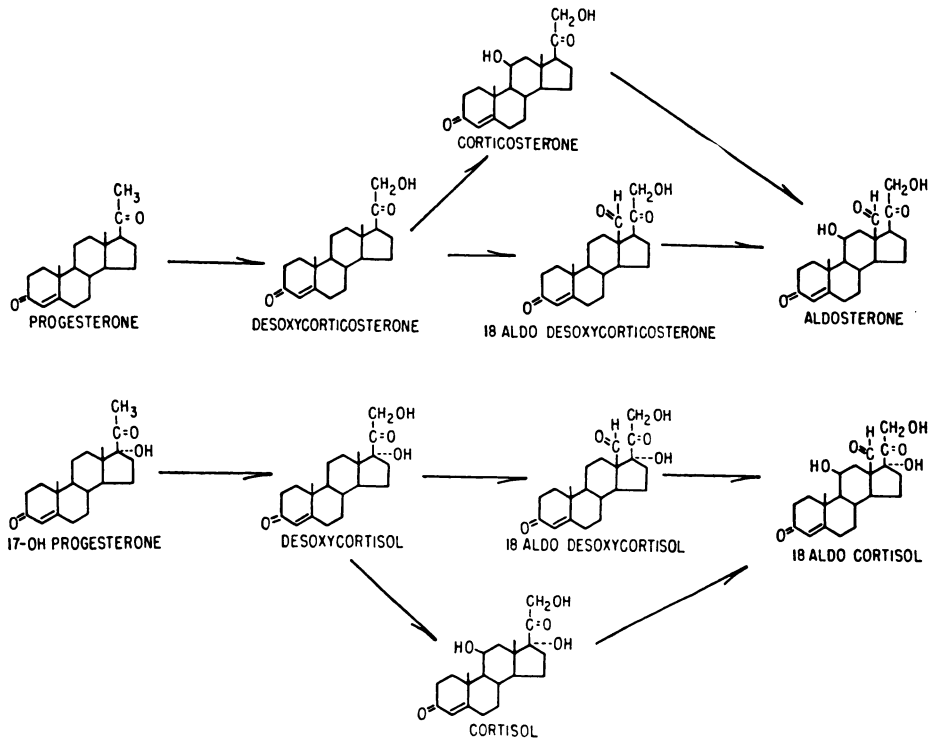


FIG. 3

The pathway of biosynthesis from progesterone to aldosterone here represented has been demonstrated. Possibility that a similar pathway from 17-OH progesterone to 18-aldocortisol may exist is here depicted.

to cortisol, but instead is transformed to corticosterone (68, 99) and aldosterone (121, 122) *via* hydroxylation at C-11 and oxidation at C-18. Progesterone, DOC, or corticosterone can be converted to aldosterone (121, 122) through oxidation at position 18 (see Fig. 3). This certainly indicates that the presence or absence of hydroxyl groups at carbons 11 or 21 or both, does not abolish oxidation at carbon 18, although the presence of a C-11β hydroxyl group partially hinders the C-18 oxidation.

17α-Hydroxyprogesterone is hydroxylated at C-21 to form 4-pregnene-17α,21-diol-3,20-dione (substance S of Reichstein), and this steroid is further hydroxylated at position 11 to yield cortisol (68, 99).

The biological significance of the hydroxylations at key positions of the steroid nucleus is demonstrated by the fact that one of these hydroxylations will change the biological activity of a steroid. For example, progesterone has high gestagenic and mild mineralocorticoid effects. Hydroxylation of progesterone at carbon 21 will change its activity to that of a more active sodium-retaining compound, deoxycorticosterone. Through addition hydroxylation of progesterone at C-17 and C-11 (forming cortisol), the molecule acquires gluconeogenic, anti-inflammatory, and lymphocytokaryorrhectic activities (39, 52). The presence of an hydroxyl group at the 11-position of substance S of Reichstein will increase its anti-inflammatory effect many fold (52). Therefore, hydroxylation of one carbon on a steroid effects not only a qualitative biological change, but also a quantitative one with respect to its functional activity.

It would be interesting to know if oxidation at carbon 18 could occur after hydroxylation at carbon 17. This would be biologically significant because 18-aldo cortisol would possess the structural features of both the most potent anti-inflammatory and the most potent electrolyte-retaining steroids (Fig. 3).

B. Biotransformation of steroids (transformations by non-endocrine organs)

1. Biotransformations producing biologically active steroid compounds. As described above, biotransformation means oxidative and reductive changes which may or may not lead to the production of biologically active steroids. This section will deal with biotransformation which results in the formation of active compounds.

The formation of androsterone from testosterone (99) or from dehydroepiandrosterone (82) (Fig. 4) produces a compound with hypocholesterolemic properties (69). However, etiocholanolone, another metabolite of testosterone (99) or of dehydroepiandrosterone (82), does not lower blood cholesterol, but instead has a pyrogenic effect in man (75). Therefore, a difference in reaction rates of these two possible biotransformations could determine the degree of occurrence of two dissimilar biological events.

Desoxycorticosterone, which has an electrolyte-retaining effect, becomes an anesthetic agent (89a) upon the addition of two hydrogens in the A ring to form the dihydro compound (see Fig. 7 for the dihydro form). Upon reduction of the 3-keto group to produce the tetrahydro compound, there is an acquisition of the pyrogenic effect (75).

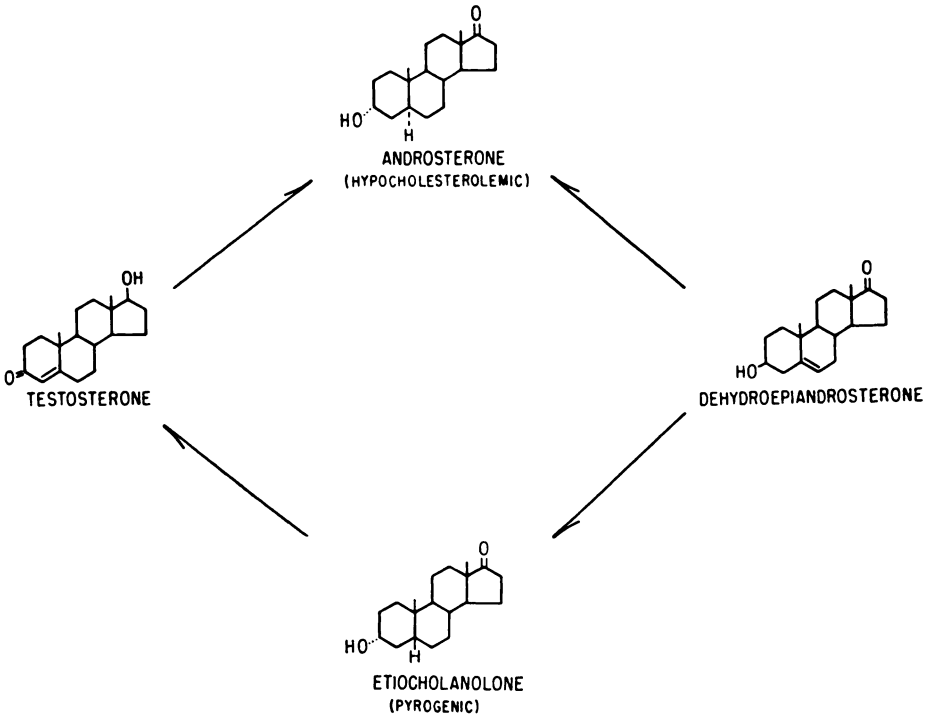


FIG. 4

Pregnanediol, the major metabolite of progesterone, is a highly effective pyrogenic steroid in man (75). The rise in temperature during the progestational phase of the menstrual cycle could very possibly be due to the peripheral conversion of progesterone to pregnanediol.

A very dramatic example of non-endocrine formation of active steroids is provided by experiments done by West *et al.* (120). These workers demonstrated the conversion of testosterone propionate to estrone and estradiol in an oöphorectomized, adrenalectomized woman with metastatic breast cancer. No estrogen could be identified in the patient's urine during a control period when she was receiving no testosterone.

It has been shown that some C_{21}-17-hydroxylated steroids, *e.g.*, cortisol, cortisone, 17-hydroxypregnenolone, *etc.*, are converted to C_{19}-steroids in non-endocrine tissues (5, 8, 11, 12, 19, 89). Thus, the oxidation of the side chain of cortisol (a C_{21}-compound) can lead to the formation of C_{19}-compounds as shown in Figure 6 (C_{19}-compounds numbered 13 and 14); cortisol, having gluconeogenic and anti-inflammatory effects (36, 42) results in the formation of compounds lacking those two activities, but possessing some androgenic activity. Jailer *et al.* (73) quoted a personal communication which stated that when applied topically to the cock's comb, 17α-hydroxyprogesterone exhibits no androgenic activity, but if the hormone is given intravenously, it is about 50%

as effective as methyltestosterone in promoting cock's comb growth. This indicates that 17α-hydroxyprogesterone, a C_{21}-compound with no reported biological activity, can be transformed to androgenic compounds.

It has become increasingly apparent that the androgens, estrogens, gestagens, and corticosteroids undergo a series of structural changes by peripheral tissues in which the relative rates of conversion determine biological potency because they establish the quantities of molecular forms having particular effects. It has been shown (Fig. 5) that reversible interconversions exist in many tissues. The reports in the literature of reversible conversions include cortisol with cortisone

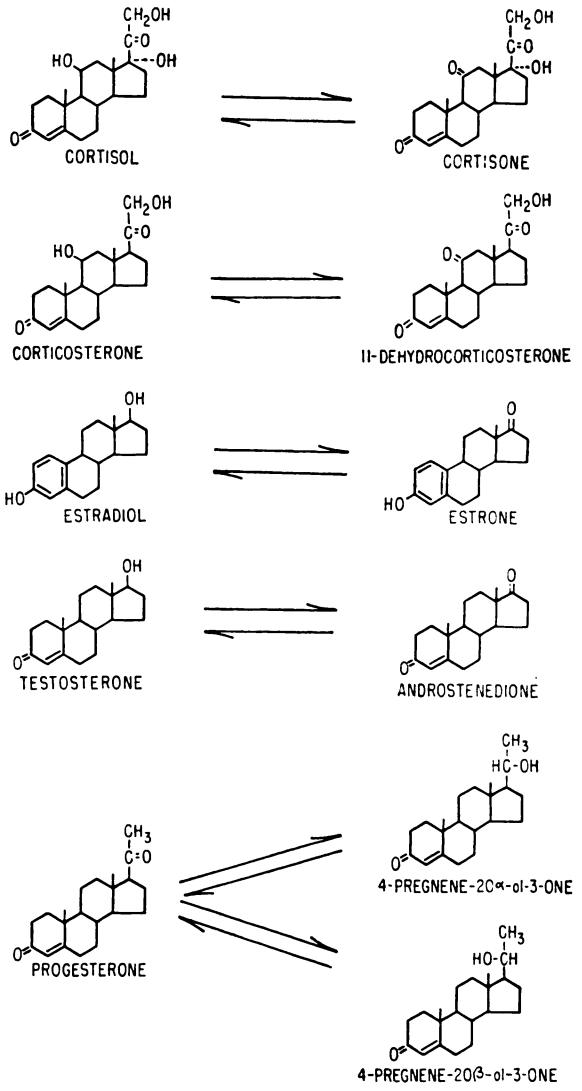


FIG. 5

(34, 48), estradiol with estrone (97), testosterone with androstenedione (27, 78), and progesterone and 4-pregnenolone, 20α (125) and 4-pregnenolone, 20β (86). These conversions represent possible ways by which a wide range of effects of different steroid hormones on different cells can be produced. Thus, the contributions of non-endocrine cells to the amounts of certain active molecular forms must be considered as well as the contributions of the secretions of endocrine organs themselves.

There is evidence that when the conversion of cortisone to cortisol is inhibited by introducing a methyl group at carbon 2, the compound loses gluconeogenic (53) and anti-inflammatory (42, 53) activity. Methylation of cortisol at carbon 2 inhibits its conversion to its C-11-keto analog (25); correspondingly, the anti-inflammatory and gluconeogenic activities of cortisol are greatly enhanced (42, 53, 58). This is a good example of the fact that increased or decreased capacity of a molecule to elicit a biological response may be due to alteration of the rate of reversible transformations of a steroid molecule which can take place in non-endocrine (peripheral) as well as in endocrine tissues.

2. Biotransformation producing compounds of no known biological activity and subsequent reactions leading to excretion of these metabolites. The liver is a major, but not the sole site of steroid metabolism. Extrahepatic tissues can carry out some of the oxidations and reductions performed by the liver, although apparently only the liver forms "tetrahydro" compounds in which the 4–5 double bond has been saturated and the 3-keto group has been converted to a 3-hydroxyl group (Fig. 6, compounds numbered 9, 7, 10, 15, 12, 8, 11, and 16). Although the kidney has the capacity to conjugate corticosteroid metabolites to glucuronides (105), the liver is thought to be the organ primarily responsible for this conjugation reaction (Fig. 7) *in vivo*.

Extrahepatic tissues have been shown *in vivo* to convert cortisol to 4-pregnene-11β,17α,20β,21-tetrol-3-one (substance E of Reichstein), 4-pregenene-17α, 20β,21-triol-3,11-dione (substance U of Reichstein), cortisone, pregnane-11β, 17α,21-triol-3,20-dione (dihydrocortisol), and 4-androstene-11β-ol-3,17-dione (12) (Fig. 6). The tetrahydro derivatives usually found as products of cortisol metabolism in intact animals and the water-soluble steroids were not detected in the hepatectomized animals used (12).

Corticosterone has been shown to be converted to 11-dehydrocorticosterone and 4-pregnene-11β,20β,21-triol-3-one (11, 60) in hepatectomized animals. Again, the techniques employed detected no water-soluble compounds. The possibility that extrahepatic tissue could conjugate a steroid which already is reduced in ring A (tetrahydro) was tested by administration of pregnane-3α, 17α-diol-11,20-dione-4C^{14} (21-deoxy tetrahydrocortisone) (60) to eviscerated, nephrectomized rats. The results indicate that no conjugation of this steroid occurred, showing again that the liver is the major organ capable of conjugating steroids. Incubation of kidneys with pregnane-3α-ol-11,20-dione-4C^{14} (a tetrahydro compound) showed that this organ can conjugate this steroid with glucuronic acid (105); however, similar incubation with cortisol and with corticosterone resulted in negligible conjugation of the steroids (105). Therefore, the

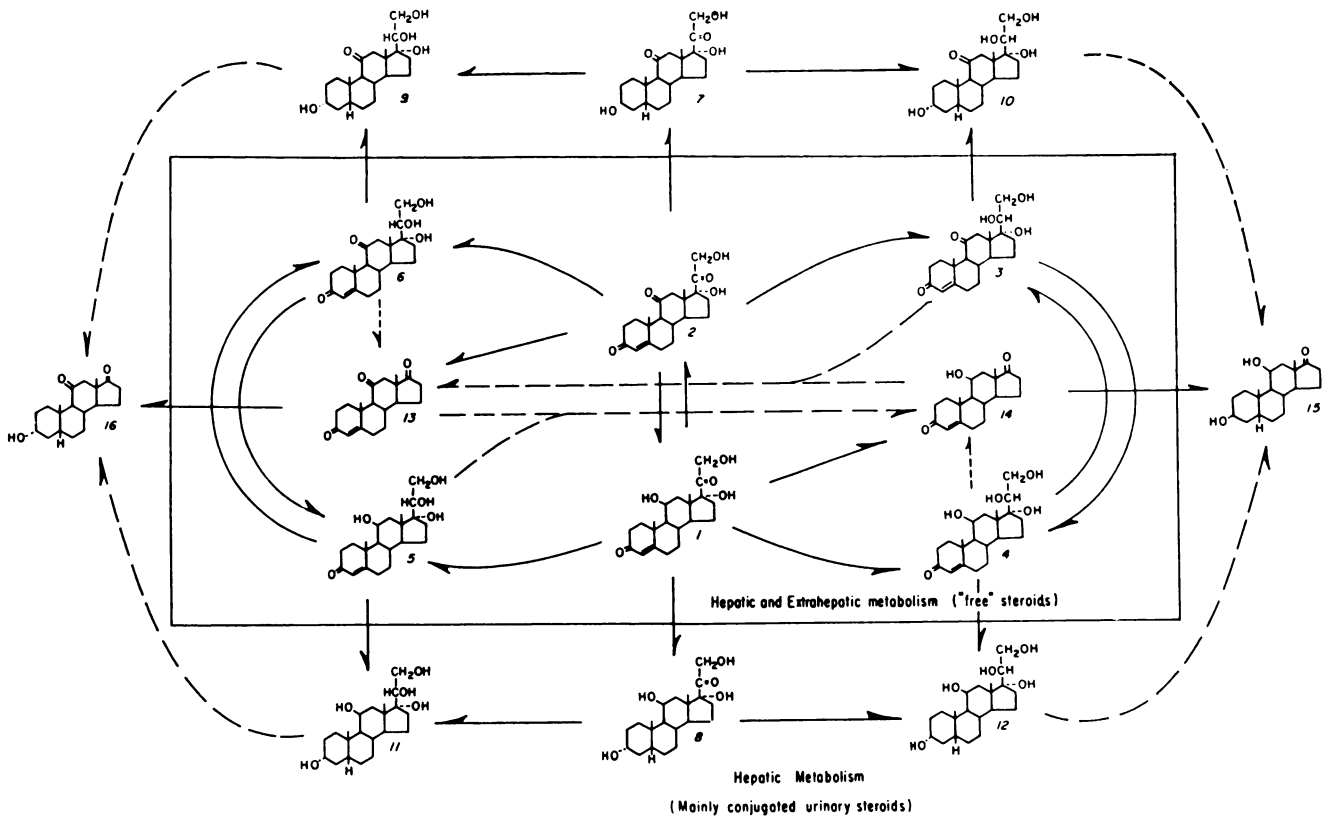


FIG. 6. Hepatic and extrahepatic metabolism of cortisol

(1) 4-Pregnene-11β,17α,21-triol-3,20-dione (cortisol); (2) 4-pregnene-17α,21-diol-3,11,20-dione (cortisone); (3) 4-pregnene-17α,20β,21-triol-3,11-dione (substance U of Reichstein); (4) 4-pregnene-11β,17α,20β,21-tetrol,3-one (substance E of Reichstein); (5) 4-pregnene-11β,17α,20α,21-tetrol,3-one (20-epi substance E of Reichstein); (6) 4-pregnene-17α,20α,21-triol-3,11-dione (20-epi substance U of Reichstein); (7) pregnane-3α,17α,21-triol-11,20-dione (tetrahydrocortisone); (8) pregnane-3α,11β,17α,21-tetrol,20-one (tetrahydrocortisol); (9) pregnane-3α,17α,20α,21-tetrol, 11-one (α-cortolone); (10) pregnane-3α,17α,20β,21-tetrol,11-one (β-cortolone); (11) pregnane-3α,11β,17α,20α,21-pentol (α-cortol); (12) pregnane-3α,11β,17α,20β,21-pentol (β-cortol); (13) 4-androstene-3,11,17-trione (andrenosterone); (14) 4-androstene-11β,ol,3,17-dione (11β-OH-androstenedione); (15) etiocholane-3α,11β-diol,17-one (11β-OH-etiocholanolone); and (16) etiocholane-3α-ol,11,17-dione (11-keto-etiocholanolone).

The compounds listed here are the major ones found; 6β-hydroxylated steroids and others not completely identified are not shown in this figure. Cortisol metabolites show the 5α ("allo") as well as the 5β configuration.

The dark lines represent pathways that we and/or other investigators have demonstrated. Pathways indicated by dotted lines have not been tested as yet in our laboratory.

It has been demonstrated recently that substance E of Reichstein is metabolized in the human to the corresponding tetrahydro derivatives (compounds 10 and 12) as well as to C-19 steroids (57b).

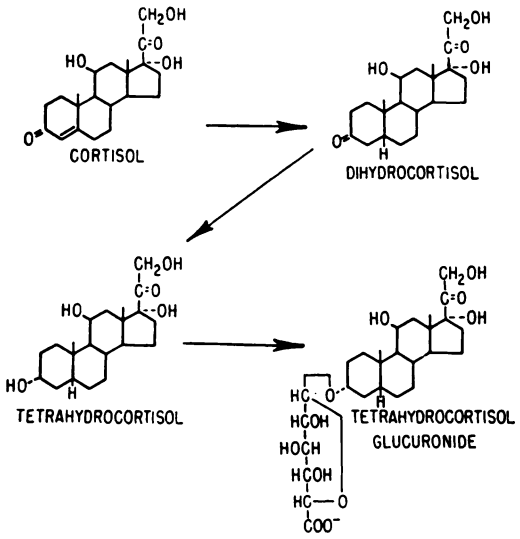


FIG. 7

dependence on the liver for conjugation is due in large part to the capacity of its cells to produce tetrahydro compounds (87). The kidney possesses no such activity but can conjugate corticosteroids previously acted upon by the liver (105).

In general the greater the state of reduction of a steroid molecule, the less active it is in biological mechanisms. In any event, as we have seen in the previous section on biotransformation, oxidative and reductive changes tend to inactivate the molecule with respect to the specific function it previously possessed. Therefore, for most corticosteroid hormone effects, there is a delicate structure-activity relationship.

Experiments have been performed which suggest that 3α- or 17-hydroxy steroids might act as coenzymes in the transhydrogenation of the pyridine coenzymes (112). Partially purified liver enzyme systems were found to catalyze reversible biotransformations at the 3- or the 17-position (99), and it was shown that the hydrogen was exchanged with a di- or a triphosphopyridine nucleotide (71). Since certain steps in lipid and six-carbon sugar synthesis are dependent upon TPNH, it was thought that the reactions of the steroid (tetrahydro form) with the pyridine nucleotides could influence lipid and sugar reactions.

Bloom (22) attempted to demonstrate the incorporation of the pyridine nucleotide hydrogen into lipid, or glycogen, or both under the influence of the ring-A-reduced steroids which had been found to be effective in the transfer of hydrogen to pyridine nucleotides in isolated organ systems. Pyridine nucleotide was tritiated intracellularly (in liver slices) to form pyridine nucleotide-4-H^3 (reduced form). There was no change in the incorporation of H^3 into lipid and glycogen in the presence of steroid. The ring-A-reduced steroids most active in hydrogen transfer are the form of steroid most rapidly conjugated to a water-

soluble moiety and disappear from the liver quite rapidly. It is not too surprising that the steroid did not enhance H^3 incorporation; the half-life of such forms is quite short in liver tissue. Accordingly, the role of steroids in hydrogen transfer (112) to pyridine nucleotides in the intact liver cell remains to be established.

Another effect of steroids on isolated enzyme systems has been described by Yielding and Tomkins (127) who showed the inhibition of DPNH oxidases (both mammalian and microbial) by steroids.

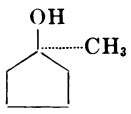
The fact that steroids enter into metabolic reactions does not explain the known specific biological actions of steroids. For example, White (74, 123), in an attempt to explain lymphocytokaryorrhectic effects, measured the ability not only of cortisol but also of non-lymphocyte-destroyers to affect lymphocyte metabolism *in vitro*. The results showed that oxygen consumption and glucose oxidation were depressed more extensively by the non-lymphocytokaryorrhectic steroids than by cortisol itself. In general it has been learned that however the steroid acts, the best way to enhance its specific biological activity is to inhibit steroid biotransformation. In the chemical synthesis of new steroids, therefore, the trend is to make a molecule which is slowly metabolized, keeping more molecules in the active state (*i.e.*, having the biological effect described for a particular steroid hormone).

All anti-inflammatory synthetic hormones have those groups intact on the steroid nucleus which were shown to be essential for anti-inflammatory activity (52). Substitution of atoms of the halide series (fluorine, chlorine, bromine) in the molecule enhances anti-inflammatory activity, though such substitution cannot create that activity in a molecule that had none to begin with.

Substance S of Reichstein (Fig. 2, no. 15) has a weak anti-inflammatory effect (52) which is due to the 17α-hydroxy group (absolutely essential for anti-inflammation) (49). The substitution of a chlorine atom at position 11 of substance S markedly potentiates the antiphlogistic influence (114). Since chlorine in position 11 enhances the anti-inflammatory effect, it seems unlikely that the action of the most active naturally occurring anti-inflammatory steroid, cortisol, could be explained by a transfer of hydrogen from the hydroxyl group at position 11.

The well-known and possibly earliest example of a molecule with increased activity due to hindrance of metabolism is the synthetic steroid 17α-methyltestosterone. The methyl group prevents the loss of hydrogen from the 17β-hydroxyl group and therefore the molecule is kept in the active androgenic state (2).

D ring of methyltestosterone



Berliner proposed the action of testosterone as a hydrogen donor (2) for the conversion of fumarate to succinate:

testosterone + fumarate ⟶ 4-androstenedione + succinate

Methyltestosterone was inactive in this test system, being unable to give up hydrogen (2).

Estradiol can also be protected from biotransformation at position 17 by substitution of an ethynyl group to yield 17α-ethynylestradiol. Again, biological activity is potentiated, in this instance estrogenic activity.

Probably the pinnacle of success in preventing hydrogen loss and subsequent biotransformations was reached with the synthesis of the extremely potent anti-inflammatory compound, dexamethasone (9α-fluoro, 16 methyl, Δ^1-cortisol), in which ring A is protected by Δ^1, 9α-fluoro inhibits the attack of the enzyme on position 11 (maintaining the 11-hydroxyl group), and 16-methyl protects the 17-, 20-, and 21-positions.

Studies performed *in vivo* (25, 81) and *in vitro* with lymphocytes and fibroblasts (42) have shown that 2α-methyl cortisol and 2α-methyl cortisone are not nearly as readily interconverted as cortisol and cortisone, *i.e.*, there is little oxidation and reduction at C-11. When oxidation and reduction at C-11 is practically abolished by substitution of a 2α-methyl group, the biological activity of a steroid is also altered (see Fig. 8).

It may be that certain biotransformations can be carried out by all living cells, whether they are target cells for the action of the steroid or not. Even bacterial, fungal, and higher plant cells which do not require steroids for growth are able to make certain transformations of the steroid which are identical with those performed by mammalian target cells (111), *i.e.*, plant cells can convert steroids into certain steroid metabolites of no known biological function.

3. Hepatic biotransformation and conjugation reactions. It has been established that the liver is the major site of steroid conjugation, and that the reason for this is the capacity of the hepatic cells to form tetrahydro compounds and to conjugate these steroid metabolites with glucuronic acid, sulfuric acid, *etc.* (9, 12, 18, 44, 59, 99), thus making the steroid metabolite water-soluble, and hence excretable. Accordingly, conjugation is the terminal step in steroid metabolism, ending biotransformation.

Figure 7 illustrates the most direct pathway possible to conjugate formation. The model compound is cortisol, a typical corticoid, possessing a ketone at C-3 and a 4–5 double bond in ring A. The first reaction involved is production of a "dihydro" compound, by reduction of the 4–5 double bond. The next reaction is production of a "tetrahydro" compound, involving the reduction of the 3-ketone. The "tetrahydro" compound is then conjugated to glucuronic acid, yielding a water-soluble compound.

Prednisolone ($\Delta^{1,4}$-cortisol), having an extra double bond in ring A, is much more slowly metabolized than cortisol (58), probably due to the inhibition of conjugation as a result of increased unsaturation in ring A.

Hepatectomized or eviscerated animals lose their ability to conjugate these steroids (12, 18, 60, 117). Recently, Bojesen and Egense have shown that the half-lives of cortisol and corticosterone are doubled in hepatectomized cats (23). Elimination of essentially all steroids from the animals was prevented when they were totally eviscerated.

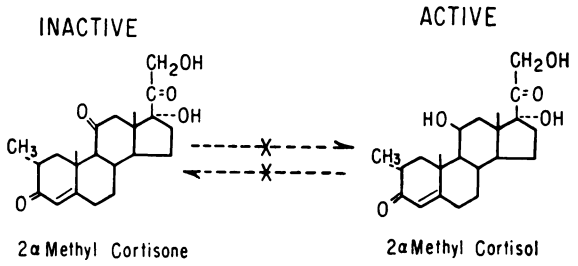


FIG. 8

These 2α-methyl steroids do not interconvert. Their biological activities (anti-inflammatory, lymphocytokaryorrhectic and gluconeogenic) are indicated, showing that hindering 11βOH-dehydrogenation can change the action of a steroid.

The cell population and the percentage distribution of the cells in liver tissue of mice and rats indicate that parenchymal cells and reticuloendothelial cells make up 94% of the total cell population (29). Hepatocytes and reticuloendothelial cells exist in approximately a two-to-one ratio (60.0:33.4%) in the liver (29), and the rest of the cells are bile duct, connective tissue and blood vessel cells. It has been demonstrated that only the hepatocytes can conjugate corticosteroids (11). The reticuloendothelial cells do not seem to have this ability; however, they are able to reduce the A ring to the tetrahydro form, reduce the C-20 position, and oxidize the C-11 position (Fig. 6) (87). They may, therefore, be essential for conjugation, since reduction of the A ring is a necessary step before conjugation can occur. We do not know whether or not hepatocytes, by themselves, can form tetrahydro compounds. The reviewers' technique for separating reticuloendothelial cells and hepatocytes leads to a quite pure preparation of reticuloendothelial cells, but the remaining portion of cells is probably a mixture of reticuloendothelial cells and hepatocytes (11).

Incubation studies *in vitro* have shown that the liver has a great capacity to oxidize and reduce the substituted groups on the steroid nucleus, with the eventual production of tetrahydro compounds (99). Studies on the liver by perfusion of radioactive steroids *in vitro* have demonstrated that the biotransformations and the conjugation step are very rapid and resemble more the results *in vivo* than the incubation studies *in vitro* in amount of metabolites formed and rate of reaction, as would be expected. Incubation of liver homogenates with cortisol has shown inhibition or abolition of conjugation, but steroid conversions taking place at positions C-11, C-17, and C-20 were increased over those found in experiments *in vivo* or in perfusion experiments *in vitro* (Fig. 6, nos. 2, 3, 4, 5, 6, *etc.*). By perfusion techniques it has been found that metabolites of cortisol are rapidly eliminated in the bile, and that the only metabolite which can be detected in circulating blood is substance E of Reichstein, which also disappears at a fairly fast rate (46).

The perfusion technique is a better method for studying liver steroid metabolism than incubation of homogenates or slices. Incubations of this type allow the testing of the ability of the incubated material to convert one steroid to

another, but they do not allow for the removal of conversion products. Perfusion allows a controlled study of just one organ without permitting abnormal accumulation of one or more metabolites. A production of metabolites in excess of the normal could cause the metabolic pattern to be altered, since larger than normal amounts of substrate would be presented to surviving enzyme systems. In addition, it is customary to add cofactors to incubations so that the rates of various reversible reactions can be changed from normal, as influenced by endogenous cofactor.

The liver and the kidney can conjugate C-19 as well as C-21 steroid metabolites as glucuronides (29). In addition to glucuronosyl transferase, the enzyme which catalyzes the coupling of glucuronic acid with tetrahydro compounds, the liver also has steroid sulfokinase which catalyzes the formation of steroid-sulfate conjugates (88). Nose and Lipmann demonstrated this enzyme in the liver, but they could not find it in the kidney or intestine (88).

C. Hormonal interrelationships which influence steroid metabolism

1. Influence of ACTH. There is no doubt that ACTH, a polypeptide produced by the anterior pituitary, stimulates the production of cortisol and corticosterone by the adrenal gland. Hypophysectomy will abolish this effect (108). The intrinsic mechanism by which ACTH affects the release of corticosteroids is still not well understood. Hechter has shown that ACTH stimulates the cleavage of the side chain of cholesterol at carbon 20 (desmolase) to form 5-pregnenolone (64, 67) (Fig. 2: conversion of compound 2 to compound 3).

Haynes has shown that ACTH stimulates phosphorylase activity in the adrenal gland (66, 66a, 66b) and that this is not indiscriminate phosphorylase stimulation, since ACTH has no effect on liver phosphorylase. Haynes has also demonstrated the mediation of ACTH-stimulation by 3′,5′-adenosine monophosphate (3′,5′-AMP). As a result of greater phosphorylase activity, more reduced pyridine coenzyme is available due to increased glycogenolysis and glycolysis, and reduced coenzyme, as previously mentioned, enters into the hydroxylation of positions 11, 17, and 21 on the steroid nucleus in corticosteroid formation (64, 67, 98).

Emberland, working with adrenal homogenates, was unable to obtain stimulation of phosphorylase with 3′,5′-AMP but obtained stimulation with 5′-AMP, a compound found by Haynes to be inactive in adrenal slices (55). Emberland suggested that it may not be valid to compare data obtained *in vitro* from studies of slices with those of homogenates.

It was recently demonstrated that TPNH is the necessary cofactor for the formation of 5-pregnenolone from cholesterol (64). Further evidence obtained from the interesting work of Koritz and Péron (79) points to a second role of ACTH in the stimulation of corticosteroid production. These investigators have shown that when quartered adrenals are maximally stimulated with TPNH and glucose-6-phosphate, an additional stimulation by ACTH is obtainable (79). The converse situation also occurs: the maximal adrenal production elicited by ACTH can be further increased by addition of TPNH and glucose-6-phos-

phate. These facts indicate that more than one mechanism is involved. Also, adrenal glands which have been frozen can be stimulated by TPNH and glucose-6-phosphate, but not by ACTH. These authors concluded that ACTH not only controls TPNH levels but makes more precursor available.

A most interesting and stimulating suggestion is that of Hechter, that ACTH might act at the mitochondrial surface to make material available to the desmolase system, or at the cell surface to make material available as substrates or cofactors, since the conversion of cholesterol to 5-pregnenolone has been shown to occur in adrenal mitochondria (83). Electron microscopic examination of adrenocortical cells of bovine adrenals stimulated during perfusion by ACTH has shown an increase in the density and number of cytoplasmic granules (83). Also, there is an increased number of pseudopodial extensions of the cell membrane into the surrounding ground substance. It is possible to correlate these findings of Hechter with the ones found in the reviewers' laboratory (10, 43, 45) in which ACTH was shown to increase the phagocytic activity of the reticuloendothelial cells of several organs, including the adrenal cortex, with respect to rate of uptake of cholesterol. Thus, more substrate (cholesterol synthesized by peripheral cells) might be made available to the adrenal cortical cell and consequently, more splitting of side chain at C-20 could occur, yielding more 5-pregnenolone.

Further evidence that ACTH action may involve regulation of cellular permeability processes has been demonstrated recently by Eichhorn *et al.* (54), who showed D-xylose to be excluded from the major fraction of cellular water in the adrenal gland of hypophysectomized rats. Administration of ACTH increased D-xylose distribution in adrenal tissue but not in muscle. Insulin, on the other hand, increased D-xylose distribution in muscle but had no effect on the adrenal (54).

The uptake of material by the adrenal gland as stimulated by ACTH may not always be beneficial. It has been demonstrated that ACTH will produce hemorrhagic necrosis of the adrenal cortex in the presence of diphtheria toxin (116). In hypophysectomized animals, diphtheria toxin does not have this effect, even in high doses. As is the case with localization of D-xylose in the previous experiment, ACTH tends to localize diphtheria toxin in the adrenal cortex. An interesting point is that the other endocrine organs (*e.g.*, testis, ovary) show no reactivity to the diphtheria toxin with ACTH treatment. However, hemorrhagic necrosis of the target gland is found after treatment with the appropriate gonadotropic hormones. Here again, we observe specificity of the hypophyseal hormones influencing the entry of substances into the target endocrine organs (116). Endocrinopathies, then, can also be produced by a specific action of a noxious agent which is dependent upon the influence of pituitary hormones and which destroys the steroid-producing cells.

It has also been shown that prolonged ACTH treatment may make quantitative changes in the way the adrenal produces steroids. When rabbits (mainly corticosterone producers) were treated with ACTH for 21 to 28 days, the ratio of cortisol to corticosterone produced in adrenal venous blood was found to be 4

(76). Normal, untreated rabbits exhibit a cortisol to corticosterone ratio of 0.05. Very recently it has been discovered that isolated adrenal cortical reticuloendothelial cells perform the hydroxylation of progesterone at position 17 (14a), and cortisol is formed as a final product. The enhanced change in the ratio of cortisol-corticosterone production by ACTH may be explained by the fact that ACTH stimulates reticuloendothelial cell activity and thus enhances the rate of 17α-hydroxylation.

It has been shown that ACTH treatment, surgical stress, and semistarvation together with water deprivation increase blood corticosteroid levels. Experiments with ACTH-infused dogs and stressed rats have shown prolonged half-lives of steroid in plasma (57). That the mechanism of this prolonged half-life of steroids may not be a simple effect of ACTH on the adrenal was demonstrated by showing that the half-lives of blood radioactive steroids in cortisol-4C^{14}-infused, adrenalectomized, ACTH-treated mice were longer than the half-lives in the non-treated controls (44). The half-life of cortisol-4C^{14} isolated from the blood of a normal human subject given ACTH was shown to be 90 to 110 minutes, as compared with 55 minutes when the same subject was given cortisol-4-C^{14} alone (44). In an Addisonian patient, administration of ACTH did not increase endogenous steroid levels but did lengthen the half-life of administered radioactive cortisol (44). Corroborative data have been obtained by De Moor *et al.* (32a) showing that ACTH has an extra-adrenal effect in prolonging the half-life of cortisol in human blood.

It has been shown that endogenous and exogenous ACTH in cortisol-4C^{14}-infused adrenalectomized animals caused a greater retention of cortisol and its metabolites in blood, kidney, thymus, and liver than in non-treated control mice infused with the same amount of cortisol-4C^{14} (13).

The effect of ACTH was shown to parallel that of stress in the inhibition of steroid conjugation in the liver. As compared with normal livers, the livers from hypophysectomized-adrenalectomized animals given ACTH, conjugated less cortisol-4C^{14} and 21-deoxytetrahydrocortisone-4C^{14} as determined by incubation studies. The tetrahydrosubstrate was more actively conjugated by livers of normal animals (11) but the livers from ACTH-treated, hypophysectomized-adrenalectomized animals showed the same decrease in capacity to conjugate steroids with the Δ^4-3-keto structure as toward tetrahydro steroids. This would indicate that the ACTH effect was on the conjugation reaction itself and not the preceding steps of ring A reduction. Furthermore, as Reid has shown, two to three weeks after adrenalectomy, at a time when endogenous ACTH levels are presumably high, the uridine nucleotide levels (including uridinediphosphoglucuronic acid which is required for glucuronide formation) in rat liver are low (95).

2. Influence of thyroid hormones. Steroid turnover in man has been shown to be accelerated in thyrotoxicosis (24, 80, 91, 92). Various steroids which were given to patients having this disease had shorter half-lives in blood than in normal human subjects (91). Tomkins (115) has shown that thyroxin given to rats increases the reduction of the double bond of cortisone. Thyroxin produces

this effect by increasing Δ^4-reductase, according to this author (115). It has also been shown in the reviewers' laboratory that D- or L-triiodothyronine (T-3) when given to mice increases the amount of conjugation of cortisol and corticosterone in their livers (9, 11, 87). It was demonstrated that this was due to an increased reduction of ring A. Furthermore, in myxedematous patients, a decreased excretion of steroids is observed (24, 80). Yates *et al.* have shown that treatment of rats with triiodothyronine increases the capacity of the liver to reduce ring A of cortisone (126). Interestingly enough, hepatic activity per gram of liver increases with T-3 treatment only in male rats, females having higher levels normally. These authors proposed that TPNH availability may be a controlling factor, as TPNH has been shown to be a rate-limiting factor in ring A reduction (118). An adrenal atrophy secondary to surgical removal of 65% of the liver has been demonstrated, indicating hepatic control of adrenal activity under these circumstances. Furthermore, these authors suggested that the capacity of the liver for corticosteroid inactivation determines the rate of ACTH secretion (118). Similarly, the work of Huggins and Yao shows that T-3 treatment increases, and thyroidectomy or hypophysectomy decreases, the levels of DPNH- and TPNH-generating enzymes in the liver (70). In female rats the levels of DPNH- and TPNH-generating enzymes are normally higher. It is interesting to note the similarity of this last feature of female rat liver function to the greater normal Δ^4-steroid hydrogenase activity found for the female by Yates *et al.* (126).

The liver, therefore, could be an organ which controls the output of ACTH by the rate at which it inactivates and eliminates corticosteroids. This process could possibly involve an antagonism of thyroid hormone actions. Thyroid hormone stimulates the reduction of ring A and therefore favors the conjugation of the steroid; ACTH, on the other hand, inhibits the rate of conjugation of steroids (11, 13, 14, 44, 79a).

3. Influence of estrogens. Estrogenic compounds influence the biotransformation and hence the stereochemistry at position 3 of the steroid molecule. They favor formation of the 3α-form when the 3-ketone is reduced to the 3-hydroxyl group. Livers of female mice form more 3α-isomers than do those of males (96). Livers of female rats contain mainly Δ^4-reductase (5α-microsomal) in contrast to those of males which contain the soluble Δ^4-5β reductase as well (57a).

Estrogens can also increase the half-life of corticosteroids in blood (93). There is evidence that the increased half-life of cortisol and corticosterone is due to an increased plasma protein-binding of these steroids, and therefore less free steroid is presented to hepatic and extrahepatic cells for metabolism. Similar findings have been made during pregnancy (26, 31, 84).

The concentration of available (unbound) corticosteroid in the blood may influence the magnitude of biological response at the periphery. Daughaday (30) showed that cortisol was bound more strongly to some other protein in the plasma than to albumin. The strongly-binding protein proved to be an α-2-globulin (different from the thyroxine-binding component) by electrophoretic study. The protein which binds cortisol was named "transcortin" by Slaunwhite

and Sandberg (102). The affinity of steroids other than cortisol for transcortin is considerably less. In pregnancy, cortisol binding is increased (26, 31, 84); estrogens have been shown to increase the binding of cortisol to transcortin (85, 93). The biological half-life of cortisol increases proportionately with the increase in transcortin levels. The hypothesis has been advanced that the transcortin-bound cortisol is biologically inactive because binding makes it unavailable to the cell, and that only unbound cortisol is active (30, 31).

Eik-Nes *et al.* (54a) found that the degree to which a steroid was bound to bovine serum albumin was related to its polarity, *i.e.*, those steroids like cortisone and DOC which have relatively high water solubility are poorly bound, and on the other end of the spectrum steroids like estradiol and 4-androstene-3-one which are not very water-soluble are much more strongly bound. These authors also suggested that the low binding capacity of the polar (hydrophilic) glucuronide conjugates explains their very rapid filtration into the urine.

The binding capacity of the albumin molecule is such that the saturation of binding sites with steroid hormones is never achieved under physiologic conditions (31).

Dialysis experiments *in vitro* with plasma show that if the amount of cortisol added to the dialysis bag is increased beyond a physiological concentration, cortisol begins to bind in significant quantities to albumin after the transcortin binding sites are saturated (31).

The binding forces between neutral steroids and proteins are low energy forces (5 to 10 kcal/mol) and therefore may be best interpreted as hydrogen bonds or van der Waals forces (120a, 120b).

D. Metabolism of cortisol by some target cells

Three of the various biological effects of cortisol (anti-inflammatory, lymphocytokaryorrhectic, and gluconeogenic) have been studied in some detail. As yet we cannot explain exactly how cortisol accomplishes these effects, but the amounts of cortisol required to produce a detectable effect, and the metabolism of the steroid in the target cells can be measured.

1. Inflammation. Inflammation takes place in connective tissue. The most prevalent cell in the connective tissue is the fibroblast, which makes up about 90% of the total cell population. The fibroblast, a mesenchymal derivative, has been shown to be a target cell for corticosteroid anti-inflammatory activity (42, 49, 51, 52).

The normal metabolism of corticosteroid by fibroblasts was determined in order to test the capacity of the cells to convert corticosteroids (5). Particular attention was paid to the metabolism of three steroid hormones which have anti-inflammatory activity—cortisol, corticosterone (8, 17), and 4-pregnene-17α,21-diol-3,20-dione (substance S of Reichstein) (5). The metabolism of these steroids was compared with that of steroids with no anti-inflammatory activity—17α-hydroxyprogesterone and progesterone—and it was shown that reduction at C-20 occurred with both the active and the inactive steroid, suggesting that metabolism of C-20 positions is not required for anti-inflammatory

activity (5, 11). Tissue cultures of pure strains of fibroblasts and fresh mouse loose connective tissue extensively oxidized and reduced the substituent groups of cortisol, corticosterone, and substance S of Reichstein. The cortisol incubation studies (Table 1) showed that mouse loose connective tissue fibroblasts preferentially reduced the C-20 ketone of cortisol to the α-hydroxy configuration, while tissue-cultured fibroblasts favored the formation of the 20β-hydroxy form. This difference is probably a quantitative one. Another quantitative difference was observed in the oxidation of the C-11-hydroxyl group of cortisol to a ketone, yielding cortisone. While loose connective tissue fibroblasts exhibited the capacity to carry out this oxidation, the incubations with tissue-culture fibroblasts produced only small amounts of cortisone (106). Incubations of cortisone with loose connective tissue resulted in the formation of large quantities of cortisol, demonstrating that this tissue can interconvert these two hormones (5, 11).

Experiments were performed *in vivo* with cortisol-4C^{14} to determine the distribution of hormone throughout the body tissues and cells of adrenalectomized mice which had been inflamed at a focal point (50). The amounts of cortisol-4C^{14} and its metabolites were determined in blood, normal loose connective tissue, and inflamed loose connective tissue at intervals following intravenous administration of the labelled steroid. It was found that the half-life of free cortisol in blood was about 40 minutes. Tetrahydrocortisol began to form almost immediately, and its conjugates were present in amounts equal to free cortisol within 20 minutes after administration (50); cortisol and the conjugates were in equilibrium in both blood and connective tissue from 40 to 60 minutes after injection of the cortisol (43). Both inflamed and non-inflamed loose connective tissue contained the labelled free cortisol and its metabolites. The amounts of these substances were greater in the inflamed connective tissue than in the non-inflamed although the same proportion of free to conjugated steroids was maintained in all connective tissue examined. Also, starting from the time of its appearance in the tissue, the curves for the rates of increase and decrease of both free and conjugated tetrahydro cortisol were essentially the same. There were no qualitative differences, as far as compounds were concerned, between the inflamed and the non-inflamed tissues. It was concluded, therefore, that there is no special mechanism which tends to concentrate cortisol in an inflamed area, but rather that the greater amount of this substance in such areas is a result of a non-specific increased localization of many substances from the blood due to edema (43, 50).

The greatest amount of glucuronide formation began at a period when there was a maximum concentration of free cortisol in the connective tissue. Therefore, at that time approximately one-half of the radioactivity present in the connective tissue was from conjugated steroid molecules, which have been shown to lack anti-inflammatory activity (46).

It seems that the mechanisms by which cortisol exerts anti-inflammatory activity are effective within the first 40 minutes after induction of inflammation. Of course, in an intact animal with capacity to supply endogenously produced cortisol an increase of that hormone in the inflamed area could occur. However,

TABLE 1

Metabolism of steroids by fibroblasts

Steroid Incubated	Steroid Isolated
Corticosterone	4-Pregnene-11β,20β,21-triol-3-one**
17OH-Progesterone	† 4-Pregnene-17α,20ξ-diol-3-one*
Progesterone	4-Pregnene-20β-ol-3-one** 4-Pregnene-20α-ol-3-one** Allopregnane-3,20-dione** Allopregnane-20α-ol-3-one**
4-Pregnene-17α,21-diol-3,20-dione	† 4-Pregnene-17α,20ξ,21-triol-3,20-dione*
Cortisol	4-Pregnene-11β,17α,20α,21-tetrol-3-one(20 epi substance "E" of Reichstein)* 4-Pregnene-11β,17α,20β,21-tetrol-3-one (substance "E" of Reichstein)** Pregnane-11β,17α,21-triol-3,20-dione (dihydrocortisol)* Pregnane-11β,17α,21-triol-3,20-dione (dihydrocortisol)* 4-Pregnene-17α,20α,21-triol-3,11-dione (20 epi substance "U" of Reichstein)* 4-Pregnene-17α,20β,21-triol-3,11-dione (substance "U" of Reichstein)** 4-Pregnene-17α,21-diol-3,11,20-trione (cortisone)* 4-Androstene-11β-ol-3,17-dione*, **

These metabolites are isolated and characterized from incubations of 4-C^{14} steroids with tissue cultures and of fibroblasts of loose connective tissue.

* Loose connective tissue of mice.

** Tissue culture fibroblasts.

† Configuration at C-20 not determined at this time.

the major point established here is that following a single administration of cortisol after induction of inflammation, the anti-inflammatory effect will last as long as six hours, despite the fact that 50% of the hormone has been converted to a metabolite which possesses no anti-inflammatory activity within 40 minutes (46). Since inflammation is suppressed after the concentration of cortisol in connective tissue has decreased, it is likely that this hormone induces alterations in the local inflamed tissue which persist and do not require a continuous hormone supply; however, we cannot discount the possibility that the minute amounts of cortisol (not detected by the reviewers' technics) remaining in the tissue were sufficient to maintain the anti-inflammatory action. The mechanism of action by which cortisol might produce its anti-inflammatory effect has been discussed in detail elsewhere (56).

2. Lymphocytokaryorrhexis. The lymphocytokaryorrhectic effect of cortisol has been studied in a similar manner. Labelled cortisol was administered intravenously to adrenalectomized mice (77). The rates of appearance, times of

maximum concentration, and rates of decrease of cortisol and its metabolites were determined for different lymphatic and non-lymphatic organs and tissues at different times after injection. Essentially, it was shown that the maximum concentration in lymphatic organs occurred within five minutes after administration of cortisol. Only 0.1% of the administered radioactivity was present in lymph nodes, 0.2% in the spleen, and 0.3% in the thymus 15 minutes after injection (77). It is surprising that the maximum amounts of these substances found in lymphatic tissue varied from 0.03 to 0.09 mμM of 4-C^{14} labelled cortisol and its metabolites when they were at their maximal concentration. Within 100 minutes, only 0.01% of the injected dose could be found in lymphatic tissues. However, of this amount less than 8% was free cortisol. However, as we have pointed out previously (37, 41), the effects of this hormone on the lymphocytes are most evident at about 45 minutes and persist, again, for as long as six hours. During this time these cells show budding of cytoplasm and interruption of mitoses, and many are destroyed. This is a direct effect of cortisol since it occurs *in vitro* also (41). The suppression of lymphocyte production and the enhanced rate of destruction occur as dose-dependent phenomena (38), and potency ratios for different molecular configurations of cortisol analogs have been published (100). In spite of this dramatic and highly quantitative dose-dependent relationship, it is surprising that exceedingly small amounts of cortisol were present in lymphatic tissues as compared to other tissues. Further, it is interesting that as is the case with the anti-inflammatory effect, the biological response persists when the particular active form of the hormone apparently is no longer present (41).

3. Gluconeogenesis. Similar findings have been reported for the gluconeogenic action of cortisol, in which it was found that gluconeogenesis proceeded for some time after the concentration of cortisol had markedly diminished in the liver (72). Cortisol-$4C^{14}$ and its metabolites disappeared from the liver approximately one hour before the significant increase in glycogen content took place, and two hours before the maximum physiological response (72). Significant increases in glycogen content of hormone-treated animals as compared to controls were found three hours after injection and four hours after intra-gastric administration.

In general, therefore, it appears that as far as these three biological effects of cortisol are concerned, this hormone triggers a mechanism in the target cells (fibroblasts, lymphocytes, and hepatocytes) which continues after the active form of this molecule diminishes to a level which is below that needed to produce the response and may, in fact, all but disappear from the site of action.

E. Abnormal peripheral metabolism

When the physiological and biochemical environment of cells is sufficiently altered by disease or experimentation they may metabolize steroids differently from cells in a normal environment. Malignant cells of various types can metabolize steroids to a greater extent than non-malignant cells. Malignant lymphocytes, fibrosarcoma cells, osteosarcoma cells, prostatic carcinoma cells, and mammary carcinoma cells demonstrate this phenomenon. Also, normal lympho-

cytes repeatedly subjected to high doses of cortisol may develop an increased capacity to metabolize the steroid.

During the course of investigations of cortisol metabolism by lymphocytes, it was found that malignant lymphocytes taken from murine lymphomas metabolized cortisol to several different compounds which were not found following incubation with normal lymphocytes (19). The point was made at the time that this difference in the metabolic activities of the malignant and normal lymphocytes was probably qualitative rather than quantitative (40). By the use of more refined techniques (3, 7, 16) it has been found that some of the metabolites produced by malignant cells can also be made by normal cells in exceedingly small amounts (47).

Normal and malignant lymphocytes were able to oxidize reversibly the substituted oxygen (hydroxyl or ketone) function at C-11 (48). Malignant lymphocytes from murine and human lymphatic leukemias can reduce the C-20 keto group and oxidize the side chain to form C-19 steroids in large amounts (19). Normal lymphocytes can also form these substances, but only in trace amounts. The compounds isolated from cortisol incubations were: cortisone, substances E and U of Reichstein, and 11βOH-androstenedione (19). None of these steroid molecules exerts lymphocyte-suppressing effects. Thus, the cell acted upon tends to produce compounds which are inactive in this respect.

We have proposed that the mechanism which allows the malignant lymphocyte to continue to divide and to resist maturation and death (karyorrhexis) is an increased capacity to inactivate cortisol (40). Immature normal lymphocytes can also inactivate cortisol but much less so than malignant lymphocytes (47, 48).

Other studies *in vitro* demonstrate that malignant cells other than malignant lymphocytes also have a greater capacity to metabolize cortisol than their normal counterparts (119). For example, murine fibrosarcoma cells metabolize cortisol to a much greater extent than normal murine fibroblasts (46). When cortisol-4C^{14} was incubated with two strains of fibroblasts, one of which was sensitive to the growth-inhibiting action of cortisol and the other of which was resistant to cortisol inhibition, both strains metabolized cortisol to the same products, but the resistant strain metabolized twice as much cortisol per unit time as did the sensitive strain (61, 62).

Malignant cells from osteosarcomas of dogs catabolized cortisol not only in tissue slices, but also in cell culture (46). They produced the same metabolites as those formed by fibroblasts, but again in much greater quantity. Surprisingly, unlike lymphocytes, these cells have the ability to form dihydro-derivatives (8, 119).

These findings suggest that the ability of a cell to inactivate a hormone which moderates its growth and enhances its maturation may be essential to malignancy. Several studies performed *in vivo* also tend to strengthen this concept. A comparison of cortisol metabolism was made between normal human subjects and a patient with acute lymphatic leukemia (4, 6, 20). It was found that the patient with leukemia who had received cortisol-4C^{14} excreted in the urine a far greater amount of 11βOH-etiocholanolone-4C^{14} than the normal subjects

(4, 20) who were similarly treated. The production of this compound is representative of the action found for lymphatic leukemia cells *in vitro*. The cells remove the side chain, thus forming 11βOH-androstenedione. This compound, when acted upon by the liver *in vivo*, is transformed to its tetrahydro form, 11βOH-etiocholanolone (Fig. 7). The latter compound was shown by Dobriner (33) many years ago to be increased in the urine of patients having lymphatic leukemia and certain other types of cancer. The meaning of Dobriner's observation is now clearer, since it is now known that the difference is quantitative rather than qualitative, and Dobriner's methods were inadequate to detect the small amounts of 11βOH-etiocholanolone in normal subjects' urine.

Recently, Slaunwhite *et al.* (101) found that after receiving cortisone, patients with carcinoma of the prostate excreted a greater amount of 11-oxygenated-17-keto steroids than did similarly treated normal individuals. The same authors reported a like finding for a patient having carcinoma of the lung (101). Injection of tritiated 17α-hydroxypregnenolone acetate to a patient with an extensively metastasizing primary adrenal tumor (104) revealed that all four C_{19} 17-ketosteroids isolated from the urine contained the isotope (4.3% of the total radioactivity injected; dehydroepiandrosterone accounted for 65% of the total urinary 17-ketosteroids). In contrast, the same study done in a normal male showed so little radioactivity in the urinary 17-ketosteroids (about 0.1% of the total injected) that unequivocal conversion was not demonstrable (104). This study also demonstrates that the presence of the tumor favors the splitting of the side-chain and the formation of C_{19} compounds.

Sokal *et al.* (103) showed that patients with advanced carcinoma of the prostate produce more 17-ketosteroids from cortisone acetate or cortisol than normal individuals. This is also seen occasionally in patients with other tumors, but rarely in individuals without evidence of cancer (103). They pointed out that this metabolic abnormality might depend on the presence of relatively large amounts of tumor cells.

Malignant cells of tumors which respond to estrogenic hormones, such as mammary cancer, are able to catabolize the hormones which regulate the growth of mammary cells (32). For example, it was found that patients with estrogen-sensitive mammary carcinoma catabolized estradiol-4C^{14} less than did patients with insensitive tumors.

In addition to these quantitative abnormalities of steroid metabolism in malignant cells, it is possible that aberrations exist which affect a steroid action other than growth inhibition. For example, it has been shown that inhibition of fibroblast destruction is a fundamental effect of cortisol and that through this mechanism there is an inhibition of the inflammatory response (42). As is the case with growth inhibition, it has been demonstrated that cortisol is the active form of the molecule which inhibits the inflammatory response, and that any conversion of this molecule to any other of the fibroblast-produced derivatives brings about an inactivation of the anti-inflammatory property possessed by cortisol (see Fig. 9). Cortisol is rapidly converted to cortisone by fibroblasts and lymphocytes (5, 11, 48). Cortisone, on the other hand, can revert to cortisol and become an active anti-inflammatory compound (42). The greater the ca-

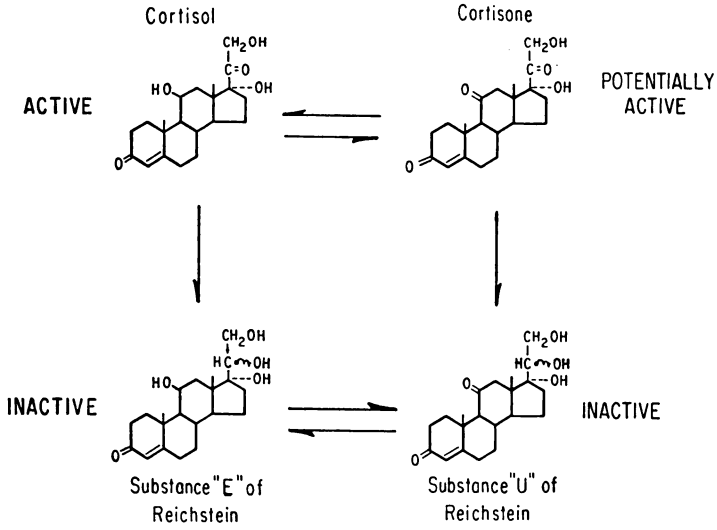


FIG. 9

pacity of the cell to maintain cortisol in this state and to enhance reversibility from cortisone, the greater the anti-inflammatory effect. The converse of this is the displacement of the equilibrium in the cortisol-to-cortisone reaction in favor of cortisone, thus diminishing the anti-inflammatory effect of cortisol (5, 42). In general, then, should the cell be able to increase the amount of conversion to inactive compounds, a chronic lack of anti-inflammatory hormone could exist. Such a situation could arise from the development of greater and greater capacities for cortisol inactivation by cells which are being acted upon by greater and greater levels of cortisol, as has been shown to be the case for lymphatic tissue (47). When mice were given very large doses of cortisol daily for a prolonged period it was found that the lymphocytes derived from their thymi had increased enormously (by 217%) their capacity for 11β-hydroxydehydrogenase activity in converting cortisol to cortisone (47). As yet it is not known whether this is also true for fibroblasts in chronic disease states.

It is evident that inflammatory disease, acute or chronic, once initiated, might be exacerbated or ameliorated as a result of deficiency of the biologically active hormone, due to alterations in cellular metabolism which could arise, in turn, from enzymatic defects or cofactor imbalances, or a combination of both. According to a theory which has been proposed elsewhere (42), peripheral cells may influence the disease process by their own capacities to metabolize cortisol, and disease may result from quantitatively or qualitatively altered hormone metabolism at the cellular level.

SUMMARY

It appears from a review of the work of numerous authors that cells of many types, including mammalian and bacterial cells, can metabolize steroid hormones. Different cells of the mammal have quantitatively and qualitatively different

capacities to perform steroid metabolism. Biosynthesis of steroids appears at present to be an exclusive property of cells derived from the genital ridge. The steroid hormones, once synthesized, have two fates. The first is that the substituted groups of the steroid nucleus are oxidized or reduced to form other steroid molecules. Some of these metabolites possess known functions. In the process of conversion, however, the transformed steroid usually loses, either quantitatively or qualitatively, its original biological activity but may acquire a different function. The conversion of one steroid hormone to another by peripheral cells other than those capable of biosynthesis has been called biotransformation. A second fate is the conjugation of steroid metabolites which can then be excreted.

Many peripheral cells perform the first type of metabolic transformation, but only the liver has been shown so far to be able to form tetrahydrocorticosteroids, which is an essential step before conjugation. The kidney can conjugate corticosteroids provided they are already in the tetrahydro form. The oxidation and reduction of steroid molecules (biotransformation) may be reversible or irreversible. The degree of reversibility thus regulates partly the necessity for replenishing the supply of the steroid hormone.

The concept of active and inactive steroid hormones is used to indicate the presence or absence of a particular biological activity which is dependent upon the presence or absence of certain substituent groups on the steroid nucleus.

Prevention of oxidation or reduction of the steroid hormone keeps it in the active state, since the original structure-activity relation is maintained. It appears, although it has not been proved in each case, that increased potency of synthetic steroid hormones is due in part to the fact that essential positions for activity are protected from normal metabolism. The point of view presented here is that the actual mechanism of action for most if not all steroid hormones is still not understood and that the metabolism of these hormones is related not to mechanism of action but to their turnover, which imposes requirements for steroid renewal.

Non-steroidal hormones which influence steroid hormone biosynthesis, such as pituitary hormones, also directly influence steroid metabolism. Other hormones may influence steroid hormone metabolism by more indirect means—increasing their biosynthesis, biotransformation and conjugation, and excretion. The major emphasis in this review has been upon the steroid metabolism of lymphocytes, fibroblasts, reticuloendothelial cells, hepatocytes, and their malignant counterparts. These cells appear to be among the most important target cells for corticosteroid activity.

Recent findings indicate that biological activity exists for metabolites which were thought to be inactive. Other metabolites may possess important functions which are at present unknown. The concept of biotransformation may have future practical and scientific importance because it may be possible to control the production of particular metabolites and thus control certain biological responses. For example, testosterone, an androgenic hormone, can be transformed to pyrogenic or hypocholesterolemic steroids. Estrogens seem to favor

the formation of the metabolite which has hypocholesterolemic effect. It is possible that thyroid hormone might have a similar effect. The control of the preferential production of the pyrogenic steroid has not been investigated. In the opinions of the reviewers, the implications of this field of steroid hormone research for a further understanding of physiological and pathological processes are enormous.

The importance of abnormal metabolism by malignant cells may lead to new points of view concerning the fundamental processes of malignant growth and are already important in the diagnosis of some malignancies. Further research along this line offers rational avenues to the development of new steroid hormones for treatment of some types of cancer.

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